CUK - H06809 - 229 - RP359

NATURE

INTERNATIONAL JOURNAL OF SCIENCE

229

Volume 225

JANUARY 3, 1970, to MARCH 28, 1970

LONDON
MACMILLAN (JOURNALS), LTD.
PRICE: 2s.

DATES and PAGINATION of WEEKLY ISSUES

Serial No.	*Date of issue*		*Pagination*
5227	January	3	1–112
5228	,,	10	113–208
5229	,,	17	209–304
5230	,,	24	305–396
5231	,,	31	397–484
5232	February	7	485–572
5233	,,	14	573–672
5234	,,	21	673–776
5235	,,	28	777–884
5236	March	7	885–984
5237	,,	14	985–1084
5238	,,	21	1085–1176
5239	,,	28	1177–1272

PP359

AUTHOR INDEX

A

Aaronson, Stuart A., and Todaro, George J.: Transformation and Virus Growth by Murine Sarcoma Virus in Human Cells, 458
Abbott, A. J., and Webb, R. A.: Achene Spacing of Strawberries as an Aid to Calculating Potential Yield, 663
Abbott, Joan: Absence of Blood-Brain Barrier in a Crustacean, *Carcinus maenus* L., 291
Abou-Chaar, Charles I.: Alkaloids of an *Ipomea* Seed known as *Xaladana* in Pakistan, 663
Acs, G.: *see under* Kapuler, A. M., 1209
Adams, J. Frank: Lectures on Lie Groups, *review* by R. W. Carter, 976
Adinolfi, M.: *see under* Glynn, A. A., 77
Adler, A., and Brenner, W.: New Durable Flame Retardant Finish for Polypropylene Textiles, 60
Agallianos, D.: *see under* Messiha, F. S., 868
Agrios, George N.: Diseases of Higher Plants, *review* by R. K. S. Wood, 107
Ajakaiye, Deborah E.: Gravity Measurements over the Nigerian Younger Granite Province, 50
Akagane, K.: *see under* Allan, G. G., 175
Alabaster, V. A.: *see under* Ferriera, S. H., 379
Alfvén, Hannes: Poor Man's Guide to Celestial Mechanics, 229
Allan, G. G., Akagane, K., Neogi, A. N., Reif, W. M., and Mattila, T.: Physical Entrapment of Polyelectrolytes within Microporous Solids: the "Jack-in-the-Box" Effect, 175
Allan, H. R., Clay, R. W., and Jones, J. K.: Frequency Spectrum of Air Shower Radio Pulses, 253
Allardyce, Randall A., Hunter, Robert D., and James, Keith: Effect of Adjuvants on the Immunosuppressive Properties of Antilymphocytic Globulin, 1141
Allen, J. R. L.: Methods for the Study of Sedimentary Structures, *review*, 666
Allen, R. J.: *see under* Davies, J., 1240
Aller, Hugh D.: Variations of Linear Polarization in Extragalactic Radio Sources, 440
Allison, A. C.: *see under* Burnstein, N. A., 1139
Alper, Paul: *see under* Armitage, Peter, 1075
Altenhoff, W. J., and Wilson, T. L.: Percentage Number of Nonthermal Sources in the Northern and Southern Halves of the Galaxy, 245
Amaldi, F.: *see under* Buongiorno-Nardelli, M., 946
Anderson, Bryan, Hodgkin, Dorothy Crowfoot, and Viswamitra, M. A.: The Structure of Thiostreptin, 233
Anderson, N. F.: *See under* Dalton, R. G., 1140
Andreou, D., and Bali, L. M.: Frequency Mixing in the Scattering of Light by Harmonically Bound Electrons, 1239
Andrews, J. G., and Varey, R. H.: Source of Electrons in a Selected Energy Range (SESER), 270
Anfinsen, Christian B.: *see under* Ontjes, David A., 189
Angrist, B. M., Schweitzer, J. W., Friedhoff, A. J., and Gershon, S.: Investigation of *p*-Methoxyamphetamine Excretion in Amphetamine Induced Psychosis, 651
Antero, G.: *see under* Su, Shirley, 62
Apparao, M. V. Krishna: Pulsars and the Universal X-ray Background, 836
Arai, Toshihiko: *see under* Watanabe, Tsutomu, 70
Arai, Yoshiko: *see under* Chihara, Goro, 943
Arakelian, M. A.: Evidence of Radio Luminosity Evolution of Quasi-stellar Radio Sources, 358
Archibald, A. R.: *see under* Hepinstall, S., 519
Armbrecht, Bernard H., Shalkop, William T., Rollins, Larry D., Pohland, Albert E., and Stoloff, Leonard: Acute Toxicity of Aflatoxin B_1 in Wethers, 1062
Armitage, Peter, Smith, Cyril, and Alper, Paul: Decision Models for Educational Planning, *review* by John Vaizey, 1075
Arnold, Elsie: *see under* Epstein, Samuel S., 1260
Arora, S. K., Varghese, T. G., and Krishnan, C. A.: Some Aspects of the Structure of Southern India based on Recent Bhadrachalam Earthquakes, 261
Arp, Halton: Redshifts of Companion Galaxies, 1033
Arranaga, A. B.: Friction Reduction Characteristics, of Fibrous and Colloidal Substances, 447
Ascher, P.: *see under* Kehoe, JacSue, 820
Asfari, A. F.: *see under* Francis, J. R. D., 725
Asher, Yael: *see under* Becker, Yechiel, 454
Asikainen, M.: *see under* Blomqvist, L., 58
Atkin, A. O. L.: Modular Forms and Dirichlet Series, *review*, 1077
Atkins, E. D. T., Mackie, W., and Smolko, E. E.: Crystalline Structures of Alginic Acids, 626
Atsatt, P. R.: Hemiparasitic Flowering Plants: Phenotypic Canalization by Hosts, 1161
Austin, J.: *see under* Briggs, Michael, 81
Axelrod, Julius, Mueller, Robert A., Henry, James P., and Stephens, Patricia M.: Changes in Enzymes involved in the Biosynthesis and Metabolism of Noradrenaline and Adrenaline after Psychosocial Stimulation, 1059
Axford, W. I.: *see under* Banks, P. M., 924
Ayer, A. J.: Metaphysics and Common Sense, *review* by John Watling, 767

B

Bach, Fritz H.: *see under* Janis, Martin, 238
Bacharach, A. D. E.: *see under* Hall, Michael O., 637
Bachman, Charles H.: *see under* Spadaro, Joseph A., 1134
Badash, Lawrence, Edit.: Rutherford and Boltwood, *review* by A. S. Russell, 201
Baddeley, A. D.: *see under* Lewis, J., 95
Baddiley, J.: *see under* Hepinstall, S., 519
Baden, Howard P.: *see under* Goldsmith, Lowell A., 1052
Bagg, D. G.: A-Levels and University Performance, 1105
Bagley, B. G.: Five-fold Pseudosymmetry, 1040
Bailey, A. S.: Organic Charge-Transfer Complexes, *review*, 299
Bailey, J. M.: Planetary Fission Events: Dynamic Constraints, 48
Baker, Francis S.: Field Emission from Silicon Carbide Whiskers, 539
Baker, R. J.: Parasitic Protozoa, *review* by D. Walliker, 205
Baker, Ralph: *see under* Hora, T. S., 1071
Bakhle, Y. S.: *see under* Ferriera, S. H., 379
Balaban, Alexandru T.: Pulses, Units, Pitts and Hertz, *correspondence*, 1270
Bali, L. M.: *see under* Andreou, D., 1239
Baller, Julie: *see under* Uhlenbeck, Olke C., 508
Banerjee, S. P.: *see under* Tobin T., 745
Banerjee, Subir K., Johnson, C. E., and Krs, Miroslav: Mossbauer Study to find the Origin of Weak Ferromagnetism in Cassiterite, 173
Banks, C. J.: *see under* Eastop, V. F., 970
Banks, P. M., and Axford, W. I.: Origin and Dynamical Behaviour of Thermal Protons in the Venusian Ionosphere, 924
Barbanti-Brodano, Giuseppe: *see under* Koprowski, Hilary, 1045
Barbier, Marcel: Induced Radioactivity, *review* by R. W. P. Drever, 1170
Barker, Clyde F., and Billingham, R. E.: Comparison of the Fates of Ag-B Locus Compatible Homografts of Skin and Hearts in Inbred Rats, 851
Barker, Lewellys F.: *see under* Mosley, James W., 953
Barlow, Peter: Précis de Biologie Végétale, *review*, 878
Barman, Thomas E.: Enzyme Handbook, *review* by K. F. Tipton, 108
Barnaby, G. F.: Tongues of Conscience, *review*, 105
Barnard, R. James: *see under* Edgerton, V. R., 866
Barnes, J., Bellamy, D. J., Jones, D. J., Whitton, B. A., Drew, E., and Lythgoe, J.: Sublittoral Reef Phenomena of Aldabra, 268
Barnes, K. S., and Peach, G.: The Shape and Shift of the Resonance Lines of Ca^+ produced by Electron Collisions, 374
Barnes, Richard A.: *see under* Levitsky, David A., 468
Barnett, Don R., Lee, Tong-Ho, and Bowman, Barbara H.: Amino-acid Sequence of the Carboxyl Terminal Octapeptide of Human Haptoglobin β Chain, 938
Barnothy, Jeno M.: *see under* Barnothy, Madelaine F., 1146
Barnothy, Madelaine F., and Barnothy, Jeno M.: Magnetic Fields and the Number of Blood Platelets, 1146
Barondes, Samuel H.: *see under* Squire, Larry R., 649
Barut, A. O., Edit.: Scattering Theory, *review* by M. H. Choudhury, 668
Bassett, C. Andrew: *see under* Sherwood, Louis, 1065
Bateson, P. P. G.: *see under* Rose, S. P. R., 650
Bartholomai, Alan, and Howie, Anne: Vertebrate Fauna from the Lower Trias of Australia, 1061
Battaglia, P. A.: *see under* Morpurgo, G., 76
Battersby, A. R.: *see under* Taylor, W. I., 1266
Bauer, S. J., Hartle, R. E., and Herman, J. R.: Topside Ionosphere of Venus and its Interaction with the Solar Wind, 533
Bautz, E. K. F.: *see under* Schmidt, D. A., 1012
Baxter, M. S., and Walton, A.: Radiocarbon Dating of Mortars, 937
Baym, Gordon: *see under* Sutherland, Peter, 353
Beals, C. S.: Impact Craters and the Relative Ages of Earth and Moon, 368
Beals, Ralph L.: Politics of Social Research, *review* by Leslie Sklair, 297
Beatty, R. A.: Understanding Media, *correspondence*, 1082
Beaumont, T. G., and Davis, K. M. C.: Charge Transfer Complexes of Tropylium and *N*-Heterocyclic Cations with Aromatic Amines, 632
Becanos-Kontos, Th.: *see under* Ignatiades, Lydia, 293
Beck, Elizabeth: *see under* Bignami, A., 194
Becker, Robert A.: *see under* Spadaro, Joseph A., 1134
Becker, Yechiel, Asher, Yael, Himmel, Nehama, and Zakay-Rones, Zichriya: Anti-trachoma Activity of Rifampicin and Rifamycin SV Derivatives, 454
Beggs, W. D. A., and Howarth, C. I.: Movement Control in a Repetitive Motor Task, 752
Békésy, Georg von: Travelling Waves as Frequency Analysis in the Cochlea, 1207
Beklemishev, W. N.: Principles of Comparative Anatomy of Invertebrates, *review* by R. P. Dales, 1079
Beleslin, D. B.: Nature of the Peristaltic Block produced by Magnesium, 383
Bell, B.: *see under* Neurath, P., 281
Bellamy, D. J.: *see under* Barnes, J., 268
Belozersky, A. N.: *see under* Vanyushkin, B. F., 948
Benda, Philippe, Tsuji, Shigeru, Daussant, Jean, and Changeux, Jean-Pierre: Localization of Acetylcholinesterase by Immunofluorescence in Eel Electroplax, 1149
Bender, E. W.: *see under* Dochinger, E. S., 476
Bennett, J. E., Mile, B., and Summers, R.: Electron Spin Resonance of Hydrogen Atoms in a Free-burning Flame at Atmospheric Pressure, 932
Bennett, Leslie R.: *see under* Byfield, John E., 859
Bennich, H. H.: *see under* Turner, M. W., 853
Benson, S. S.: *see under* Patton, Stuart, 754
Berenbaum, M. C.: Immunosuppression by L-Asparaginase, 550
Bergel, F.: Keep off the Grass, *correspondence*, 979
Bergh, Sydney van den: Extra-galactic Scale, 503
Berkhuijsen, Elly M., Haslam, C. G. T., and Salter, C. J.: The Galactic Continuum Spurs and Neutral Hydrogen, 364
Berl, S.: *see under* Puszkin, S., 558
Berman, M. C., Harrison, G. G., Bull, A. B., and Kench, J. E.: Changes underlying Halothane-induced Malignant Hyperpyrexia in Landrace Pigs, 653
Bernard, B. de: *see under* Stagni, N., 942
Bernard, J., Paul, R., Boiron, M., Jacquillat, Cl., and Maral, R., Edit.: Rubidomycin, *review* by F. G. J. Hayhoe, 879
Bernoulli, Daniel: Hydrodynamics, *review* by J.R. Ravetz, 1167
Bertrand, Mireille: The Behavioural Repertoire of the Stumptail Macaque, *review* by Y. Spencer-Booth, 1080
Bétuel, H., Richard, G. B., Colobert, L., and Revol, L.: Effect of A:S Globulins on Amino-acid Incorporation by Human Peripheral Lymphocytes, 459
Bevan, N. E.: *see under* Timbrell, V., 97
Beverton, R. J. H.: NERC Support for University Research, *correspondence*, 481
Beynon, W. J. G.: Introduction to Ionospheric Physics, *review*, 568
Bezanilla, F.: *see under* Rojas, E., 747
Bignami, A., Beck, Elizabeth, and Parry, H. B.: Neurosectrion-like Material in the Hindbrain of Ageing Sheep and Sheep Affected with Natural Scrapie, 194
Billingham, R. E.: *see under* Barker, Clyde, F., 851
Bird, Robert E., Lark, Karl G., Curputte, Basil, and Maxfield, John E.: Exchange of Bound Tritium, between DNA and Water at Different Temperatures, 1043
Birnbaum, Allan: Statistical Methods in Scientific Inference, 1033

INDEX

Boiron, M.: see *under* Bernard, J., 879
Bishop, D. G.: *see under* Roberts, D. G., 170
Bishop, Yvonne: *see under* Arnold, Elsie, 1260
Bitensky, Lucille: Lysosomes in Biology and Pathology, *review*, 877
Bitensky, Lucille: *see under* Chayen, J., 1050
Black, Ira B.: Induction of Hepatic Tyrposine Aminotransferase mediated by a Cholinergic Agent, 648
Blaich, R.: see *under* Curio, E., 750
Blake, Richard D., Fresco, Jaques R., and Langridge Robert: High-resolution X-ray Diffraction by Single Crystals of Mixtures of Transfer Ribonucleic Acids, 32
Blakemore, Colin, and Pettigrew, John D.: Eye Dominance in Visual Cortex, 426
Blanpied, William A.: Physics, *review* by E. J. Wenham, 392
Blaug, Mark, Layard, Richard, and Woodhall, Maureen: The Causes of Graduate Unemployment in India, *review* by John Vaizey, 297
Blomqvist, L., and Asikainen, M.: Tungsten-181 in Fallout in Finland, 58
Blood, Frank R., Edit.: Essays in Toxicology, *review* by R. T. Williams, 669
Bloor, Kenneth: Adding a Ventricle instead of transplanting a heart, 1142
Bodsworth, C.: Electro-Slag Refining, *review*, 478
Bollen, W. B.: see *under* Bulla, L. A., jun., 664
Bonhamn, K. G.: *see under* Lewis, J., 95
Bonnor, W. B.: Charge Moving with the Speed of Light, 932
Bonomo, F. S.: see *under* Goldman, A., 443
Bonser, G. M.: see *under* Carters, R. L., 1081
Borsellino, Antonio, Pierantoni, Ruggero, Schieti-Cavazza, Barbara: Survival in Adult Mealworm Beetles (*Tenebrio molitor*) of Learning acquired at the Larval Stage, 963
Bos, R. C. van den, and Planta, R. J.: Structural Comparison of 26S and 17S Ribsonal RNA of Yeast, 183
Bose, Ajay K.: see *under* Manhas, Maghar, S., 976
Bosia, G., Castagnoli, C., Dardo, M., and Marangoni, G.: Observation of Structure in Čerenkov Pulses from Extensive Air Showers using Fast Techniques, 532
Bott, M. H. P., and Watts, A. H.: Deep Sedimentary Basins proved in the Shetland-Hebridean Continental Shelf and Margin, 265
Bottema, O., Djordjević, R. Z., Janić, R. R., Mitrinović, D. S., and Vasic, P. M.: Geometric Inequalities, *review* by James Hirschfield, 1268
Bough, Edward W.: Stereoscopic Vision in the Macaque Monkey: a Behavioural Demonstration, 42
Boulton, Alan A.: *see under* Majer, J. R., 658
Bouma, Arnold H.: Methods for the Study cf Sedimentary Structures, *review* by J. R. L. Allen, 666
Bourne, W. R. P.: The Third London Airport, *correspondence*, 773
Boustead, I.: Molecular Oxygen as an Electron Trap in the Thermoluminescence of Saturated Hydrocarbons, 846
Bowman, Barbara H.: *see under* Barnett, Don R., 938
Bownds, D., and Gaide-Huguenin, A. C.: Rhodopsin Content of Frog Photoreceptor Outer Segments, 870
Bowyer, C. S., Mack, J., and Lampton, M.: X-ray Scattering by Grains in the Direction of the Crab Pulsar and Sco XR-1, 1125
Bowyer, C. Stuart, and Field, George B.: *reply* to 'Intensity of the Soft X-ray Background Flux: Reply to Bowyer and Field (R. C. Henry, G. Fritz, J. F. Meekins, H. Friedman and E. T. Byram), 362
Bradbury, E. M., Carpenter, B. G., Crane-Robinson, C., and Goldman, H.: Orientation of Polypeptide Side Chains in Solution, 64
Bradfield, R. B., and Jelliffe, R. F. P.: Early Assessment of Malnutrition, 283
Bradley, D. J., Gale, G. M., and Smith, P. D.: Self-induced Transparency and Dispersion Delays in Potassium Vapour, 719
Bradshaw, Ralph A.: *see under* Neurath, Hans, 881
Brain, C. K.: New Finds at the Swartkrans Australopithecine Site, 1112
see under Clarke, R. J., 1219
Brain, M. C.: *see under* White, J. M., 939
Bramwell, Cherrie D., and Whitfield, G. R.: Flying Speed of the Largest Arial Vertebrate, 660
Brandie, G. W.: Excess Surface Density of Extragalactic Radio Sources at 8 GHz, 352
Bren, Donna: see *under* Flurry, R. L., jun., 729
Brenner, W.: *see under* Adler, A., 60
Brereton, J. Le Gay: *see under* Webb, L. J., 768
Brereton, R. G.: *see under* Burke, J. D., 1234
Brèsciani, F.: *see under* Puca, G. A., 1251
Bresler, S., Mosevitsky, M., and Vyacheslavov, L.: Complete Mutagenesis in a Bacterial Population induced by Thymine Starvation on Solid Media, 764
Brewer, D. F.: Helium-3 and Helium-4, *review*, 771
Bridle, A. H.: Low-frequency Spectrum of the Crab Nebula, 1035
and Caswell, J. L.: Decametric Variability of Cas A and 3C 84, 356
Briggs, M. H.: Hormonal Contraception, *review*, 299
Briggs, Michael Austin J., and Staniford, Maxine: Oral Contraceptives and Copper Metabolism, 81
Bright, D., and Truter, Mary R.: Crystal Structure of Cyclic Polyether Complex of Alkali Metal Thiocyanate, 176
Britton, H. G.: Exchange Diffusion, 746
Brocklehurst, M.: Level Population of Excited States of Hydrogen in Gaseous Nebulae, 618
Brooks, John, R. V.: Engineering Geology and Rock Mechanics, *review*, 667
Broten, N. W.: *see under* Galt, J. A., 530
Brown, Dorothy J.: *see under* Eisner, Thomas, 661
Brown, L. V.: *see under* Kaplan, A. E., 384
Bruce, D. J., Evans, C. R., Fenwick, P. B. C., and Spencer, Valerie: Effect of Presenting Novel Verbal Material during Slow-wave Sleep. 873
Bruck, M. T., Lawrence, L. C., Nandy, K. N., Thackeray, A. D., and Wood, R.: The Extinction Law in the Magellanic Clouds, 531
Bruere, A. N.: *see under* Kilgour, R., 71
Buck, K. W., and Kempson-Jones, Gillian F., 945
Buckingham, A. D.: Elementary Molecular Bonding Theory, *review*, 478
Buerger, A. A., and Fennessy, A.: Learning of Leg Position in Chronic Spinal Rats, 751
Buettner-Janusch, John: The Social Sciences, *correspondence*, 775
Bull, A. B.: *see under* Berman, M. C., 653
Bulla, L. A. jun., Gilmour, C. M., and Bollen, W. B.: Non-biological Reduction of Nitrite in the Soil, 664
Bullough, K., and Sagredo, J. L.: Longitudinal Structure in the Plasma Pause: VLF Goniometer Observations of Knee-whistlers, 1038
Buneman, O. P.: *see under* Willshaw, D. J., 177
Bunkenburg, J.: *see under* Chance, B., 705
Buongiorno-Nardelli, M., and Amaldi, F.: Autoradiographic Detection of Molecular Hybrids between rRNA and DNA in Tissue Sections, 946
Burch, G. E., Sohal, R. S., and Fairbanks, L. D.: Senescent Changes in the Heart of *Drosophila repleta* Wollaston, 286
Burch, P. R. J.: New Approach to Cancer, 512
Burke, J. D., Brereton, R. G., and Muller, P. M., Desert Stream Channels resembling Lunar Sinuous Rilles, 1234
Burns, B. Delisle, and Webb, A. C.: Spread of Responses in the Cerebral Cortex to Meaningful Stimuli, 469
Burstein, N. A., and Allison, A. C.: Effect of Antilymphocyte Serum on the Appearance of Reticular Neoplasms in SJL/J mice, 1139
Burstein, Sumner H., Menezes, Franco, Williamson, Ethel, and Mechoulam, Raphael: Metabolism of $\triangle^1$(6)-Tetrahydrocannabinol, an Active Marihuana Constituent, 87
Burnstein, T.: *see under* Byington, D. P., 554
Burrows, E. M.: Marine Algae, *review*, 106
Burtin, F.: *see under* Rivat, Liliane, 1136
Butcher, G. A.: *see under* Cohen, S., 732
Butler, William T.: *see under* Mittal, Kamal, K., 1255
Buvat, Roger: Plant Cells, *review* by D. H. Northcote, 391
Byers, Sanford, O.: *see under* Freidman, Meyer, 464
Byfield, John E., Lee, Young C., and Bennett, Leslie R.: Bonding of Small Molecules to DNA following Ionizing Radiation, 859
Byington, D. P., Castro, A. E., and Burnstein, T.: Adaptation to Hamsters of Neurotropic Measles Virus from Subacute Sclerosing Panencephalitis, 554
Byram, E. T.: *see under* Henry, R. C., 362

C

Cahn, R. W.: Case Histories of Innovations, 693
Calder, A. B.: Photometric Methods of Analysis, *review* by T. S. West, 202
Caldwell, R. C.: *see under* Pruitt, K. M., 1249
Cameron, A. G. W.: *see under* Truran, J. W., 710
Campbell, D. B., Heiles, C., and Rankin, J. M.: Pulsar *NP* 0532: Average Polarization and Daily Variability at 430 MHz, 527
Campbell, I. D.: *see under* Chapman, G. E., 639
Campbell, K. S. W., Edit. Stratigraphy and Palaeontology, *review* by J. Shirley, 1078
Cannon, D. F.: *see under* Davies, J., 1240
Canterford, J. H., and Colton, R.: Halides of the Second and Third Row Transition Metals, *review* by D. J. Machin, 203
see under Colton, R., 203
Canuto, V., Chiu, H. Y., Chiuderi, C., and Lee, H. J.: New Source of Intense Magnetic Fields in Neutron Stars, 47
Canuto, Vittorio: *see under* Chiu, Hong-Yee, 1230
Cariff, Lawrence J., and Scargle, Jeffrey D.: Coherent Synchrotron Emission in the Crab Nebula, 168
Carpenter, B. G.: *see under* Bradbury, E. M., 64
Carr, Ronald E., and Siegel, Irwain M.: Action Spectrum of the Human Early Receptor Potential 88
Carrel, James: *see under* Eisner, Thomas, 661
Carstairs, G. M.: Psychiatry in a Changing Society, *review*, 565
Carter, R. L.: *see under* Roe, F. J. C., 1081
Carter, R. W.: Lectures on Life Groups, *review*, 976
Carter, S. B.: Cell Movement and Cell Spreading: a Passive or an Active Process? 858
Carter, C. O.: An ABC of Medical Genetics, *review* by E. H. R. Ford, 480
Carthy, J. D.: Communication, *review*, 481
Caskey, T.: *see under* Scolnick, E., 152
Castagnoli, C.: *see under* Bosia G., 532
Castenholtz, Richard W.: *see under* Halfen, Lawrence, N., 1163
Castro, A. E.: *see under* Byington, D. P., 554
Caswell, J. L.: see *under* Bridle, A. H., 356
Cavenor, M. C.: Wall Precursor Effects in Gaseous Detonation, 718
Chamberlain, A. C.: Deposition and Uptake of Airborne Particles by Cattle, 99
Chambers, W. H., Fehlau, P. E., Fuller, J. C., Kunz, W. E.: Anisotropic Atomic Hydrogen Distribution in Interplanetary Space, 713
Chance, B., McCray, J. A., and Bunkenburgh, J.: Fast Spectrophotometric Measurement of H^+ Changes in *Chromatium* Chromatophores activated by a Liquid Dye Laser, 705
Changeux, Jean-Pierre: see *under* Benda, Phillippe, 1149
Chapman, G. E., Campbell, I. D., and McLauchlin, K. A.: NMR Investigation cf Deuterated Collagen, 639
Chatterjee, B. K., Murthy, G. T., Murthy, P. V. Ramana, Sreekantan, B. V., and Tonwar, S. C.: Search for High Energy Gamma Rays from Four Pulsars, 839
Chayen, J., Bitensky, Lucille, and Poulter, L. W.: Histochemistry of some Lysosomal "Proteolytic Enzymes of Human Rheumatoid Synovia, 1050
Chen, David, and Osborne, Daphne J.: Ribosomal Genes and DNA Replication in Germinating Wheat Embryos, 336
Chen, L., and Watkins, J. F.: Evidence against the Presence of H2 Histocompatibility Antigens in Ehrlich Ascites Tumour Cells, 734
Chenery, D. H.: see *under* Kennard, Olga, 333
Chenoweth, M. B.: see *under* Schneider, C. W., 1262
Cherry, Sheldon: *see under* Lisgar, Fredrica, 280
Chessman, Mary: *see under* Horai, Ki-Iti, 264
Chihara, Goro, Hamuro, Junji, Maeda, Yukiko, Arai, Yoshiko, and Fukuoka, Fumiko: Antitumour Polysaccharide derived Chemically from Natural Glucan (Pachyman), 943
Chiu, Hong-Yee, and Canuto, Vittorio: Bremsstrahlung Radiation in an Intense Magnetic Field and Emission from Pulsars, 1230
Warasila, Robert L., and Reno, John L., edit.: Stellar Astronomy, *review* by B. E. J. Pagel, 202
see under Canuto, V., 47
Chiuderi, C.: *see under* Canuto, V., 47
Chlap, Z.: *see under* Ptak, W., 655
Cho, Seung-Am, and Wuensch, B. J.: Crystal Chemistry of the Plagionite Group, 444
Chopra, D. P., and Simnett, J. D.: Stimulation of Mitosis in Amphibian Kidney by Organ Specific Antiserum, 657
Choquet, Gustave: Lectures on Analysis, *review* by D.M.A. Edwards, 880
Chothia, Cyrus: Interaction of Acetylcholine with Different Cholinergic Nerve Receptors, 36
Choudhury, M. H.: Scattering Theory, *review*, 668
Chow, Tsaihwa J.: Lead Accumulation in Roadside Soil and Grass, 295
Christiansen, J. M.: *see under* Slotin, Cecilia A., 385
Christiansen, W. N., and Högbom, J. A.: Radiotelescopes, *review* by R. C. Jennison, 974
Christou, A.: Influence of Shock Deformation on the Transverse Magnetoresistivity of Polycrystalline Iron, 372
Chu, Hsien Ming: *see under* Kok, Loke Tuck, 661
Chua, S. E.: Phytokinin-like Activity of 8-Quinolinol Sulphate, 101
Ciaranfi, E.: *see under* De-Angeli, Lavinia Carta, 549
Ciferri, A.: see *under* Volpin, D., 382
Cikes, Matko: Antigenic Expression of a Murine Lymphoma during Growth *in vitro*, 645
Clapham, A. R.: The Environmental Revolution, *review*, 389
Clark, Allen W., Mauro, Alexander, Longenecker, Herbert E., jun., Hurlbut, William P.: Effect on the Fine Structure of the Frog Neuromuscular Junction ,703
see under Longenecker, Herbert E., jun., 703
Clarke, Geoffrey M.: Statistics and Experimental sign, *review* by K. L. Q. Read, 770
Clarke, M. C., and Haig, D. A.: Evidence for the Multiplication of Scrapie Agent in Cell Culture, 100
Clarke, R. J., Howell, F. Clark, and Brain, C. K.: More Evidence of an Advanced Hominid at Swartkrans, 1219
Clarke, R. H.: Microbarograph Observation of Acoustic Gravity Waves, 535
Clausen, J.: Immunochemical Techniques for the Identification and Estimation of Macromolecules, *review* by N. Shaw, 976
Clay, R. W.: see *under* Allan, H. R., 253
Clinton, D.: *see under* Wilson, A. D., 272
Cloot, P. L.: The Critical Path Method, *review*, 668
Cloudsley-Thompson, J. L.: Deserts of the World, *review*, 768
Clower, C.: *see under* Messiha, F. S., 868
Cocking, E. C.: *see under* Power, J. B., 1016
Coetzee, J. F., and Ritchie, Calvin D., edit.: Solute-Solvent Interactions, *review* by J. A. Salthouse, 1170
Cohen, S., and Butcher, G. A.: Properties of Protective Malarial Antibody, 732

Cohn, Melvin: see *under* Schubert, David, 154
Cole, T. W., Hesse, H. K., and Page, C. G.: Long Term Variations of Pulsar Intensities, 712
Colgate, Stirling A.: Ejection of Companion Objects by Supernovae, 247
Collieu, A. McB.: see *under* Hulme, H. R., 479
Collins, G. G. S., Sandler, M., Williams, E. D., Youdim, M. B. H.: Multiple Forms of Human Brain Mitochondrial Monoamine Oxidaze, 817
Colobert, L.: *see under* Bétuel, H., 459
Colton, R., and Canterford, J. H.: Halides of the First Row Transition Metals, *review* by D. J. Machin, 203
  see *under* Canterford, J. H., 203
Comfort, Alex: The Immunologic Theory of Ageing, *review*, 1265
Comline, R. S., Silver, Marian, and Silver, I. A.: Effect of Foetal Hyphopysectomy on Catecholamine Levels in the Lamb Adrenal during Prolonged Gestation, 739
Cooke, G. W.: Population and Food Supply, *review*, 105
Cooper, J. D., Woore, J., and Young, D. A.: Electronic Properties of Well Oriented Graphite, 721
Coppola, C.: see *under* Kennard, Olga, 333
Cordle, M.: see *under* Henderson, A., 967
Cornillon, R.: *see under* Vasilescu, D., 635
Costa, G.: *see under* Stagni, N., 942
Cotchin, E.: see *under* Carter, R. L., 1081
Cottrell, G. A.: Direct Postsynaptic Responses to Stimulation of Serotonin-containing Neurones, 1060
  and Osborne, N. N.: Subcellular Localization of Serotonin in an Identified Serotonin-containing Neurone, 470
Courts, A.: Secondary Amines in Cooking, *correspondence*, 301
Cousins, Frank W.: Sundials, *review* by F. A. B. Ward, 665
Cox, Joyce M.: *see under* Watson, H. C., 806
Crane-Robinson, C.: see *under* Bradbury, E. M., 64
Crick, Francis: Diffusion in Embryogenesis, 420
Crossley, S., and Zuill, E.: Courtship Behaviour of some *Drosophila melanogaster* Mutants, 1064
Crowson, R. A.: Formal and Transendental Logic, *review*, 477
Crozier, G. F.: see *under* Patton, Stuart, 754
Cruchaud, A.: see *under* Rivat, Liliane, 1136
Csillik, Bertalan, and Knyihár, Elizabeth: Distribution of ^{14}C-Thiosemicarbazide in the Rat Brain: an Attempt to localize Sites of Aminobutyric Acid Production, 562
Culberson, Chicita F.: Chemical and Botanical Guide to Lichen Products, *review* by D. C. Smith, 977
Cullen, A. L.: Microwaves, *review*, 392
Cummins, S. E.: see *under* Power, J. B., 1016
Curio, E., Blaich, R., and Rieder, N.: Two Innate Releasing Mechanisms subserving the Same Motor Pattern, System, 750
Curnutte, Basil: see *under* Bird, Robert E., 1043
Curtis, D. R., Johnston, G. A. R.: Strychnine, Glycine and Vertebrate Postsynaptic Inhibition, 1258
Cutler, D. F.: Anatomy of Monocotyledons, review by D. H. Valentine, 1078

D

Dacie, J. V.: *see under* White, J. M., 860
Dale, J.: see *under* Hughes, R. Elfyn, 756
Dale, P. F.: Simple Photogrammetry, *review*, 769
Dales, R. P.: Principles of Comparative Anatomy of Invertebrates, *review*, 1078
Dalton, R. G., Tourarine, J. L., Anderson, N. F., and Woodruff, M. F. A.: Effect of Route Administration of Antilymphocytic Globulin on Skin Allograft Survival in Rats, 1140
Daniel, Joseph C., jun., Culture of Rabbit Embryo in Circulating Medium, 193
Dardo, M.: *see under* Bosia, G., 532
Daussant, Jean: see *under* Benda, Philippe, 1149
Dausset, J.: see *under* Fellous, M., 191
Davie, Earl W.: see *under* Su, Shirley, 62
Davies, A. J. S.: *see under* Mosier, D. E., 276
Davies, A. S.: see *under* Timbrell, V., 97
Davies, D.: The Seismicity of the Earth, *review*, 1268
  McKenzie, D. P., and Turner, J. S.: "Evolution"—"Development"—Anatomical and Cerebral Features and the Pathological Consequences, 636
  SI Units, *correspondence*, 978
Davis, K. M. C.: see *under* Baeumont, T. G., 632
Davies, J., Cannon, D. F., and Allen, R. J.: Measurements of Electrical Potential for detecting Crack Extension in COD Tests, 1240
Davis, Bernard D.: Ribosome Ambiguity, *correspondence*, 206
de Jager, G.: Cassiopeia A Absorption Spectrum and the Interarm Medium, 622
De Wiest, J. M., Edit.: Flow Through Porous Media, *review* by M. J. Hall, 1267
De-Angeli, Lavinia Carta, Pocchiari, F., Russi, Serena, Tonolo, A., Zurita, Victoria E., Ciaranfi, E., and Perin, A.: Effect of L-Asparaginase from *Aspergillus terreus* on Ascites Sarcoma in the Rat, 549
Deeming, T. J.: Apparent Periodicities in the Redshifts of Quasi-stellar Objects, 620
DeFeudis, F. V., and Delgado, J. M. R.: Effects of Lithium on Amino-acids, in Mouse brain *in vivo*, 749
DeFries, J. C.: *see under* Klein, T. W., 555
Delgado, J. M. R.: see *under* DeFeudis, J. M. R., 749
Demarest, Robert J., and Sciarra, John J.: Conception, Birth and Contraception, *review* by D. M. Potts, 299
Denney, Ronald C.: Named Organic Reactions, *review* by E. J. Forbes, 108
DeRemer, K.: see *under* Neurath, P., 281
Derr, Ina: see *under* Parger, Morton D., 952
Derwent, F. W.: Numerical Notation: Decimalization *correspondence*,, 483
Desbazeille, G.: see *under* Kaufman, A.: 668
Deuchar, Elizabeth M.: Diffusion in Embryogenesis, *correspondence*, 671
Deuser, W. G.: Isotopic Evidence for Diminishing Supply of Available Carbon during Diatom Bloom in the Black Sea, 1069
Dewar, A. J., and Reading, H. W.: Nervous Activity and RNA Metabolism in the Visual Cortex of Rat Brain, 869
Dewey, J. F., and Horsfield, Brenda: Plate Tectonics, Orogeny and Continental Growth, 521
Dew-Hughes, David: A Guide to Superconductivity, *review*, 109
Diamond, J.: *see under* Roper, S., 1259
Dicker, R. M.: Mathematical Logic and Hilbert's ε symbol, *review*, 1077
Diener, Erwin, Shortman, Ken, and Russell, Pamela: Induction of Immunity and Tolerance *in vitro* in the Absence of Phagocytic Cells, 731
Dietrich, Felix M., and Rittel, Werner: Antigenic Site(s) of Synthetic Human Calcitonin *M*, 75
Dingle, J. T., and Fell, Honor B., Edit.: Lysosomes in Biology and Pathology, *review* by Lucille Bitensky, 877
Djordjević, R. Z.: see *under* Bottema, O., 1268
Dochinger, L. S., Bender, E. W., Fox, F. L., and Heck, W. W.: Chlorotic Dwarf of Eastern White Pine caused by an Ozone and Sulphur Dioxide Interaction, 476
Dodge, J. A.: Production of Duodenal Ulcers and Hypertrophic Pyloric Stenosis by Administration of Pentagastrin to Pregnant and Newborn Dogs, 284
Dohrenwend, Barbara Snell: *see under* Dohrenwend, Bruce P., 879
Dohrenwend, Bruce P., and Dohrenwend, Barbara Snell: Social Status and Psychological Disorder, *review*, Peter Kelvin, 879
Donaghay, R. H.: see *under* Essen, C. G. van, 847
Doolittle, R. F., and Mross, G. A.: Identity of Chimpanzee with Human Fibrinopeptides, 643
Doty, Paul: see *under* Lewis, James B., 510
  see *under* Uhlenbeck, Olke C., 508
Dowding, B.: see *under* Tärnocky, A. L., 742
Downing, A. L.: The Biological Aspects of Water Pollution, *review*, 106
Dowson, D.: *see under* Walker, P. S., 956
Drever, R. W. P.: Induced Radioactivity, *review*, 1170
Drew, Charles M.: see *under* Nagy, Bartholomew, 1028
Drew, E.: see *under* Barnes, J., 268
Dubois, Reuben S.: see *under* Roy, Claude, C., 1055
Duckham, A. N.: Can Primitive Farming be Modernized? *review*, 298
Duckworth, W. E., and Hoyle, G.: Electro-Slag Refining, *review* by C. Bodsworth, 478
Duke, C. B.: Tunnelling in Solids, *review* by A. F. G. Wyatt, 667
Duncan, Neil: Engineering Geology and Rock Mechanics, *review* by John R. V. Brooks, 667
Duplan, J. F.: see *under* Legrand, E., 737
Dutton, M. F.: Aflatoxin, *review*, 1171
Dyer, K. R.: Linear Erosional Furrows in Southampton Water, 56
Dyson, E. D., Jackson, C. K., and Whitehouse, W. J.: Scanning Electron Microscope Studies of Human Trabecular Bone, 957

E

Eagers, R. Y.: Toxic Properties of Inorganic Fluorine Compounds, *review* by L. Magos, 1171
Easley, C. W.: Basic Radiation Protection, *review* by M. Ebert, 880
Eastlund, Bernard J.: Low Mode Coherent Synchrotron Radiation and Pulsar Phenomena, 430
Eastop, V. F., and Banks, C. J.: Suspected Insecticide Resistance Mechanism in the Peach-Potato Aphid, 970
Ebel, J. P.: *see under* Fellner, P., 26; 1131
Ebert, M.: *see under* Ealey, C. W., 880
Edge, D. O.: Career Choices by Science Studies Students, 506
Eden, G. E.: Urban Planning Aspects of Water Pollution Control, *review*, 767
Edgerton, V. R., Simpson, Donald, Barnard, R. James, and Peter, J. B.: Phophorylase Activity in Acutely Exercised Muscle, 866
Edwards, D. A.: Lectures on Analysis, *review*, 880
Edwards, Phyllis I.: A Short History of Botany in the United States, *review*, 1078
Eggleton, R. A.: Silicon Tetrahedral Sheet in Stilpnomelane, 625
Ehmann, W. D., and Morgan, J. W.: Twin Terrestrial Impact Craters, 255
Ehresmann, C.: *see under* Fellner, P., 26
Eidelberg, E., and Schwarts, A. S.: Possible Mechanism of Action of Morphine on Brain, 1152
Eilber, F. R., and Morton, D. L.: Demonstration in Sarcoma Patients of Antitumour Antibodies which Fix only Human Complement, 1137
Eisner, Thomas, Zahler, Stanley A., Carrel, James, E. Brown, Dorothy J., and Lones, George W.: Absence of Antimicrobial Substances in the Egg Capsules of Millipedes, 661
Elder, J. W.: Lunar Continental Migration and Maria Spreading, 842
Elek, Stephen R.: see *under* Freidman, Meyer, 454
Eley, D. D.: Understanding Chemical Thermodynamics, *review*, 203
Ellis, Stanley: *see under* McDonald, J. Ken, 1048
Ellory, J. C.: *see under* Lew, V. L., 865
English, W.: The Textile Industry, *review* by C. S. Whewell, 973
Énomé, Shinzo: *see under* Tanaka, Harua, 435
Epstein, Samuel S., Joshi, Sewa R., Arnold, Elsie, Page, Elaine C., and Bishop, Yvonne: Abnormal Zygote Development in Mice after Paternal Exposure to a Chemical Mutagen, 1260
  see under Lijinsky, William, 21
Erdös, E. G.: *see under* Seki, T., 864
Erhan, Semih, Reisher, Sam, Franko, Elizabeth A., Kamath, S. A., and Rutman, R. J.: Evidence for "The Wedge", Initiator of DNA Replication, 340
Erikson, Barrett H., Naugler, Frederic P., and Lucas William H.: Emperor Fracture Zone a Newly Discovered Feature in the Central North Pacific, 53
Ervin, Frank R.: see *under* Levy, Charles K., 463
Essen, C. G. van, Schulson, E. M., and Donaghay, R. H.: Electron Channelling Patterns from Small (10 μm) Selected Areas in the Scanning Electron Microscope, 847
Evans, C. R.: *see under* Bruce, D. J., 873
Evans, G. R., and Penston, M. V.: Interstellar and Atmospheric Clouds, 357
Evans, M. C. W.: see *under* Smith, R. V., 1253
Evans, L. T., Edit.: The Induction of Flowering, *review* by P. F. Wareing, 567
Evetts, K. D., Uretsky, N. J., Iversen, L. L., and Iversen, S. D.: Effects of 6-Hydroxydopamine in CNS Catecholamines, Spontaneous Motor Activity and Amphetamine Induced Hyperactivity in Rats, 961
Ewan, Joseph, Edit.: Short History of Botany in the United States, *review* by Phyllis I. Edwards, 1078

F

Fairbanks, L. B.: see *under* Burch, G. E., 286
Fairbrother, Nan: New Lives, New Landscapes, *review* by R. J. S. Hookway, 1075
Farnsworth, P. N., Nadel, M. R., and Stoll, B. J.: Surface Ultramicroscopy of Sickle Cells, 190
Fawley, Dorothea E.: see *under* Schneiderman, Lawrence J., 1041
Fazio, G. G.: High-energy Gamma-ray Astronomy, 905
Fehlau, P. E.: see *under* Chambers, W. H., 713
Feldman, P. A.: *see under* Gribbin, J. R., 1123
Fell, Honor B.: *see under* Dingle, J. T., 877
Fellner, P., and Ebel, J. P.: Observations on the Primary Structure of the 23S Ribosomal RNA from *E. coli*, 1131
  Ehresmann, C., and Ebel, J. P.: Nucleotide Sequences Present within the 16S Ribosomal RNA of *Escherichia coli*, 26
Fellous, M., and Dausset, J.: Probable Haploid Expression of *HL-A* Antigens on Human Spermatozoon, 191
Fennessy, A.: see *under* Buerger, A. A., 751
Fernand, Philippon: see *under* Roy, Claude C., 1055
Fernandez, Linda A.: *see under* Hempel, Karl H., 955
Fernandez-Campa, J., and King Engel, W.: Histochemical Differentiation of Motor Neurones and Interneurones in the Anterior Horn of the Cat Spinal Cord, 748
Ferreira, S. H., Greene, L. J., Alabaster, V. A., Bakhle, Y. S., and Vane, J. R.: Activity of Various Fractions of Bradykinin Potentiating Factor against Antiogensin I Converting Enzyme, 379
Field, George B.: see *under* Bowyer, C. Stuart, 362
Fielden, E. M., Stevens, G. C., Phillips, J. M., Scholes, G., and Willson, R. L.: Effects due to Non-equilibrium pH Conditions in the Radiolysis of Aqueous Systems, 632
Findley, James S.: see *under* Wilson, Don E., 1155
Finean, J. B.: Structural and Functional Aspects of Lipoproteins in Living Systems, *review*, 877
Finkelstein, Simon: see *under* Walford, Roy L., 461
Firman, I. D.: Possible Side Effects of Fungicides on Banana and Coffee Diseases, 1161
Fischer, L.: An Introduction to Gel Chromatography *review* by Shaw, N., 976
Fishlock, David, Edit.: A Guide to Superconductivity *review* by David Dew-Hughes, 109

Finland, M.: *see under* Sabath, L. D., 1074
Fitch, F. W.: *see under* Mosier, D. E., 276
Fitter, Richard: The Last of the Lands, *review*, 768
Flaschka, Hermann: *see under* Schwarzenbach, Gerold, 202
Fleischmann, P.: The Chemical Basis of Inheritance, 30
Fletcher, K. E.: see *under* Wilson, A. D., 272
Flurry, R. L., jun., and Breen, Donna: Hückel-level Calculations on the Band Structure of Ply-AT, 729
Forbes, E. J.: Named Organic Reactions, *review*, 108
Ford, E. H. R.: An ABC of Medical Genetics, *review*, 480
Foreman, J. K., Palframan, J. F., and Walker, E. A.: Gas Chromatography Determination of N-Alkyl Nitroamines, 554
Forrest, W. W.: Entropy of Microbial Growth. 1165
Foschi, Gabriel V., and Manson, Lionel A.: Radioimmune Assay for Histocompatibility Antigens, 353
Foster, R.: Organic Charge-Transfer Complexes, *review* by A. S. Bailey, 299
Foulkes, S. H., and Prince, G. Stewart, Edit.: Psychiatry in a Changing Society, *review* by G. M. Carstairs, 565
Fox, F. L.: *see under* Dochinger, L. S., 476
Foy, Henry, Kondi, Athena, Linsell, C. A., Parker, Alison M., and Sizaret, P.: Positive α_1-Foetoprotein Tests in Pyridoxine Deprived Baboons: Relevance to Liver Carcinoma in Africans, 952
Fraenkel-Conrat, Heinz: The Chemistry and Biology of Viruses, *review* by Roscoe, D. H., 565
Frame, Marion, Mollison, P. L., and Terry, William D.: Anti-Rh Activity of Human γ G4 Proteins, 641
Francis, J. R. D., and Asfari, A. F.: Visualization of Spiral Motion in Curved Open Channels of Large Width, 725
Franko, Elizabeth A.: *see under* Ehram, Semih, 340
Freidhoff, A. J.: *see under* Angrist, B. M., 651
French, J. E.: *see under* Sheppard, B. L., 1054
Fresco, Jaques R.: *see under* Blake, Richard D., 32
Friedman, H.: *see under* Henry, R. C., 362
Friedman, Herman, and Goldner, Herman: Effect of a DNA Tumour Virus on Antibody Forming Cells in Neonatally Infected Hamsters, 455
Freidman, Meyer, Byers, Sanford O., and Elek, Stephen R.: Pituitary Growth Hormone Essential for Regulation of Serum Cholesterol, 464
Fritz, G.: *see under* Henry, R. C., 362
Fröhlich, H.: Superconductivity, *review*, 300
Fudenberg, H. H.: *see under* Vyas, G. N., 275
Fukuoka, Fumiko: *see under* Chihara, Goro, 943
Fukutomi, Takeo: *see under* Miwa, Keiji, 271
Fuller, A. J. Baden: Microwaves, *review* by A. L. Cullen, 392
Fuller, J. C.: *see under* Chamber, W. H., 713
Furey, Michael J.: Iodine as a Boundary Lubricant: A Contrary View, 630

G

Gadd, G. E.: Pulsatile Flow for detecting Viscoelasticity in Dilute Polymer Solutions, 724
Gaide-Huguenin, A. C.: *see under* Bownds, D.: 870
Gale, G. M.: *see under* Bradley, D. J., 719
Galloway, Robert L.: A History of Coal Mining in Great Britain, *review* by F. F. Roxborough, 665
Galt, J. A., Broten, N. W., Legg, T. H., Locke, J. L., and Yen, J. L.: Long Baseline Interferometer Measurements of CP 0329, 530
Galzigna, Lauro: Complexes between Acetylcholine and Catecholamines and their Tolerance to Mental Illness, 1058
Ganguly, S., and Rao, Manoranjan: Penetration of Solar Particles to Ionospheric Heights at Low Lattitudes, 169
Ganson, A.: Fingerprint Classification, *correspondence*, 205
Garcia, John: *see under* Levy, Charles K., 463
Garriott, Owen K.: *see under* Rishbeth, Henry, 568
Garner, Barry J.: Urban Decay, *review*, 974
Gasser, R. P. H., and Vaight, P. R.: Catalytic Inhibition by Adsorbed Oxygen, 933
Gershon, S.: *see under* Angrist, B. M., 651
Gerstein, D. A.: *see under* Sabath, L. D., 1074
Gertner, Melvin: *see under* Lisgar, Fredrica, 280
Gervais, A. G.: Transplantation Antigens in the Central Nervous System, 647
Gewecke, M.: Antennae: Another Wind-sensitive Receptor in Locusts, 1263
Ghiśelin, Michael T.: The Triumph of the Darwinian Method, *review* by Sydney Smith, 390
Gibbs, Julian H.: *see under* Stein, Joseph M., 743
Gibson, Ian, and Hewitt, Godfrey: Isolation of DNA from *B* Chromosomes in Grasshoppers, 67
Gill, Sydney: Sir William Grove, *correspondence*, 774
Gillespie, T., and Hulme, M. A.: Kinetics of Growth of Filamentous Fungi with particular Reference to Wood Decay, 102
Gilmour, C. M.: *see under* Bulla, L. A., jun., 664
Gilvarry, J. J.: Thermal History of the Moon, 623
Glassman, E., Henderson, A., Cordle, M., Moon, H. M., and Wilson, J. E.: Effect of Cycloheximide and Actinomycin *D* on the Behaviour of the Headless Cockroach, 967
Glickman, Stephen E., and Milner, Peter M., edit.: The Neurological Basis of Motivation, *review* by J. L. Malcolm, 205
Glover, R. S.: Goldfinger no Longer, *correspondence*, 570
Glynn, A. A., Martin, W., and Adinolfi, M.: Levels of Lysozyme in Human Foetuses and Newborns, 77
Glynn, I. M.: *see under* Lew, V. L., 865
Goldblatt, Leo A., edit.: Aflatoxin, *review* by M. F. Dutton, 1171
Goldman, A., Murcray, D. G., Murcray, F. H., Williams, W. J., and Bonomo, F. S.: Identification of the $\nu 3$ NO_2 Band in the Solar Spectrum observed from a Balloon Borne Spectrometer, 443
Goldman, H.: *see under* Bradbury, E. M., 64
Goldner, Herman: *see under* Friedman, Herman, 455
Goldsmith, Lowell A., and Baden, Howard P.: Uniquely Oriented Epidermal Lipid, 1052
Goodall, M. C.: Ion Dependent Conductance: Switching in Lipid Bilayers, 1257
Goodman, David A., and Weinberger, Norman M.: Possible Relationships between Orienting and Diving Reflexes, 1153
Gordon, A. H.: Electrophoresis of Proteins in Polyacrylamide and Starch Cells, *review* by N. Shaw, 976
Gordon, Benjamin L.: Dyscrasias of Immunoglobulin Production, *review* by David S. Rowe, 204
Gotoh, Keishi: Migration of a Neutrally Buoyant Particle on Poiseuille Flow: a Possible Explanation, 848
Gourlay, R. N.: Isolation of a Virus infecting a Strain of *Mycoplasma laidlawii*, 1165
Graham, D. A., Lyne, A. G., and Smith, F. G.: Polarization of the Radio Pulses from the Crab Nebula Pulsar, 526
Grass, P.: Should Slides be Seen Blind? *correspondence*, 1269
Grasso, P., and Rostron, Christine C.: Micro invasion of Epidermis caused by Substituted Anisoles, 188
Grava, Sigurd: Urban Planning Aspects of Water Pollution Control, *review* by G. E. Eden, 767
Gray, John S.: *see under* Lewis, John R., 1081
Greenaway, Frank: The Periodic System of Chemical Elements, *review*, 477
Green, W. V., and Zukasa, E. G.: Stored Energy on Creep Deformed Graphite, 628
Greene, L. J.: *see under* Ferriera, S. H., 379
Gresham, G. A.: Tissue Cultures, *review*, 977
Gribbin, J. R., Feldman, P. A., and Plageman, S. H.: Short-term Periodic Variations following an Optical Flare of Sco X-1, 1123
Groos, Ole V.: Cover-to-cover Translation, *correspondence*, 492
Gruber, William H., and Marquis, Donald G., Edit.: Factors in the Transfer of Technology, *review* by John Langrish, 201
Grummer, R. H.: *see under* Rapacz, J., 941
Grüneberg, Hans: Is there a viral component in the Genetic Background? 39
Gurtsevich, V. E., Mazurenko, N. P., Jarova, E. I., Probatova, N. A., and Stepanova, G. N.: Surface Leukaemic Antigen and Malignancy of Cells on Mice infected with Mazurenko Virus, 1047
Gutfreund, H.: Theoretical Biochemistry, *review*, 1267

H

Haig, D. A.: *see under* Clarke, M. C., 100
Hale, D. K.: Dielectric and Anelastic Effects in Inorganic Glasses, 930
Halfen, Lawrence N., and Castenholtz, Richard W.: Gliding in a Blue–Green Alga: a Possible Mechanism, 1163
Hall, Elizabeth R., and Hupp, E. W.: Localization of Irradiation Damage in Rat Seminiferous Tubules, 85
Hall, M. J.: Flow Through Porous Media, *review*, 1267
Hall, Michael O., and Bacharach, A. D. E.: Linkage of Retinal to Opsin and Absence of Phospholipids in Purified Frog Visual Pigment, 500, 637
Hallam, A.: *see under* Smith, A. Gilbert, 139
Haller, Jürgen: Hormonal Contraception, *review* by M. H. Briggs, 299
Halsey, M. J., Smith, E. B., and Wood, T. E.: Effects of General Anaesthetics on Na^+ Transport in Human Red Cells, 1151
Hamilton, Howard B.: *see under* Kadotani, Tetsuji, 735
Hamilton, Paul B.: *see under* Nagy, Bartholomew, 1028
Hamuro, Junji: *see under* Chihara, Goro, 943
Hansen, J. R., and Lawson, K. D.: Magnetic Relaxation in Ordered Systems, 542
Harmer D. L., and Pagel, B. E. J.: ν Indi: A Weak-lined Halo Star with Excess Deficiency of Nitrogen, 349
Harnarine-Singh, Deowatte, and Hyde, John Baskerville: Post-natal Growth of the Arterial Net in the Human Cerebral Pia Mater, 86
Harris, Fredric A.: Population Analysis of Somatosensory Thalamus in the Cat, 559
Harris, Robin K.: see *under* Lynden-Bell, Ruth M., 1169
Harrison, E. R.: Bouncing-core Theory of Pulsars, 44[illegible]
  Radiation Catastrophe, 245
Harrison, G. G.: *see under* Berman, M. C., 653
Harrison, J. S.: *see under* Rose, Anthony H., 1171
Harrison, Ross Granville: Organization and Development of the Embryo, *review* by D. R. Newth, 480
Harry, Owen G.: Gregarines: their Effect on the Growth of the Desert Locust (Schistocerca-gregaria), 964
Hartle, R. E.: *see under* Bauer, S. J., 533
Hartley, B. S.: *see under* Shotton, D. M., 802
Harwit, M.: *see under* Vanýsek, V., 1231
Harwood, R. S.: Essentials, of Meteorology, *review*, 974
Haslam, C. G. T.: *see under* Berkhuijsen, Elly M., 364
Hasler, Judith: *see under* Rapacz, J., 941
Hastings, G. W.: *see under* Samways, C., 632
Hatch, Milford H.: *see under* Mosley, James W., 953
Hatherton, Trevor: Symmetry of Crustal Earthquakes above Benioff Zones, 844
Hattersley-Smith, G., Keys, J. E., S[illegible]rson, H., Mielke, James E.: Density Stratified Lakes in Northern Ellesmere Island, 55
Hattori, Takanori: *see under* Watanabe, Tsutomu, 70
Haughton, S. H.: Geological History of Southern Africa, *review* by R. M. Shackleton, 975
Hayashi, H.: *see under* Yoshinaga, M., 1138
Haydon, D. A.: *see under* Hladky, S. B., 451
Hayes, Robert: *see under* Jenrasiak, Gordon L.: 278
Hayhoe, F. G. J.: Rubidomycin, *review*, 879
Hayward, A. T. J.: Mechanical Pump with a Suction Lift of 17 Metres, 376
Heap, R. B.: *see under* Slotin, Cecilia A., 385
Heck, W. W.: *see under* Dochinger, L. S., 476
Heckenlively, Donald B.: Intensity of Aggression in the Crayfish, *Orconectes virilis* (Hagen), 180
Heerden, P. J. van: Models for the Brain, *reply* by D. J. Willshaw, H. C. Longuet-Higgins and O. P. Buneman, 177
Heikkila, W. J.: Satellite Observations of Soft Particle Fluxes in the Auroral Zone, 369
Heiles, C.: *see under* Campbell, D. B., 527
Hein, Piet: Grooks, *review* by Nicholas Wade, 390
  More Grooks, *review* by Nicholas Wade, 390
Heller, Joram: Absorptivity and Quantum Yield of Bleaching in Bovine Visual Pigment_{500}, 636
Hempel, Karl H., Fernandez, Linda A., Persellin, Robert H.: Effect of Pregnancy Sera in Isolated Lysosomes, 955
Henderson, A.: *see under* Glassman, E., 967
Henry, J.: *see under* Jurion, F., 298
Henry, James P.: *see under* Axelrod, Julius, 1059
Henry, R. C., Fritz, G., Meekins, J. F., Friedman, H., and Byram, E. T.: Intensity of the Soft X-ray Background Flux: Reply to Bowyer and Field, reply by C. Stuart Bowyer and George B. Field, 362
Hennig, W., and Walker, P. M. W.: Variations in the DNA from Two Rodent Families (Cricetidae and Muridae), 915
Hepinstall, S., Archibald, A. R., and Baddeley, J.: Teichoic Acids and Membrane Function in Bacteria, 519
Herberman, Ronald B.: *see under* Oren, Mark E., 951
Herman, J. R.: *see under* Bauer, S. J., 533
Herrman, Ingeborg: *see under* Sherwood, Louis, 1056
Herz, R. H.: The Photographic Action of Ionizing Radiations on Dosimetry and Medical, Industrial, Neutron, Auto- and Microradiography, *review* by J. E. Roberts, 1266
Heslop-Harrison, J.: *see under* Knox, R. B., 1066
Hesse, K. H.: Secondary Periodicities in Pulsars, 837
  see under Cole, T. W., 712
Hesse, Rosanne I.: *see under* Julez, Bela, 243
Hesstvedt, Eigil: Vertical Distribution of CO near the Tropopause, 50
Hewitt, Godfrey: *see under* Gibson, Ian, 67
Heywood, Stuart M.: Formation of the Initiation Complex using Muscle Messenger RNAs, 696
Hibbard, David J., and Leedale, Gordon F.: Eustigmatophyceae—a New Algal Class with Unique Organization of the Motile Cell, 758
Hicks, J.: Theory of Economic History, *review* by John Vaizey, 973
Hide, R.: Equatorial Jets in Planetary Atmospheres, 254
  and Malin, S. R. C.: Novel Correlations between Global Features of the Earth's Gravitational and Magnetic Fields, 605
Highnam, Kenneth C., and Hill, Leonard: The Comparative Endocrinology of the Invertebrates, *review*, V. B. Wigglesworth, 1265
Hignett, R. C.: *see under* Kirkham, D. S., 388
Hill, Leonard: *see under* Highnam, Kenneth C., 1265
Hillman, Nina: *see under* Tasca, Richard J., 1022
Hills, J. G.: Dynamic Relaxation of Planetary Systems and Bode's Law, 840
Himmel, Nehama: *see under* Becker, Yechiel, 454
Hinder, R. A.: Observations of Atmospheric Turbulence with a Radio Telescope at 5 GHz, 614

INDEX

Hindley, Keith B., and Houlden, Michael A.: The 11 Canis Minorids—a New Meteor Stream Probably associated with Comet Mellish 1917 I, 1232
  and Miles, Howard G.: Fireball and Meteorite of April 25, 1969, 255
Hipkin, L. J.: Gonadotrophin Inhibition by the Synergistic Action of Vasopressin and Oxytocin, 740
Hirschfield, James: Geometric Inequalities, 1268
Hirschorn, Kurt: *see under* Lisgar, Fredrica, 280
Hladky, S. B., and Haydon, D. A.: Discreteness of Conductance Change in Bimolecular Lipid Membranes in the Presence of Certain Antibiotics, 451
Hodge, Paul W., and Wright, Francis W.: Meteoritic Spherules in the Soil Surrounding Terrestrial Impact Craters, 717
Hodgkin, Dorothy Crowfoot: *see under* Anderson, Bryan, 233
Hogbom, J. A.: *see under* Christiansen, W. N., 974
Holborow, E. J.: Fluorescent Antibody Techniques and their Applications, *review*, 878
Hollweg, Joseph V., and Volk, H. J.: Two New Plasma Instabilities in the Solar Wind, 441
Honig, E. P.: Solid State Chemistry and Colloid Chemistry, 537
Hookway, R. J. S.: New Lives, New Landscapes, *review*, 1075
Hooijer, D. A.: Pleistocene South-east Asiatic Pygmy Stegodonts, 474
Hook, W. Alexander van: *see under* Jancsi, Gabor, 723
Hoppe, Heinz A.: *see under* Levring, Tore, 106
Hora, T. S., and Baker, Ralph: Volatile Factor in Soil Fungistasis, 1071
Horai, Ki-Iti, Chessman, Mary, and Simmons, Gene: Heat Flow Measurements on the Reykjanes Ridge, 264
Horn, Ann L. D.: *see under* Rose, S. P. R., 650
Horn, G. R.: *see under* Rose, S. P. R., 650
Horne, R. A.: Marine Chemistry, *review* by J. P. Riley, 567
Horsfield, Brenda: *see under* Dewey, J. F., 521
Hotine, Martin: Mathematical Geodesy, *review* by A. R. Robbins, 666
Hough, S.: The Yeasts, *review*, 1171
Houlden, Michael A.: *see under* Hindley, Keith B., 1232
Howarth, C. I.: *see under* Beggs, W. D. A., 752
Howell, F. Clark: *see under* Clarke, R. J., 1219
Howie, Anne: *see under* Bartholomai, Alan, 1061
Howlett, J.: The Economics of Computers, review, 1168
Hoyle, G.: *see under* Duckworth, W. E., 478
Hoyle, F., and Narliker, J. V.: Quantization of Wheeler-Feynman Electrodynamics, 1233
Hsu, Lillian Y.: *see under* Lisgar, Fredrica, 280
Hubel, D. H., and Wiesel, T. N.: Cells sensitive to Binocular Depth in Area 18 of the Macaque Monkey Cortex, 41
Huebner, Robert J.: *see under* Levy, Jay A., 949
Huffman, Donald R.: An Explanation for the Broad Supernova Bands, 833
Hughes, R. Elfyn, and Dale J., Trends in Montane Grasslands, expressed in Terms of "Relative Entropy", 756
Hughes, S. R. C.: Non-Aqueous Solvents, *review*, 1077
Hulett, H. R.: Non-enzymatic Hydrolysis of Adenosine Phosphates, 1248
Hulme, H. R., and Collieu, A. McB.: Nuclear Fusion, *review* by Daphne F. Jackson, 479
Hulme, M. A.: *see under* Gillespie, T., 102
Hunter, James H., jun., and Lü, Phillip K.: Reply to Kinman concerning 3C 345, 366
Hunter, Philippa: *see under* Munro, Alan, 277
Hunter, Robert D.: *see under* Allardyce, Randall A., 1141
Hupp, E. W.: *see under* Hall, Elizabeth R., 85
Hurlbert, William P.: *see under* Clarke, Allen W., 703
  see under Longenecker, Herbert E., jun., 703
Hurtt, W.: *see under* Reid, C. P. P., 291
Husserl, Edmund: Formal and Transcendental Logic, *review* by R. A. Crowson, 477
Hutchinson, Sir Joseph, Edit.: Population and Food Supply, *review* by G. W. Cooke, 105
Hyde, John Baskerville: *see under* Harnarine-Singh, Deowatter, 86

I

Ignatiades, Lydia, and Becanos-Kontos, Th.: Ecology of Fouling Organisms in a Polluted Area, 293
Isaacs, N. W.: *see under* Kennard, Olga, 333
Ise, Norio: Catalysis of Polynucleotides in Ionic Reactions, 66
Israel, H. W.: *see under* Steward, F. C., 760
Issidorides, M.: *see under* Stefamis, C. N., 963
Itzkan, Irving: *see under* Myer, James A., 544
Iversen, L. L.: *see under* Evetts, K. D., 961
Iversen, S. D.: *see under* Evetts, E. D., 961

J

Jackson, C. K.: *see under* Dyson, E. D., 957
Jackson, Daphne F.: Nuclear Fusion, *review*, 479
Jackson, J. F.: Atomic Shield, 1947/52, *review*, 1167
Jackson, Michael B., and Osborn, Daphne J.: Ethylene, the Natural Regulator of Leaf Abscission, 1019
Jacob, S. T., Sajdel, E. M., and Munro, H. N.: Specific Action of —Amanitin on Mammalian RNA Polymerase Protein, 60
Jacquillat, Cl.: *see under* Bernard, J., 879
Jaeger, J. C.: Elasticity, Fracture and Flow with Engineering and Geological Application, *review* by J. G. Oldroyd, 568
Jahn, W.: Umbellulidae Distribution extended in the Atlantic, 1068
James, Keith: *see under* Allardyce, Randall A., 1141
James, William H.: Schizophrenia as a Genetic Morphism, 1153
Jamieson, A. D.: *see under* Pruitt, K. M., 1249
Jamieson, J. C., and Smith, J. V.: Chemical Homogeneity of Groups of Terrestrial Planets, 354
Jancovico, Bernard: Thermodynamiques et Physique Statistique, Premier Cycle, *review* by G. L. Sewell, 568
Jancso, Gabor, Pupezin, Jovan, and Hook, W. Alexander van: Vapour Pressure of $H_2{}^{18}O$ Ice (—17° C to 0° C) and $H_2{}^{18}O$ Water (0° C to 16° C), 723
Janič, R. R.: *see under* Bottema, O., 1268
Janis, Martin, and Bach, Fritz H.: Potentiation of *in vitro* Lymphocyte Reactivity, 238
Jarova, E. I.: *see under* Gurtsevich, V. E., 1047
Jarvik, L.: *see under* Neurath, P., 281
Jaschek, Mercedes, and Malaroda, Stella: Heavy Elements in 73 Draconis, 246
Jeffreys, Harold: Imperfections of Elasticity and Continental Drift, 1007
  Some Needs of Geophysics, 1008
Jelliffe, R. F. P.: *see under* Bradfield, R. B., 283
Jendrasiak, Gordon L., Hayes, Robert: Spin-Label Study of the Iodine-Lecithin Interaction, 278
Jennings, B. R.: The Scattering of Light and Other Electromagnetic Radiation, *review*, 300
Jennison, R. C.: Radiotelescopes, *review*, 974
Jensen, T. A.: *see under* Seki, T., 864
Jöbsis, F. F.: *see under* Mills, E., 1147
Johnson, A.: Information Explosion, *correspondence*, 1173
Johnson, C. E.: *see under* Banerjee, Subir K., 173
Johnson, Robert T.: *see under* Rao, Potu N., 159
Johnston, G. A. R.: *see under* Curtis, D. R., 1258
Jollès, P.: *see under* Landureau, J. C., 968
Jones, D. J.: *see under* Barnes, J., 268
Jones, J. K.: *see under* Allan, H. R., 253
Jones, K. W.: Chromosomal and Nuclear Location of Mouse Satellite DNA in Individual Cells, 912
Jones, S.: British Rail Advanced Passenger Train, 417
Jörnvall, Hans: Differences in E and S Chains from Isoenzymes of Horse Liver Alcohol Dehydrogenase, 1133
Joshi, Sew R.: *see under* Epstein, Samuel S., 1260
Joule, J. A.: The Chemistry of the Isoquinoline Alkaloids, *review*, 479
Jukes, Thomas H.: Alarums and Excursions, *correspondence*, 301
  Attitudes to Conservation, *correspondence*, 1173
Julesz, Bela, and Hesse, Rosanne I.: Inability to Perceive the Direction of Rotation of Line Segments, 243
Junga, Irene G.: *see under* Schneiderman, Lawrence J., 1041
Jurion, F., and Henry, J.: Can Primitive Farming be Modernised? *review* by A. N. Duckham, 298

K

Kadotani, Tetsuji, Ohama, Koso, Sofuni, Tishio, and Hamilton, Howard B.: Aberrant Karyotypes and Spontaneous Abortion in a Japanese Family, 735
Kalashnikov, V. N., and Kudin, A. M.: Karman Vortices in the Flow of Drag-reducing Polymer Solutions, 445
Kalucy, Elizabeth C., McMillan, B.: Transmission of *Plasmodium berghei* (NK65 Strain) by *Anopheles annulipes* Walker, 97
Kamath, S. A.: *see under* Ehram, Semih, 340
Kametani, Tetsuji: The Chemistry of the Isoquinoline Alkaloids, *review* by J. A. Joule, 479
Kang, Soo-Sang: Temperature Sensitive Alteration of 30S Subunits demonstrated *in vitro* Reassociation of Functional Ribosomes, 1132
Kao, K. N.: *see under* Kasha, K. J., 874
Kaplan, A. E., Brown, L. V., and Salk, J.: Increases in Serum Lactate Dehydrogenase in Experimental Allergic Encephalomyelitis, 384
Kapuler, A. M., Mendelsohn, N., Klett, H., and Acs, G.: Four Base-specific Nucleoside 5′-Triphosphatases in the Subviral Core of Reovirus, 1209
Kasai, T.: *see under* Schmidt, D. A., 1012
Kasha, K. J., and Kao, K. N.: High Frequency Haploid Production in Barley (*Hordeum vulgare* L.), 874
Kato, T.: *see under* Neurath, P.: 281
Katz, Amnon, Ornan, Ezuz, and Schwartze, Moshe: Hoyle-Narliker Quantization of Wheeler-Feynman Electrodynamics, 534
Katz, Michael: *see under* Koprowski, Hilary, 1045
Kaufman, A., and Desbazielle, G.: The Critical Path Method, *review* by P. L. Clot, 668
Kaufman, Stanley, Steim, Joseph M., and Gibbs, Julian H.: Nuclear Relaxation on Phospholipids and Biological Membranes, 743
Kausch-Blecken, H. H., Schmeling, Von, and Tschoegl, N. W.: Osculatory Packing of Finite Areas with Circles, 1119
Kawamura, Akiyoski, jun., Edit.: Fluorescent Antibody Techniques and their Applications, *review* by E. J. Holborow, 878
Kehoe, JacSue, and Ascher, P.: Re-evaluation of the Synaptic Activation of an Electrogenic Sodium Pump, 820
Keller, A., Pedemonte, E., and Willmouth, F. M.: Macro-lattice from Segregated Amorphous Phases of a Three Block Copolymer, 538
Keller, William E.: Helium-3 and Helium-4, *review* by D. F. Brewer, 771
Kelly, A.: The Mechanical Properties of Matter, *review*, 1267
Kelvin, Peter: Social Status and Psychological Disorder, *review*, 879
Kempson-Jones, Gillian F.: *see under* Buck, K. W., 945
Kench, J. E.: *see under* Berman, M. C., 653
Kennard, Olga, Isaacs, N. W., Coppola, J. C., Kirby, A. J., Warren, Stuart, Motherwell, W. D. S., Watson, D. G., Wampler, D. H., Chenery, D. H., Larson, A. C., Kerr, K. Ann, and Riva de Sanseverino, L.: Three Dimensional Structure of Adenosine Triphosphate, 333
Kent, B. E.: *see under* Wilson, A. D., 272
Kerker, Milton: The Scattering of Light and other Electromagnetic Radiation, *review* by B. R. Jennings, 300
Kerr, K. Ann: *see under* Kennard, Olga, 333
Keverne, E. B.: *see under* Michael, Richard, 84
Keys, J. E.: *see under* Hattersley-Smith, G., 55
Kibrik, Sidney: *see under* Polgar, Peter R., 857
Kierstead, E.: *see under* Myer, James A., 544
Kikuchi, H.: Harmonic Ion Cyclotron Resonances observed by the OGO-4 Satellite, 257
Kilgour, R., and Bruere, A. N.: Behaviour Patterns in Chromatin-positive Klinefelter's Syndrome of Sheep, 71
Kimbrell, G. McA., Weinrott, Mark R., Morris, Edward K., jun., Scheid, Jennifer, and Sangston, Deborah: Alarm Pheromone and Avoidance Conditioning in Goldfish, *Carassius auratus*, 754
King Engel, W.: *see under* Fernandez-Campa, J., 748
Kingman, J. F. C.: Probability Theory, *review*, 1169
Kinzey, W. G.: Rates of Evolutionary Change in the Hominid Canine Teeth, 296
Kirby, A. J.: *see under* Kennard, Olga, 333
Kirkham, D. S., and Hignett, R. C.: Antagonism between the Effects of Kinetin and Extracellular Metabolites of the Pathogen in Apple Scab Disease, 388
Kirschner, Stanley, Edit.: Coordination Chemistry, *review* by D. W. A. Sharp, 202
Klein, T. W., and DeFries, J. C.: Similar Polymorphism of Taste Sensitivity to PTC in Mice and Men, 555
Klett, H.: *see under* Kapuler, A. M., 1209
Knox, R. B., Heslop-Harrison, J., and Reed, C.: Localization of Antigens associated with the Pollen Grain Wall by Immunofluorescence, 1066
Knyihár, Elizabeth: *see under* Csillik, Bertalan, 562
Kok, Loke Tuck, Norris, Dale M., Chu, Hsien Ming: Sterol Metabolism as a Basis for a Mutualistic Symbiosis, 661
Komesaroff, M. M.: Possible Mechanism for the Pulsar Radio Emission, 612
Kondi, Athena: *see under* Foy, Henry, 952
Konrad, Pat: *see under* Walford, Roy L., 462
Koprowski, Hilary, Barbanti-Brodano, Giuseppe, and Katz, Michael: Interaction between Papova-like Virus and Paramyxovirus in Human Brain Cells: a Hypothesis, 1045
Korf, J.: *see under* Praag, H. M. van, 1259
Korge, G.: Dosage Compensation and Effect for RNA Synthesis in Chromosome Puffs of *Drosophila melanogaster*, 386
Kovács, István: Rotational Structure in the Spectra of Diatomic Molecules, *review* by W. G. Richards, 569
Kreiči, J.: *see under* Pick, E., 236
Krishnan, G. A.: *see under* Arora, S. K., 261
Krs, Miroslav: *see under* Banerjee, Subir K., 173
Kuchowicz, B.: Primordial Synthesis of Superheavy Nuclei in the Galaxy, 440
Kudin, A. M.: *see under* Kalashnikov, V. N., 445
Kuechler, Ernst, and Rich, Alexander: Position of the Initiator and Peptidyl Sites in the *E. coli* Ribosome, 920
Kumar, Vijai: *see under* Varghese, T. G., 259
Kunz, W. E.: *see under* Chambers, W. E., 713
Kynoch, P. A. M.: *see under* Ogawa, S., 1042

L

Labhsetwar, Anant P.: Role of Oestrogen in Spontaneous Ovulation demonstrated by the Use of an Antagonist of Oestrogen, ICI 46,474, 80
Lakin, A.: *see under* Tárnoky, A. L., 742
Lampton, M.: *see under* Bowyer, C. S., 1125
Landmark, B. J.: *see under* Thomas, J. O., 975
Landsberg, P. T.: Thermodynamics in Progress 1204

Landureau, J. C., and Jollès, P.: Lytic Enzyme produced *in vitro* by Insect Cells: Lysozyme or Chitinase, 968
Lang, Kenneth R., and Rickett, B. J.: Size and Motion of the Interstellar Scintillation Pattern from Observations of CP 1133, 528
Langridge, Robert: *see under* Blake, Richard D., 32
Langrish, John: Factors in the Transfer of Technology, *review*, 201
Lanks, Karl W.: Derivation of Partial Sequence of Human β Chain Messenger RNA from Mutation Data, 1244
Large, M. I.: *see under* Vaughan, A. E., 167
Lark, Karl G.: *see under* Bird, Robert E., 1043
Larson, A. C.: *see under* Kennard, Olga, 333
Laughton, A. S.: *see under* Roberts, D. G., 170
Lawson, K. D.: *see under* Hansen, J. R., 542
Lawrence, L. C.: *see under* Brück, M. T., 531
Lawrence, P. A.: *see under* Willis, J. H., 81
Lawton, J. H.: *see under* McNeill, S., 472
Layard, Richard: *see under* Blaug, Mark, 297
Leaf, C. D.: *see under* Sabath, L. D., 1074
Leakey, L. S. B.: "Newly" Recognized Mandible of *Ramapithecus*, 199
Leakey, M. D.: Stone Artefacts from the Swartkrans, 1222
Lebowitz, Paul, and Singer, Marcus: Neurotrophic Control of Protein Synthesis in the Regenerating Limb of the Newt, *Triturus*, 824
Lee, H. J.: *see under* Canuto, V., 47
Lee, Tong-Ho: *see under* Barnett, Don R., 938
Lee, Young C.: *see under* Byfield, John E., 859
Leedale, Gordon F.: *see under* Hibbard, David J., 758
Legg, T. H.: *see under* Galt, J. A., 530
Leggio, T.: *see under* Morpurgo, G., 76
Legrand, E., and Duplan, J. F.: Presence of an Additional Metacentric Chromosome in AKR/TIALD Leukaemic Cells, 737
Lehmann, H.: *see under* Ogawa, S., 1042
  see under White, J. M., 939
Leibovitz, Clement: An Oscillating State as an Alternative to Gravitational Collapse 711
Leisenring, A. C.: Mathematical Logic and Hilbert's ϵ-symbol, *review* by R. M. Dicker, 10[illegible]7
Lenherr, A. D., and Ormerod, M. G.: Electron Reactions in β-Irradiated Thymidine and Cystamine, 546
Levin, A. S.: *see under* Vyas, G. N., 275
Levin, Y.: *see under* Seki, T., 864
Levitsky, David A., and Barnes, Richard H.: Effect of Early Malnutrition on the Reaction of Adult Rats to Early Stimuli, 468
Levring, Tore, Hoppe, Heinz A., and Schmid, Otto J.: Marine Algae, *review* by E. M. Burrows, 106
Levy, Charles K., Ervin, Frank R., and Garcia, John: Effect of Serum from Irradiated Rats on Gastrointestinal Function, 463
Levy, Jay A., and Huebner, Robert J.: Association of Murine Leukaemia Virus from a Mouse Lymphoma (2731/L) with Reovirus Type 3 Infections, 949
Lew, V. L., Glynn, I. M., and Ellory, J. C.: Net Synthesis of ATP by Reversal of the Sodium Pump, 865
Lewis, H. A. G., Edit.: Times Atlas of the Moon, The, *review* by Edward Phillips, 770
Lewis, Heather: *see under* Oswald, Ian, 959
Lewis, J., Baddeley, A. D., Bonham, K. G., and Lovett, D.: Traffic Pollution and Mental Efficiency, 95
Lewis, J. Parry: *see under* Medhurst, Franklin, 974
Lewis, J. R.: Goldfinger No Longer, *correspondence*, 774
Lewis, James B., and Doty, Paul: Derivation of the Secondary Structure of 5S RNA from its Binding of Complementary Oligonucleotides, 510
Lewis, John R., Gray, John S., Pollock, Leland W., and Moore, P. Geoffrey: Can a Scientific Article be Libellous? *correspondence*, 1081
Libby, L. Marshall: Age of Cosmic Rays and Abundance of Antimatter in the Galaxy, 166
Lidsky, Martin D.: *see under* Mittal, Kamal K., 1255
Lijinsky, William, and Epstein, Samuel S.: Nitrosamines as Environmental Carcinogens, 21
Lim, T. C., and Musgrave, M. J. P.: Stonely Waves in Anisotropic Media, 372
Lindley, D. V.: Models on Regression and Related Topics, *review*, 770
Lindop, Patricia J., and Rotblat, J.: Sternglass's Assumptions, *correspondence*, 979
Lindström, M.: Faunal Provinces in the Ordovician of North Atlantic Areas, 1158
Linsell, C. A.: *see under* Foy, Henry, 952
Linsell, J. L.: *see under* Slotin, Cecilia A., 385
Lisgar, Fredrica, Gertner, Melvin, Cherry, Sheldon, Hsu, Lillian Y., and Hirschorn, Kurt: Prenatal Chromosome Analysis, 280
Locke, J. L.: *see under* Galt, J. A., 530
Löfroth, Göran: DDT in Milk, *correspondence*, 881
Lones, George W.: *see under* Eisner, Thomas, 661
Long, P. L.: Development (Schizogony) of *Eimeria tenella* in the Liver of Chickens treated with Corticosteroid, 290
Longenecker, Herbert E., jun., Hurlbut, William P., Mauro, Alexander, and Clark, Allen W.: Effects on End-plate Potential, Miniature End-plate Potential and Nerve Terminal Spike, 701
  see under Clark, Allen, W., 703
Longfield, M. D.: *see under* Walker, P. S., 956
Longuet-Higgins, H. C.: *see under* Willshaw, D. J. 177
Lorkin, P. A.: *see under* White, J. M., 939
Lowe, G.: Synthesis of Penicillin, Cephalosporin C and Analogs, *review*, 976
Louwagie, A. C., and Verwilhgen, R. L.: Growth of Haematopoietic Spleen Colonies after Grafting of Human Bone Marrow in Mice, 383
Lovering, W. F.: Magnets in Electromagnetic Theory, 1127
Lovett, D.: *see under* Lewis, J., 95
Lü, Benjamin C.-Y., Yu, Paul, and Poon, David P. L.: Liquid Phase Inversion, 1128
Lu, Phillip K.: *see under* Hunter, James B., jun., 366
Lucas, William H.: Erikson, Barrett H., 53
Ludford, P. M.: *see under* Steward, F. C., 760
Ludlaw, F. H.: Atmospheric Circulating Systems, *review*, 479
Luria, S. E.: On Which Side are the Angels? *correspondence*, 301
Lux, B.: *see under* Minkoff, I., 540
Lynden-Bell, Ruth M., and Harris, Robin K.: Nuclear Magnetic Resonance Spectroscopy, *review* by D. E. Webster, 1169
Lyne, A. G.: *see under* Graham, D. A., 526
Lythgoe, J.: *see under* Barnes, J., 268
Lynts, George W.: Conceptual Model of the Bahamian Platform for the Last 135 Million Years, 1226

M

McCall, G. J. H.: Lunar Rilles and a Possible Terrestrial Analogue, 714
McCarroll, Bruce, and McKee, D. W.: Interaction of Atomic Hydrogen and Nitrogen with Graphite Surfaces, 722
McConaghey, Paddy, and Sledge, Clement B.: Production of "Sulphation Factor" by the Perfused Liver, 1249
McCormac, Billy M., and Omholt, Anders, Edit.: Atmospheric Emissions, *review* by David W. Hughes, 392
MacCullum, J. R., Tanner, J.: Derivation of Rate Equations used in Thermogravimetry, 1127
McCray, J. A.: *see under* Chance, B.: 705
McCrea, W. H.: Groups of Terrestrial Planets—a Reply, 355
  Planetary Fission Events—Reply to J. M. Bailey, 49
McDonald, J. Ken, Zeitman, Benjamin B., and Ellis, Stanley: Leucine Naphthylamide : an Appropriate Substrate for the Histochemical Detection of Chathepsins B and B′, 1048
McDougall, J. K.: Effects of Adenoviruses on the Chromosomes of Normal Human Cells and Cells Trisomic for an *E* Chromosome, 456
McEwan, William S.: *see under* Nagy, Bartholomew, 1028
McGinnies, William G.: Deserts of the World, *review* by J. L. Cloudsley-Thompson, 768
Machin, D. J.: Halides of the First Row Transition Metals, *review*, 203
  Halides of the Second and Third Row Transition Metals, *review*, 203
McIntosh, D. H., and Thom, A. S.: Essentials of Meteorology, *review* by R. S. Harwood, 974
Mack, J.: *see under* Bowyer, C. S., 1125
MacKay, D. M.: Elevation of Visual Threshold by Displacement of Retinal Image, 90
  Interocular Transfer of Suppressive Effects of Retinal Image Displacement, 872
McKee, D. W.: *see under* McCarroll, Bruce, 722
McKenzie, D. P.: *see under* Davies, D., 636
McKerns, Kenneth W.: Steroid Hormones and Metabolism, *review* by May Reed, 204
Mackie, W.: *see under* Atkins, E. D. T., 626
McLaughlin, K. A.: *see under* Chapman, G.E., 639
McLean, A. E. M., and McLean E. K.: Miscellaneous Intelligence, *correspondence*, 206
McLean, E. K.: *see under* McLean, A. E. M., 206
McLintock, I. S., and Orr, J. C.: Catalytic Oxidation of SO_2 on Carbon Films studied by Electrical Resistance Measurements, 629
McMillan, B.: *see under* Kalucy, Elizabeth C., 97
McNeill, S., and Lawton, J. H.: Annual Production and Respiration in Animal Populations, 472
Macpherson, Ian: *see under* Zavada, Jan, 24
Maddison, R. E. W.: Life of the Honourable Robert Boyle, F.R.S., The, *review* by A. J. Turner, 1076
Maeda, Yukiko: *see under* Chihara, Goro, 943
Maglio, Vincent J.: Early Elephatidae of Africa and a Tentative Correlation of African Plio-Pleistocene Deposits, 328
Magos, L.: Toxic Properties of Inorganic Fluorine Compounds, *review*, 1171
Mahanty, H. K.: Host Response of Tobacco to *Verticillium dahliae* (tob.) Kleb.—a Cytological Method of assaying Resistance, 755
Maher, Veronica M., and Summers, William C.: Mutagenic Action of Aflatoxin B_1 on Transforming DNA and Inhibition of DNA Template Activity *in vitro*, 68
Majer, J. R., and Boulton, Alan A.: Absolute Unambiguous Ultramicroanalysis of Metabolites Present in Complex Biological Extracts, 658
Majumdar, A. J.: *see under* Vekey, R. C. de, 172
Malaroda, Stella: *see under* Jeschek, Mercedes, 246
Malcolm, J. L.: The Neurological Basis of Motivation, *review*, 205
Malcolmson, Jean F.: Vegetative Hybridity in *Phytophthora infestans*, 971
Malin, S. R. C.: *see under* Hide, R., 605
Mallet, G.: *see under* Vasilescu, D., 635
Manchester, R. N.: Improved Parameters for Seven Pulsars, 1124
Mandava, N.: *see under* Mitchell, J. W., 1065
Mandel, Lazaro J.: Active Ionic Transport across Biological Membranes: Possible Role of Electrons and Protons, 450
Manhas, Maghar S., and Bose, Ajay K.: Synthesis of Penicillin, Cephalosporin C and Analogs, *review* by G. Lowe, 976
Manson, Lionel A.: *see under* Foschi, Gabriel V., 853
Maral, R.: *see under* Bernard, J., 879
Marangoni, G.: *see under* Bosia, G., 532
Marcker, K. A.: *see under* Smith, A. E., 184
Mark, R. F.: Chemospecific Synaptic Repression as a Possible Memory Store, 178
Marschall, K. J.: Introduction of a New Virus Disease of the Coconut Rhinoceros Beetle in Western Samoa, 288
Marshall, W. A.: Human Growth after Birth, *review*, 108
Martin, Ian: View that may Upset Scientists, *correspondence*, 882
Martin, P. G.: The Darwin Rise Hypothesis of the Biogeographical Dispersal of Marsupials, 197
Martin, W.: *see under* Glynn, A. A., 77
Martini, E., and Worsley, T.: Standard Neogene Calcareous Nannoplankton Zonation, 289
  see under Worsley, T., 1242
Marzullo, G.: Production of Chick Chimaeras, 72
Mathews, M. B.: *see under* Smith, A. E., 184
Matthews, A. D., and Riley, J. P.: Occurrence of Indium in Seawater and some Marine Sediments, 1242
Matthews, D. H.: *see under* Roberts, D. G., 170
Matthews, L. Harrison: The Life of Mammals, *review* by R. J. Harrison, 107
Mattila, T.: *see under* Allan, G. G., 175
Mauro, Alexander: *see under* Clark, Allen W., 703
Mauro, William P.: *see under* Longenecker, Herbert E., jun., 703
Maxfield, John E.: *see under* Bird, Robert E., 1043
Maxwell, Thomas R. A.: *Hydramoeba hydroxena* (Entz) discovered on *Hydra graysoni* sp. nov., 1068
Mayumi, M.: *see under* Yoshinaga, M., 1138
Mazaitis, A. J.: *see under* Schmidt, D. A., 1012
Mazurenko, N. P.: *see under* Gurtsevich, V. E., 1047
Meadows, A. J.: Lockyer as Astronomer, 230
Mearns, A. M., and Morris, A. J.: Production of Oxygen Atoms in Quartz and Polytetrafluoroethylene Discharge Tubes, 60
Mechoulam, Raphael: *see under* Burstein, Sumner H., 87
Medhurst, Franklin, and Lewis, J. Parry: Urban Decay; *review* by Barry J. Garner, 974
Meekins, J. F.: *see under* Henry, R. C., 362
Melrose, G. J. H., Muller, H. K., and Vagg, W. J.: Correlation of Bradykinin Structure with ability to increase Vascular Permeability, 547
Meltzer, Bernard: Generation of Hypothesis and Theories, 972
Mendelsohn, N.: *see under* Kapuler, A. M., 1209
Menezes, Franco: *see under* Burstein, Sumner H., 87
Merrington, Judith: *see under* Oswald, Ian, 959
Mesley, R. J.: *see under* Wilson, A. D., 272
Messiha, F. S., Agallianos, D., and Clower, C.: Dopamine Excretion in Affective States and following Li_2CO_3 Therapy, 868
Mestroni, G.: *see under* Stagni, N., 942
Michael, Richard P., and Keverne, E. B.: Primate Sex Pheromones of Vaginal Origin, 84
Mielke, James H.: *see under* Hattersley-Smith, G., 55
Mikikits, W., Mortara, A., and Spector, R. G.: Effect of Drugs on Red Cell Fragility, 1150
Mile, B.: *see under* Bennett, J. E., 932
Miles, Howard G.: *see under* Hundley, Keith B., 255
Miller, R. P.: *see under* Wilson, A. D., 272
Mills, A. A.: Transient Lunar Phenomena and Electrostatic Glow Discharges, 929
Mills, E., and Jöbsis, F. F.: Simultaneous Measurement of Cytochrome a_3 Reduction and Chemoceptor Afferent Activity in the Carotid Body, 1147
Milman, G.: *see under* Scolnick, E., 152
Milner, Peter M.: *see under* Glickman, Stephen E., 205
Minkoff, I., and Lux, B.: Ribbed Growth of Graphite in Ni-C Solutions containing Boron, 540
Mitchell, J. W., Mandava, N., Worley, J. F., Plimmer, J. R., and Smith, M. V.: Brassins—a New Family of Plant Hormones from Rape Pollen, 1065
Mitrinovic, D. S.: *see under* Bottema, O., 1268
Mittal, Kamal K., Rossen, Roger D., Sharp, John T., Lidsky, Martin D., and Butler, William T.: Lymphocyte Cytotoxic Antibodies in Systemic Lupus Erythematosus, 1255
Miwa, Keiji, and Fukutomi, Takeo: Oriented Grain Growth of Chlorinated Naphthalene induced by an Electric Field, 271
Modzeleski, Vincent: *see under* Nagy, Bartholomew, 1028
  see under Scott, Ward M., 1129
Mollison, P. L.: *see under* Frame, Marion, 6[illegible]
Moon, H. M.: *see under* Glassman, E., 967
Moore, Geoffrey P.: *see under* Lewis, John R., 1081

INDEX

Morgan, J. W.: see *under* Ehmann, W. D., 255
Morpurgo, G., Battaglia, P. A., and Leggio, T.: Negative Bohr Effect in Newt Haemolysates and its Regulation, 76
Morris, A. J.: see *under* Mearns, A. M., 60
Morris, Edward K., jun.: see *under* Kimbrell, G. McA., 754
Mortara, A.: see *under* Mikikits, W., 1150
Morton, D. L.: see *under* Eilber, F. R., 1137
Mosevitsky, M.: see *under* Bresler, S., 764
Mosier, D. E., Fitch, F. W., Rowley, D. A., and Davies, A. J. S.: Cellular Deficit in Thymectomized Mice, 276
Mosley, James W., Barker, Lewellys F., Shulman, N. Raphael, and Hatch, Milford H.: Failure to Detect an Antigen associated with Hepatitis in a Community Epidemic, 953
Motherwell, W. D. S.: see *under* Kennard, Olga, 333
Mott, R. L.: see *under* Steward, F. C., 760
Mross, G. A.: see *under* Doolittle, R. F., 643
Mueller, Robert A.: see *under* Axelrod, Julius, 1059
Muirhead, Hilary: see *under* Watson, H. C., 806
Muller, H. K.: see *under* Melrose, G. J. H., 547
Muller, Heinrich Dieter, and Steinback, Friedrich: Decomposition of Isopropylalcohol photosensitized by Zinc Oxide, 728
Muller, P. M.: see *under* Burke, J. D., 1234
Munday, D. E.: see *under* Timbrell, V., 97
Munro, Alan, and Hunter, Philippa: *In vitro* Reconstitution of the Immune Response of Thymus-deprived Mice to Sheep Red Blood Cells, 277
Munro, H. N.: see *under* Jacob, S. T., 60
Murcray, D. G.: see *under* Goldman, A., 443
Murcray, F. H.: see *under* Goldman, A., 443
Murthy, G. T.: see *under* Chatterjee, B. K., 839
Murthy, P. V. Ramana: see *under* Chatterjee, B. K., 839
Musgrave, M. J. P.: see *under* Lim, T. C., 372
Mutt, Victor: see *under* Said, Sami I., 863
Myer, J. A., and Nicholls, R. W.: Resonance Fluorescence of the CN Free Radical, 928
Myer, James A., Itzkan, Irving, and Kierstead, E.: Dye Lasers in the Ultraviolet, 544

N

Nadel, M. R.: see *under* Farnsworth, P. N., 190
Nagy, Bartholomew, Scott, Ward M., Modzeleski, Vincent, Nagy, Lois Anne, Drew, Charles M., McEwan, William S., Thomas, Joseph E., Hamilton, Paul B., and Urey, Harold C.: Carbon Compounds in Apollo 11 Lunar Samples, 1028
  see *under* Scott, Ward M., 1129
Nagy, Lois Anne: see *under* Nagy, Bartholomew, 1028
Nahas, Gabriel G.: see *under* Triner, Lubos, 282
Nandy, K., and Wolstencroft, R. D.: Origin of the Optical Polarization in the Nucleus of N.G.C. 1068
Nandy, K. N.: see *under* Brück, M. T., 531
Naranan, S., and Shah, G. A.: Scattering of X-rays from Pulsar NP 0532 by Interstellar Grains, 834
Narlikar, J. V.: see *under* Hoyle, F., 1233
Natvig, J. B., and Turner, M. W.: Rheumatoid Anti Gm Factors with Specificity for the pFc′ Subfragment of Human Immunoglobulin G, 855
  see *under* Turner, M. W., 853
Naugler, Frederic P.: see *under* Erikson, Barrett H., 53
Neerhout, Robert: see *under* Walford, Roy L., 461
Neidle, S., and Rogers, D.: Assignment of Absolute Configuration from Anomalous Dispersion by Oxygen Atoms, 376
Neogi, A. N.: see *under* Allan, G. G., 175
Netter, Hans: Theoretical Biochemistry, *review* by H. Gutfreund, 1267
Neu, Harold C., and Winshell, Elaine B.: Lack of Synergy EDTA with Antimicrobials in Resistant Enterobacteriaceae, 763
Neurath, Hans, Walsh, Kenneth A., and Bradshaw, Ralph A.: Enzymes in Crystals, *correspondence*, 881
Neurath, P., DeRemer, K., Bell, B., Jarvik, L., and Kato, T.: Chromosome Loss compared with Chromosome Size, Age and Sex of Subjects, 281
Neville, A. C.: "Airborne Organism" Identified, 199
Newth, D. R.: Organization and Development of the Embryo, *review*, 480
Newton, C. W.: see *under* Palmén, E., 479
Ng, K. K. F., and Vane, J. R.: Some Properties of Angiotensin Converting Enzyme in the Lung *in vivo*, 1142
Nicholls, R. W.: see *under* Myer, J. A., 928
Nichols, J. L.: Nucleotide Sequence from Polypeptide Chain Termination Region of the Coat Protein Cistron in Bacteriophage R17 RNA, 147
Nicholson, Max: The Environmental Revolution, *review* by A. R. Clapham, 389
Nieduszynski, I., and Preston, R. D.: Crystallite Size in Natural Cellulose, 273
Noble-Nesbitt, J.: Water Uptake from Subsaturated Atmospheres: its Site in Insects, 758
Nogami, Hiroshi: see *under* Urist, Marshall R., 1051
Norman, Colin: Education for Democracy, *review*, 1076
Norris, Dale M.: see *under* Kok, Loke Tuck, 661
Norris, Grace, and Reporter, Minocher: Hydrolysis of Phospholipid Esters localized in Lysosomes and Related Structures of Rat Muscle Cells in Culture, 1246
Northcote, D. H.: Plant Cells, *review*, 391
Nougarède, A.: Précis de Biologie Végétale, *review* by Peter Barlow, 878

O

Oatley, Keith: Brain Mechanisms and Motivation, 797
O'Connell, Robert F., and Verma, Satya Dev: X-Ray Stars and Infrared Excess, *correspondence*, 671
O'Connor, F. B.: Population Dynamics, *review*, 1266
Ogawa, S., Shulman, R. G., Kynock, P. A. M., Lehmann, H.: High Resolution Nuclear Magnetic Resonance Studies of Haemoglobin J Capetown, 1042
Ogg, Andrew: Modular Forms and Dirichlet Series, *review* by A. O. L. Atkin, 1077
Ohama, Koso: see *under* Kadotani, Tetsuji, 735
Okamoto, T.: see *under* Sugiura, M., 598
Oldroyd, J. G.: Elasticity, Fracture and Flow with Engineering and Geological Application, *review*, 568
Oliver, James A.: American Museum of Natural History at 100, 1199
Omenn, Gilbert S., Ontjes, David A., and Anfinsen, Christian B.: Fractionation of Antibodies against Staphylococcal Nuclease on 'Sepharose' Immunoadsorbents, 189
Omholt, Anders: see *under* McCormac, Billy M., 392
Ontjes, David A.: see *under* Omenn, Gilbert S., 189
Oren, Mark E., Herberman, Ronald B., and Rogentine, G. Nicholas, jun.; Effect of Heparin on the Detection of Human Isoantigens by the Cytotoxicity Technique, 951
Ormerod, M. G.: see *under* Lenherr, A. D., 546
Ornan, Ezuz: see *under* Katz, Amnon, 534
Orr, J. C.: see *under* McLintock, I. S., 629
Osborne, Daphne J.: Physiology of Plant Growth and Development, *review*, 480
  see *under* Chem, David, 336
  see *under* Jackson, Michael B., 1019
Osborn, J. W.: New Approach to Zahnreihen, 343
Osborne, N. N.: see *under* Cottrell, G. A., 470
Oswald, Ian, Merrington, Judith, and Lewis, Heather: Cyclical "On Demand" Oral Intake by Adults, 959
Ottow, J. C. G.: Bacterial Mechanisms of Gley Formation in Artificially Submerged Soil, 103
Overweg, N. I. A.: see *under* Triner, Lubos, 282
Owen, D. F.: Inheritance of Sex Ratio in the Butterfly *Acraea encedon*, 662

P

Page, C. G.: see *under* Cole, T. W., 712
Page, Elaine C.: see *under* Arnold, Elsie, 1260
Pagel, B. E. J.: Stellar Astronomy, *review*, 202
  see *under* Harmer, D. L., 349
Paleg, L. G.: Differential Inhibition of Rat Liver Cholesterol Biosynthesis by Plant Growth Retardants, 1252
Palframan, J. F.: see *under* Foreman, J. K., 554
Palmén, E., and Newton, C. W.: Atmospheric Circulation Systems, *review* by F. H. Ludlam, 479
Parker, Alison M.: see *under* Foy, Henry, 952
Parks, R. D., Edit.: Superconductivity, *review* by H. Fröhlich, 300
Parry, H. B.: see *under* Bignami, A., 194
Patterson, B. D.: see *under* Wills, R. B. H., 199
Paton, James: Climate of the Free Atmosphere, *review*, 769
Patton, Stuart, Crozier, G. F., and Benson, A. A.: Serum Lipids and the Death of Spawning Pacific Salmon, 754
Paul, R.: see *under* Bernard, J., 879
Peach, G.: see *under* Barnes, K. S., 374
Pedemonte, E.: see *under* Keller, A., 538
Penston, M. V.: see *under* Evans, G. R., 357
Perkins, Peter G.: Elementary Molecular Bonding Theory, *review* by A. D. Buckingham, 478
Perin, A.: see *under* De-Angeli, Lavinia Carta, 549
Perry, A. R.: Flowers of Europe, *review*, 566
Persellin, Robert H.: see *under* Hempel, Karl H., 955
Peter, J. B.: see *under* Edgerton, V. R., 866
Pethick, Christopher: see *under* Sutherland, Peter, 353
Pettigrew, John D.: see *under* Blakemore, Colin, 426
Pfleiderer, Jörg: Pulsar Periods and Rapid Changes in the Terrestrial Rotation Rate: a Reply, 437
Phillips, Edward: The Times Atlas of the Moon, *review*, 770
  Think, *review*, 1168
Phillips, J. M.: see *under* Fielden, E. M., 632
Phondke, G. P., Sundaram, K., and Sundaresan, P.: Effect of Anti-lymphocyte Serum on the Electrophoretic Mobility of the Lymph Node Cells of the Rat, 79
Pick, E., Kreiči, J., and Turk, J. L.: Release of Skin Reactive Factor from Guinea-pig Lymphocytes by Mitogens, 236
Pickles, M. M.: Autoimmunization and the Autoimmune Hemolytic Anemias, *review*, 566
Pickles, V. R.: Prostaglandins—an Experiment, 636
Pierantoni, Ruggero: see *under* Borsellino, Antonio, 963
Pilbeam, David: *Gigantopithecus* and the Origins of Hominidae, 516
Pimentel, George C., and Spratley, Richard D.: Understanding Chemical Thermodynamics, *review* by D. D. Eley, 203
Pines, David: see *under* Sutherland, Peter, 353
Piper, J., Vane, J. R., and Wyllie, J. H.: Inactivation of Prostaglandins by the Lungs, 600
Piper, Priscilla J., Said, S. I., and Vane, J. R.: Effects on Smooth Muscle Preparations of Unidentified Vasoactive Peptides from the Intestine and Lung, 1144
Pirofsky, Bernard: Autoimmunization and the Autoimmune Hemolytic Anemias, *review* by M. M. Pickles, 566
Pisolkar, V. G.: Effect of Drag Reducing Additives on Pressure Loss across Transitions, 936
Place, Marquis de la: Celestial Mechanics, *review* by L. Rosenfeld, 880
Plageman, S. H.: see *under* Gribbin, J. R., 1123
Planta, R. J.: see *under* Bos, R. C. van den, 183
Plimmer, J. R.: see *under* Mitchell, J. W., 1065
Pocchiari, F.: see *under* De-Angeli, Lavinia Carta, 549
Pohland, Albert E.: see *under* Armbrecht, Bernard D., 1062
Polgar, Peter R., and Kibrick, Sidney: Origin of Small Lymphocytes following Blastogenesis induced by Short-term PHA Stimulation, 857
Pollack, O. J.: Tissue Cultures, *review* by Gresham, G. A., 977
Pollock, J. N.: Sperm Transfer by Spermatophores in *Glossina austeni* Newstead, 1063
Pollock, Leland W.: see *under* Lewis, John R., 1081
Polunin, Oleg: Flowers of Europe, *review* by A. R. Perry, 566
Poon, David P. L.: see *under* Lu, Benjamin C.-Y., 1128
Porwit-Bòbr, Z.: see *under* Ptak, W., 655
Potts, D. M.: Birth Control, *review*, 299
  Conception, Birth and Contraception, *review*, 299
Poulter, L. W.: see *under* Chayen, J., 1050
Power, J. B., Cummins, S. E., and Cocking, E. C.: Fusion of Isolated Plant Protoplasts, 1016
Praag, H. M. van, Korf, J., and Puite, J.: 5-Hydroxyindoleacetic Acid Levels in the Cerebrospinal Fluid of Depressive Patients treated with Probenecid, 1259
Prager, Morton D., and Derr, Ina: Inhibition of Primary Antibody Response by *E. coli* Asparaginase, 952
Pratt, J. G.: see *under* Stevenson, I., 394
Prentice, A. J. R.: On the Connexion between Pulsars and Supernova, 438
Preston, R. D.: see *under* Nieduszynski, I., 273
Priest, Robert E.: Formation of Epithelial Basement Membrane is Restricted by Scurvy *in vitro* and is Stimulated by Vitamin C, 744
Prince, G. Stewart: see *under* Foulkes, S. H., 565
Probatova, N. A.: see *under* Gurtsevich, V. E., 1047
Prohorov, Yu. V., and Rozanov, Yu. A.: Probability Theory, *review* by J. F. C. Kingman, 1169
Prokhovnik, S. J.: Cosmological Theory of Gravitation, 359
Pruitt, K. M., Jamieson, A. D., and Caldwell, R. C.: Possible Basis for Cariostatic Effect of Inorganic Phosphates, 1249
Psotka, J.: see *under* Stromeyer, C. F., 11, 346
Ptak, W., Porwit-Bobr, Z., and Chlap, Z.: Transformation of Hamster Macrophages into Giant Cells with Antimacrophage Serum, 655
Puca, G. A., and Bresciani, F.: Binding Activity of Oestrogen Receptors destroyed by Iodination, 1251
Puite, J.: see *under* Praag, H. M. van, 1259
Pupezin, Jovan: see *under* Jancso, Gabor, 723
Puszkin, S., and Berl, S.: Actin-like Properties of Colchicine Binding Protein isolated from Brain, 558
Pye, J. D., and Roberts, L. H.: Ear Movements in a Hipposiderid Bat, 285

R

Rabinowitz, Zelig, and Sachs, Leo: Control of the Reversion of Properties in Transformed Cells, 136
Raff, M. C., Sternberg, M., and Taylor, R. B.: Immunoglobulin Determinants on the Surface of Mouse Lymphoid Cells, 553
Rahman, A., and Wilson, H. R.: Molecular Conformation of 5-Iodouridine, 64
Rankin, J. M.: see *under* Campbell, D. B., 527
Rao, Manoranjan, see *under* Ganguly, S., 169
Rao, Potu N., and Johnson, Robert T.: Mammalian Cell Fusion: Studies on the Regulation of DNA Synthesis and Mitosis, 159
Rapacz, J., Grummer, R. H., Hasler, Judith, and Shackelford, R. M.: Allotype Polymorphism of Low Density Lipoproteins in Pig Serum (LDLpp 1, LDLpp 2), 941
Rastogi, R. G.: New Type of Ionospheric Disturbance, 258
Ravetz, J. R.: Hydrodynamics, *review*, 1167
Read, K. L. Q.: Statistics and Experimental Design, *review*, 770
Reading, H. W.: see *under* Dewar, A. J., 869

INDEX

Reddish, V. C.: Interstellar Solid Hydrogen: How Much and Where? 367
Reed, C.: *see under* Knox, R. B., 1066
Reed, May: Steroid Hormones and Metabolism, *review*, 204
Regan, D., and Spekreijse, H.: Electrophysiological Correlate of Binocular Depth Perception in Man, 92
Reid, C. P. P., and Hurtt, W.: Root Exudation of Herbicides by Woody Plants: Allelopathic Implications, 291
Reid, R. W.: Tongues of Conscience, *review* by G. F. Barnaby, 105
Reif, W. M.: *see under* Allan, G. G., 175
Reisher, Sam: *see under* Erham, Semih, 340
Remo, John L.: *see under* Chiu, Hong, Yee, 202
Reporter, Minocher: *see under* Norris Grace, 1245
Revol, L.: *see under* Bétuel, H., 459
Rex, D. F., Edit.: Climate of the Free Atmosphere, *review* by James Paton, 769
Richard, G. B.: *see under* Bétuel, H., 459
Richardson, John P.: Reinitiation of RNA Chain Synthesis *in vitro*, 1109
Robbins, A. R.: Mathematical Geodesy, *review*, 666
Roberts, J. E.: The Photographic Action of Ionizing Radiations on Dosimetry and Medical, Industrial, Neutron, Auto- and Microradiography, *review*, 1266
Robinson, Jack H.: Sunrise and Moonrise at Stonehenge 1236
Rodgers, William: Think, *review* by Edward Phillips, 1168
Roe, F. J. C., Carter, R. L., Cotchin, E., and Bonser, G. M.: Should Slides be Seen Blind?, *correspondence*, 1081
Rojas, E., Bezanilla, F., and Taylor, R. E.: Demonstration of Sodium and Potassium Conductance Changes during a Nerve Action Potential, 747
Roman, Ann: *see under* Schubert, David, 154
Ropartz, C.: *see under* Rivat, Liliane, 1136
Roper, S., and Diamond, J.: Strychnine Antagonism and Glycine: a Reply, 1259
Rose, Anthony H., and Harrison, J. S., Edit.: The Yeasts, *review* by S. Hough, 1171
Rose, S. P. R., Bateson, P. P. G., Horn, Ann L. D., and Horn G.: Effects of an Imprinting Procedure on Regional Incorporation of Tritiated Uracil into Chick Brain RNA, 650
Rosen, D.: *see under* Sutton, A. M., 274
Rosenberg, C. W. Von, jun.: *see under* Tear, J. D., 240
Roslansky, John D., Edit.: Communication, *review* by J. D. Carthy, 481
Rosman, M.: *see under* Scolnick, E., 152
Rossen, Roger D.: *see under* Mittal, Kamal K., 1255
Rossum, G. D. V. van: Relation of Intracellular Ca^{2+} to Retention of K^{+} by Liver Slices, 639
Rozanov, Yu. A.: *see under* Prohorov, Yu. V., 1169
Rich, Alexander: *see under* Kuechler, Ernst, 920
Richards, W. G.: Rotational Structure in the Spectra of Diatomic Molecules, *review*, 569
Richmond, C. R.: *see under* Rundo, J., 83
Richmond, Rollin C.: Non-Darwinian Evolution: a Critique, 1025
Richter, C. F.: Seismicity of the Earth, 170
Rickett, B. J.: *see under* Lang, Kenneth, R., 528
Rieder, N.: *see under* Curi, E., 750
Riley, J. P.: Marine Chemistry, *review*, 567
  see under Matthews, A. D., 1242
Rishbeth, Henry, and Garriott, Owen K.: Introduction to Ionospheric Physics, *review* by W. J. G. Beynon, 568
Ritchie, Calvin D.: *see under* Coetzee, J. F., 1170
Rittel, Werner: *see under* Dietrich, Felix M., 75
Riva di Sanseverino, L.: *see under* Kennard, Olga, 33
Rivat, Liliane, Ropartz, C., Burtin, P., Cruchaud, A.: Genetic Control of Deficiencies in γG Subclasses observed among Families with Hypogammaglobulinaemia, 1136
Roberts, D. G., Bishop, D. G., Laughton, A. S., Ziolkowski, A. M., Scrutton, R. A., and Matthews, D. H.: New Sedimentary Basin on Rockall Basin, 170
Roberts, L. H.: *see under* Pye, J. D., 285
Robinson, J. T.: Two New Hominid Vertebrae from Swartkrans, 1217
Robinson, William S.: Self-annealing of Subgroup 2 Myxovirus RNAs, 944
Rodnight, R.: *see under* Weller, M., 187
Rogentine, G. Nicholas, jun.: *see under* Oren, Mark E., 951
Rogers, D.: *see under* Neidle, S., 376
Rollins, Larry D.: *see under* Armbrecht, Bernard D., 1062
Roscoe, D. H.: The Chemistry and Biology of Viruses, *review*, 565
Rosman, M.: *see under* Scolnick, E., 152
Ross, John E.: Abundance of Iron in the Solar Photosphere, 610
Rostron, Christine C.: *see under* Grasso, P., 188
Rotblat, J.: *see under* Lindop, Patricia J., 977
Rothe, J. P.: The Seismicity of the Earth, *review* by D. Davies, 1268
Rowe, David S.: Dyscrasias of Immunoglobulin Production, *review*, 204
Rowe, M. W.: ^{81}Kr Radiation Ages of Stone Meteorites, 368
Rowlands, George: Plasma Physics and Controlled Nuclear Fusion Research, *review*, 478
Rowley, D. A.: *see under* Mosier, D. E., 276
Rowlinson, J. S.: Probability, Information and Entropy, 1196
Roxborough, F. F.: A History of Coal Mining in Great Britain, *review*, 665
Roy, Claude C., Dubois, Reuben S., and Fernand, Philippon: Inhibition of Bile Salts of the Jejunal Transport of 3-O-Methyl Glucose, 1055
Rubin, Bernard, and Saffir, Arthur: Calcification and the Ground Substance: Precipitation of Calcium Phosphate Crystals from a Nutrient Gel, 78
Rubinoowicz, A.: Quantum Mechanics, *review* by S. Zienau, 109
Rubinstein, David, and Stoneman, Colin, Edit.: Education for Democracy, *review* by Colin Norman, 1076
Rudakov, George: Transformations of Subsurface Petroleum, 370
Ruderman, Malvin: Long Period Oscillations in Rotating Neutron Stars, 619
  Pulsar Wobble and Neutron Starquakes, 838
Rundo, J., and Richmond, C. R.: Altitude Effect on the Biological Half Life of Caesium in Man, 83
Russell, A. S.: Rutherford and Boltwood, *review*, 201
Russell, Pamela: *see under* Diener, Erwin, 731
Russi, Serena: *see under* De-Angeli, Lavinia Carta, 549
Rutman, R. J.: *see under* Erham, Semih, 340
Rweyemamu, M. M.: Probable Occurrence of Infectious Bovine Rhinotracheitis Virus in Tanzania in Wildlife and Cattle, 738

S

Sabath, L. D., Leaf, C. D., Gerstein, D. A. and Finland, M.: Altered Cell Walls of *Staphylococcus aureus* resistant to Methicillin, 1074
Sachs, Leo: *see under* Rabinowitz, Zelig, 136
Saffir, Arthur: *see under* Rubin, Bernard, 78
Sagredo, J. L.: *see under* Bullough, K., 1038
Said, Sami I., and Mutt, Victor: Potent Peripheral and Splanchnic Vasodilator Peptide from Normal Gut, 863
  see under Piper, Priscilla J., 1144
Sajdel, E. M.: *see under* Jacob, S. T., 60
Salanki, J., Edit.: Neurobiology of Invertebrates, *review* by R. de G. Weevers, 391
Salk, J.: *see under* Kaplan, A. E., 384
Salter, C. J.: *see under* Berkhuijsen, Elly M., 364
Salthouse, J. A.: Solute-Solvent Interactions, *review*, 1170
Saltpeter, E. E.: Difficulties with Fusion Catalysis by Quarks, 165
Samuel, G. D.: *see under* Whiteside, T. C. B., 94
Samways, C., and Hastings, G. W.: Radiation Grafting Polysulphides, to Wood, 634
Sandley, M.: *see under* Collins, G. G. S., 817
Sanford, F. Bruce: Information Explosion, *correspondence*, 979
Sangston, Deborah: *see under* Kimbrell, G. McA., 754
Santamour, Frank S., jun.: Hybrid Vigour in Seedlings of Re-created London Plane, 1159
Scanu, A. M.; *see under* Tria, E., 877
Scargle, Jeffrey D.: *see under* Caroff, Lawrence J., 168
Scheid, Jennifer: *see under* Kimbrell, G. McA., 754
Schieti-Cavazza, Barbara: *see under* Borsellino, Antonio, 963
Schmeling, von: *see under* Kausch-Blecken, H. H., 1119
Schmid, Otto J.: *see under* Levring, Tore, 106
Schmidt, D. A., Mazaitis, A. J., Kasai, T., and Bautz, E. K. F.: Involvement of a Phage T4 σ Factor and an Anti-terminator Protein in the Transcription of Early T4 Genes *in vivo*, 1012
Schneider, C. W., and Chenoweth, M. B.: Effects of Hallucinogenic and other Drugs on the Nest-building Behaviour of Mice, 1262
Schneiderman, Lawrence J., Junga, Irene G., and Fawley, Dorothea E.: Effect of Phosphate and Non-phosphate Buffers on Thermolability of Unstable Haemoglobins, 1041
Scholes, G.: *see under* Fielden, E. M., 632
Schubert, David, Roman, Ann, and Cohn, Melvin: Anti-Nuclear Acid Specificities of Mouse Myeloma, 154
Schulson, E. M.: *see under* Essen, C. G. van, 847
Schwartz, A. S.: *see under* Eidelberg, E., 1152
Schwartz, Gerald P.: *see under* Trakatellis, Anthony C., 548
Schwarz, Helmut: Light Carrying Electrons, *correspondence*, 1173
Schwartze, Moshe: *see under* Katz, Amnon, 534
Schwarzenbach, Gerold, and Flaschka, Hermann, Complexometric Titrations, *review* by T. S. West, 202
Schweitzer, J. W.: *see under* Angrist, B. M., 651
Sciarra, John J.: *see under* Demarest, Robert J., 299
Scolnick, E., Milman, G., Rosman, M., and Caskey, T.: Transesterification by Peptidyl Transferase, 152
Scott, Peter: Anonymous Bounty, *correspondence*, 1174
Scott, Ward M., Modzeleski, Vincent E., and Nagy, Bartholomew: Pyrolysis of Early Pre-Cambrian Onverwacht Organic Matter ($>3\times10^{9}$ yr old), 1129
  see under Nagy, Bartholomew, 1028
Scrutton, R. A.: *see under* Roberts, D. G., 170
Sear, Stanley: Delayed Hypersensitivity in *Trypanosoma cruzi* Infection, 1256
Seeber, Siegfried, and Weser, Ulrich: Inhibition of ^{3}H-Thymidine Incorporation into Rat Liver Nuclei by *E. coli* L-Asparaginase, 652
Seki, T., Jenssen, T. A., Levin, Y., and Erdös, E. G.: Active Water-insoluble Derivative of Renin, 864
Self, Colin H., and Weitzman, P. D. J.: Separation of Isoenzymes by Zonal Centrifugation, 644
Seller, Mary J.: Lack of Porosity of the Mouse Placenta to Maternal Cells, 1254
Sen, A. K.: *see under* Tobin, T., 745
Seneta, E.: Population Growth and the Multi-type Galton–Watson Process, 766
Seppälä, Markku, and Tolonen, Matti: Histocompatibility and Human Placentation, 950
Serson, H.: *see under* Hattersley-Smith, G., 55
Sewell, G. L.: Thermodynamique et Physique Statistique, Premier Cycle, *review*, 568
Shackleford, R. M.: *see under* Rapacz, J., 941
Shackleton, R. M.: Geological History of Southern Africa, *review*, 975
Shah, G. A.: *see under* Naranan, S., 834
Shalkop, William T.: *see under* Armbrecht, Bernard H., 1061
Shanbrom, Edward: *see under* Walford, Roy L., 461
Sharp, D. W. A.: Coordination Chemistry, *review*, 202
Sharp, J. A.: Axenic Mammalian Cell Reactions, *review*, 772
Sharp, John T.: *see under* Mittal, Kamal K., 1255
Sharpe, William F.: The Economics of Computers, *review* by J. Howlett, 1168
Shaw, N.: An Introduction to Gel Chromatography, *review*, 976
  Electrophoresis of Proteins in Polyacrylamide and Starch Gels, *review*, 976
  Immunochemical Techniques for the Identification and Estimation of Macromolecules, *review*, 976
Shen, B. S. P.: *see under* Usher, P. D., 365
Sheppard, B. L., and French, J. E.: Platelet Adhesion in Rabbit Arteries observed by Scanning and Transmission Electron Microscopy, 1054
Sherwood, Louis M., Herrman, Ingeborg, and Bassett, C. Andrew: Parathyroid Hormone Secretion *in vitro*: Regulation by Calcium and Magnesium Ions, 1056
Shirley, J.: Stratigraphy and Palaeontology, *review*, 1078
Shitov, Yu. P.: *see under* Vitkevich, V. V., 248
Shklovsky, I. S.: Age of Pulsar *PSR* 0833-45, 252
  Nature of Emission from Pulsar *NP* 0532, 251
Shortman, Ken: *see under* Diener, Erwin, 731
Shotton, D. M., and Hartley, B. S.: Amino-acid Sequence of Porcine Pancreatic Elastase and its Homologies with other Serine Proteinases, 802
  and Watson, H. C.: Three-dimensional Structure of Tosyl-elastase, 811
  see under Watson, H. C., 806
Shulman, N. Raphael: *see under* Mosley, James W., 953
Shulman, R. G.: *see under* Ogawa, S., 1042
Siegel, Irwin M.: *see under* Carr, Ronald E., 88
Sikorski, J.: *see under* Walker, P. S., 956
Silver, I. A.: *see under* Comline, R. S., 739
Silver, Marian: *see under* Comline, R. S., 739
Silverstein, R. M.: *see under* Wood, D. L., 557
Simkins, Thomas E.: Effect of Rotational Phase Transformations on Dry (Coulomb) Friction, 446
Simmons, Gene: *see under* Horai, Ki-Iti, 264
Simnett, J. D.: *see under* Chopra, D. P., 657
Simpson, Donald: *see under* Edgerton, V. R., 866
Sinclair, David: Human Growth after Birth, *review* by W. A. Marshall, 108
Singer, Marcus: *see under* Lebowitz, Paul, 824
Singh, R. N.: *see under* Singh, R. P., 49
Singh, R. P., and Singh, R. N.: VLF Wave Generation by the Čerenkov Process in the Inner Magnetosphere, 49
Sizaret, P.: *see under* Foy, Henry, 952
Skarnes, Robert C.: L-Amino-acid Oxidase, a Bactericidal System, 1072
Sklair, Leslie: Politics of Social Research, *review*, 297
Slater, Eliot: Attitudes to Conservation, *correspondence*, 773
Sledge, Clement: *see under* McConaghey, Paddy, 1249
Slobin, L. I.: Antigenic Properties of a Homogeneous Tobacco Mosaic Virus-Hapten Conjugate, 698
Slotin, Cecilia A., Heap, R. B., Christiansen, J. M., and Linzell, J. L.: Synthesis of Progesterone by the Mammary Gland of the Goat, 385
Smith, A. Gilbert, and Hallam, A.: The Fit of the Southern Continents, 139
Smith, A. E., Marcker, K. A., and Mathews, M. B.: Translation of RNA from Encephalomyocarditis Virus in a Mammalian Cell-free System, 184
Smith, Cyril: *see under* Armitage, Peter, 1075
Smith, D. C.: Chemical and Botanical Guide to Lichen Products, *review*, 977
Smith, David S.: "Airborne Organism" Identified, 199
Smith, E. B.: *see under* Halsey, M. J., 1151
Smith, F. G.: *see under* Graham, D. A., 526
Smith, J. David: Tin in Organisms and Water in the Gulf of Naples, 103
Smith, J. V.: *see under* Jamieson, J. C., 354

INDEX

Smith, John Maynard: Natural Selection and the Concept of a Protein Space, 563
Smith, Lindsay Hamilton: see *under* Woodruff, Michael, 377
Smith, M.: see *under* White, J. M., 939
Smith, M. V.: see *under* Mitchell, J. W., 1065
Smith, P. D.: see *under* Bradley, D. J., 719
Smith, R. S.: University Reform, *correspondence*, 206
Smith, R. V., and Evans, M. C. W.: Soluble Nitrogenase from Vegetative Cells of the Blue-Green Alga *Anabaena cylindrica*, 1253
Smith, Sydney: The Triumph of the Darwinian Method, *review*, 390
Smolko, E. E.: see *under* Atkins, E. D. T., 626
Sofuni, Tishio: see *under* Kadotani, Tetsuji, 735
Sohal, R. S.: see *under* Burch, G. E., 286
Solomon, Maurice E.: Population Dynamics, *review* by F. B. O'Connor, 1266
Spadaro, Joseph A., Becker, Robert A., and Bachman, Charles H.: Size-specific Metal Complexing Sites in Native Collagen, 1134
Spector, R. G.: see *under* Mikikits, W., 1150
Speden, I. G.: Palaeontological Data, *correspondence*, 302
Spekreijse, H.: see *under* Regan, D., 92
Spencer-Booth, Y.: The Behavioural Repertoire of the Stumptail Macaque, *review*, 1080
Sprackling, M. T.: The Mechanical Properties of Matter, *review* by A. Kelly, 1267
Spratley, Richard D.: see *under* Pimentel, George C., 203
Sprent, Peter: Models on Regression and Related Topics, *review* by D. V. Lindley, 770
Spronsen, J. W. van: The Periodic System of Chemical Elements, *review* by Frank Greenaway, 477
Squire, Larry R., and Barondes, Samuel H.: Actinomycin-D: Effects on Memory at Different Times after Training, 649
Sreekantan, B. V.: see *under* Chatterjee, B. K., 839
Stagni, N., Bernard, B. de, Costa, G., and Mestroni, G.: Biological Properties of Model Molecules for Vitamin B_{12}, 942
Stark, Lawrence: Proposed New Unit of Frequency, *correspondence*, 394
Staniford, Maxine: see *under* Briggs, Michael, 81
Stefamis, C. N., and Issidorides, M.: Aberrant mRNA in Parkinsonism: Support for Hypothesis from Studies with Chloramphenicol, 963
Steim, Joseph M.: see *under* Kaufman, Stanley, 743
Steinbach, Friedrich: see *under* Müller, Heinrich Dieter, 728
Stepanova, G. N.: see *under* Gurtsevich, V. E., 1047
Stephens, George R.: see *under* Waggoner, Paul E., 1160
Stephens, Patricia M.: see *under* Axelrod, Julius, 1059
Sternberg, M.: see *under* Raff, M. C., 553
Stevens, G. C.: see *under* Fielden, E. M., 632
Stevenson, I., and Pratt, J. G.: Identification of Concealed Randomized Objects, *correspondence*, 394
Steward, F. C., Mott, R. L., Israel, H. W., and Ludford, P. M.: Proline in the Vesicles and Sporelings of *Valonia ventricosa* and the Concept of Cell Wall Protein, 760
Stoll, B. J.: see *under* Farnsworth, P. N., 190
Stoloff, Leonard: see *under* Armbrecht, Bernard D., 1062
Stoneman, Colin: see *under* Rubinstein, David, 1076
Stromeyer, C. F., II, and Psotka, J.: The Detailed Texture of Eidetic Images, 346
Stuart-Harris, C. H.: Virus of the 1918 Influenza Pandemic, 850
Stull, Mark A.: Pk 0048-09: a Possible Radio Variable Galaxy, 833
Su, Shirley, Weinberg, Fanyella, So, Antero G., and Davie, Earl W.: RNA Polymerase and the Shut-off of Host RNA and Protein Synthesis in T4 Phage Infection, 62
Sugiura, M., Okamoto, T., and Takanami, M.: RNA Polymerase σ Factor and the Selection of Initiation Site, 598
Summers, R.: see *under* Bennett, J. E., 932
Summers, William C.: see *under* Maher, Veronica M., 68
Sundaram, K.: see *under* Phondke, G. P., 79
Sundaresan, P.: see *under* Phondke, G. P., 79
Sussman, Maurice: Model for Quantitative and Qualitative Control of mRNA Translation in Eukaryotes, 1245
Sutherland, Peter, Baym, Gordon, Pethick, Christopher, and Pines, David: Can Pulsar Masses be Determined?, 353
Sutton, A. M., and Rosen, D.: Effect of X-rays on the Electrical Conductance of Phospholipid Bilayer Membranes: a Retraction, 274
Szabo, K. T.: Teratogenic Effect of Lithium Carbonate in the Foetal Mouse, 73
Szanto, Stephen, and Yudkin, John: Dietary Sucrose and Platelet Behaviour, 467

T

Takanami, M.: see *under* Sugiura, M., 598
Tait, A.: Genetics of NADP Isocitrate Dehydrogenase in *Paramecium aurelia*, 180
Tanaka, Haruo, and Énomé, Shinzo: High Resolution Observations of Solar Microwave Bursts, 435
Tanemura, Shigegi: see *under* Yourno, Joseph, 422
Tanner, J.: see *under* MacCullum, J. R., 1137
Tärnoky, A. L., Dowdong, B., and Lakin, A.: Eight Types of Bisalbuminaemia, 742
Tasca, Richard J., and Hillman, Nina: Effects of Actinomycin D and Cycloheximide on RNA and Protein Synthesis in Cleavage Stage Mouse Embryos, 1022
Taylor, Philip L.: Sternglass's Assumptions, *correspondence*, 881
Taylor, R. E.: see *under* Rojas, E., 747
Taylor, R. L.: see *under* Teare, J. D., 240
Taylor, R. B.: see *under* Raff, M. C., 553
Taylor, W. I., and Battersby, A. R.: Cycloentanoid Terpene Derivatives, *review* by W. B. Whalley, 1266
Teare, J. D., Taylor, R. L., and Rosenberg, C. W. von, jun.: Observations of Vibration–Vibration Energy Pumping between Diatomic Molecules, 240
Tëbcis, A. K.: Effects of Histamine on the Toad Spinal Cord, 196
Terry, William D.: see *under* Frame, Marion, 641
Thackeray, A. D.: see *under* Brück, M. T., 531
Thirlaway, H. I. S.: Earthquakes and Explosions: Comment on Molnar *et al.*, 1237
Thom, A. S.: see *under* McIntosh, D. H., 974
Thomas, J. O., and Landmark, B. J., Edit.: Plasma Waves in Space and in the Laboratory, *review* by M. K. Wallis, 975
Thomas, Joseph E.: see *under* Nagy, Bartholomew, 1028
Thomas, Lowell P.: Another *Acanthaster* Disaster, *correspondence*, 1269
Thompson, P. A.: Characterization of the Germination Response to Temperature Species and Ecotypes, 827
Thorne, D. J.: Strength–Modulus Relation for Carbonized Acrylic Fibres, 1039
Thorpe, W. H.: see *under* White, Sheila J., 1156
Timbrell, V., Bevan, N. E., Davies, A. S., and Munday, D. E.: Hollow Casts of Lungs for Experimental Purposes, 97
Tipton, K. F.: Enzyme Handbook, *review*, 108
Tkacheva, S. G.: see *under* Vanyushin, B. F., 948
Tobin, T., Banerjee, S. P., and Sen, A. K.: Allosteric Interactions in ($Na^+ + K^+$)-ATPase, 745
Todaro, George J.: see *under* Aaronson, Stuart A., 458
Tolonen, Natti: see *under* Seppala, Markku, 950
Tomlinson, P. B.: Anatomy of Monocotyledons, *review* by D. H. Valentine, 1078
Tonolo, A.: see *under* De-Angeli, Lavinia Carta, 549
Tonwar, S. C.: see *under* Chatterjee, B. K., 839
Touraine, J. L.: see *under* Dalton, R. G., 1140
Trakatellis, Anthony C., and Schwartz, Gerald P.: Biosynthesis of Insulin in Anglerfish Islets, 548
Travers, Andrew A.: Positive Control of Transcription by a Bacteriophage Sigma Factor, 1009
Tria, E., and Scanu, A. M., Edit.: Structural and Functional Aspects of Lipoproteins in Living Systems, *review*, J. B. Finean, 877
Triner, Lubos, Overweg, N. I. A., and Nahan, Gabriel G.: Cyclic 3′,5′-AMP and Uterine Contractivity, 282
Tritsch, George L., Edit.: Axenic Mammalian Cell Reactions, *review*, by J. A. Sharp, 772
Truran, J. W., and Cameron, A. G. W.: The Galactic Halo, 710
Truter, Mary R.: see *under* Bright, D., 176
Tschoegl, N. W.: see *under* Kausch-Blecken, H. H., 1119
Tsuji, Shigeru: see *under* Benda, Philippe, 1149
Turk, J. L.: see *under* Pick, E., 236
Turner, A. J.: The Life of the Honourable Robert Boyle, F.R.S., *review*, 1076
Turner, J. S.: see *under* Davies, D.: 636
Turner, M. W., Bennich, H. H., and Natvig, J. B.: Simple Method of Subtyping Human G-Myeloma Proteins based on Sensitivity to Pepsin Digestion, 853
see *under* Natvig, J. B., 855

U

Uhlenbeck, Olke C., Baller, Julie, and Doty, Paul: Complementary Oligonucleotide Binding to the Anticodon Loop of fMet-transfer RNA, 508
Umminger, Bruce L.: Osmoregulation of the Killifish, *Fundulus heteroclitus*, in Fresh Water at Temperatures near Freezing, 294
Uretsky, N. J.: see *under* Evetts, E. D., 961
Urey, Harold C.: see *under* Nagy, Bartholomew, 1028
Urist, Marshall R., and Nogami, Hiroshi: Morphogenic Substratum for Differentiation of Cartilage in Tissue Culture, 1051
Usher, P. D., Shen, B. S. P., and Wright, F. W.: Yearly Variations of 3C 120, 365

V

Vagg, W. J.: see *under* Melrose, G. J. H., 547
Vaight, P. R.: see *under* Gasser, R. P. H., 933
Vaizey, John: A Theory of Economic, History *review*, 973
Decision Models for Educational Planning, *review*, 1075
The Causes of Graduate Unemployment in India, *review*, 297
Valencio, D. A., and Vilas, J. F.: Palaeomagnetism of some Middle Jurassic Lavas from South-east Argentina, 262
Valentine, D. H.: Anatomy of Monocotyledons, *review*, 1078
Vane, J. R.: see *under* Ferriera, S. H., 379; Ng, K. K. F., 1142; Piper, J., 600; Piper, Priscilla J., 1144;
Vanysek, V., and Harwit, M.: Possibility of a Terrestrial Component in the Doppler Shifted Zodiacal Light, 1231
Vanyushin, B. F., Tkacheva, S. G., and Belozersky, A. N.: Rare Bases in Animal DNA, 948
Varey, R. H.: see *under* Andrews, J. G., 270
Varghese, T. G., and Kumar, Vijai: Detection and Location of an Atmospheric Nuclear Explosion by Microbarograph Arrays, 258
see *under* Arora, S. K., 261
Varricchio, F.: *comment* on Ribosome Ambiguity by Bernard D. Davies, *correspondence*, 206
Vasic, P. M.: see *under* Bottema, O., 1268
Vasilescu, D., Cornillon, R., and Mallet, G.: Piezoelectric Resonances in Amino-acids, 635
Vaughan, A. E., and Large, M. I.: Five New Pulsars, 167
Vdovykin, G. P.: Interstellar Dust and Aromatic Carbon, 254
Vekey, R. C. de, and Majumdar, A. J.: Effect of Electric Field on Phase Separation of Glass, 172
Verma, Satya Dev: see *under* O'Connell, Robert F., 671
Verwilghen, R. L.: see *under* Louwagie, A. C., 383
Vila, Samuel C.: Origin of the Ultra-short Period Binary WZ Sagittae, 1229
Vilas, J. F.: see *under* Valencio, D. A., 262
Villet, R. H.: Genetic Curing of Blindness in *Phycomyces blakesleeanus*: a Quantitative Assessment of Dominance, 453
Viswamistra, M. A.: see *under* Andreson, Bryan, 233
Vitkevich, V. V., and Shitov, Yu. P.: Short Duration Pulsations of CP 0808 and Main Features of its Radio Emission in the Metre Band, 248
Volk, H. J.: see *under* Hollweg, Joseph V., 441
Volpin, D., and Ciferri, A.: Thermoelasticity of Elastin, 382
Vyacheslavov, L.: see *under* Bresler, S., 764
Vyas, G. N., Levin, A. S., and Fudenberg, H. H.: Intrauterine Isoimmunization caused by Maternal IgA crossing the Placenta, 275

W

Waddington, T. C.: Non-Aqueous Solvents, *review* by S. R. C. Hughes, 1077
Wade, Nicholas: Grooks, *review*, 390
More Grooks, *review*, 390
Waggoner, Paul E., and Stephens, George R.: Transition Probabilities for a Forest, 1160
Walford, Roy L.: The Immunologic Theory of Ageing, *review* by Alex Comfort, 1265
Finkelstein, Simon, Neerhout, Robert, Konrad, Pat, and Shanbrom, Edward: Acute Childhood Leukaemia in Relation to the HL-A Human Transplantation Genes, 461
Walker, P. M. W.: see *under* Hennig, W., 915
Walker, P. S., Sikorski, J., Dowson, D., Longfield, M. D., and Wright, V.: Features of the Synovial Fluid Film in Human Joint Lubrication, 956
Walker, E. A.: see *under* Foreman, J. K., 554
Wallace, A. R.: Education, *letter*, *100 Years Ago*, 114
Walliker, D.: Parasitic Protozoa, *review*, 205
Wallis, M. K.: Plasma Waves in Space and in the Laboratory, *review*, 975
Walsh, Kenneth A.: see *under* Neurath, Hans, 881
Walton, A.: see *under* Baxter, M. S., 937
Wampler, D. L.: see *under* Kennard, Olga, 333
Warasila, Robert L.: see *under* Chiu, Hong-Yee, 202
Ward, F. A. B.: Sundials, *review*, 665
Wareing, P. F.: The Induction of Flowering, *review*, 567
Warren, J. W.: Black Velvet, *correspondence*, 206
Capacity for Misunderstanding, *correspondence*, 1082
Warren, S.: see *under* Kennard, Olga, 333
Wasuwat, S.: Extract of *Ipomoea pes-caprae* (Convolvulaceae) Antagonistic to Histamine and Jelly-fish Poison, 758
Watanabe, Tsutomu, Arai, Toshikiho, and Hattori, Takanori: Effects of Cell Wall Polysaccharide on the Mating Ability of *Salmonella typhimurium*, 70
Watkins, J. F.: see *under* Chen, L., 734
Watling, John: Metaphysics and Common Sense, *review*, 767
Watson, D. G.: see *under* Kennard, Olga, 333
Watson, H. C., Shotton, D. M., Cox, Joyce M., and Muirhead, Hilary: Three-dimensional Fourier Synthesis of Tosyl-elastase at 3·5 Å Resolution, 806
see *under* Shotton, D. M., 811
Watts, A. H.: see *under* Bott, M. H. P., 265
Webb, A. C.: see *under* Burns, B. Delisle, 469
Webb, E. C.: Communications in Biochemistry, 132
Webb, L. J., Whitelock, D., and Brereton, J. Le Gay, Edit.: The Last of Lands, *review* by Richard Fitter, 768
Webb, R. A.: see *under* Abbott, A. J., 663
Webster, D. E.: Nuclear Magnetic Resonance Spectroscopy, *review*, 1169

Weevers, R. de G.: Neurobiology of Invertebrates, *review*, 391
Wegmann, Thomas G.: Enzyme Patterns in Tetraparental Mouse Liver, 462
Weinberg, Fanyella: *see under* Su, Shirley, 62
Weinberger, Norman M.: *see under* Goodman, David A., 1153
Weinrott, Mark R.: *see under* Kimbrell, G. McA., 754
Weitzman, P. D. J.: *see under* Self, Colin H., 644
Weller, M., and Rodnight, R.: Stimulation by Cyclic AMP of Intrinsic Protein Kinase Activity in Ox Brain Membrane Preparations, 187
Wellman, T. R.: Gaseous Species in Equilibrium with the Apollo II Holocrystalline Rocks during their Crystallization, 716
Wenham, E. J.: Physics, *review*, 392
Weser, Ulrich: *see under* Seeber, Siegfried, 652
Wesselink, A. J.: How Significant are the Sharp Peaks in the Frequency Distribution of QSO Redshifts, 927
West, T. S.: Complexometric Titrations, *review*, 202
  Photometric Methods of Analysis, 202
Whalley, W. B.: *see under* Taylor, W. I., 1266
Wheeler, K. P., and Whittam, R.: ATPase Activity of Sodium Pump needs Phosphatidylserine, 449
Whewell, C. S.: The Textile Industry, *review*, 973
White, J. M., and Dacie, J. V.: *In vitro* Synthesis of Hb Hammersmith (CDI Phe→Ser), 860
  Brain, M. C., Lorkin, P. A., Lehmann, H., and Smith, M.: Mild "Unstable Haemoglobin Haemolytic Anaemia" caused by Haemoglobin Shepherd's Bush (B74(E18)Gly→Asp), 939
White R. E. G.: *see under* White, Sheila J., 1156
White, S.: Ionic Diffusion in Quartz, 375
White, Sheila J., White, R. E. C., and Thorpe, W. H.: Acoustic Basis for Individual Recognition by Voice in the Gannet, 1156
Whitfield, G. R.: *see under* Bramwell, Cherrie D., 660
Whitehouse, W. J.: *see under* Dyson, E. D., 957
Whitelock, D.: *see under* Webb, L. J., 768
Whiteside, T. C. B., and Samuel, G. D.: Blur Zone, 94
Whittam, R.: *see under* Wheeler, K. P., 449
Whitton, B. A.: *see under* Barnes, J., 268
Wichterle, O.: Science and Government, *correspondence*, 773
Wickramasinghe, N. C.: Retention of Dust Grains near Galactic Nuclei, 145
Wiesel, T. N.: *see under* Hubel, D. H., 41
Wigglesworth, V. B.: The Comparative Endocrinology of the Invertebrates, *review*, 1265
Wilber, Charles G.: The Biological Aspects of Water Pollution, *review* by A. L. Downing, 106
Wilkins, M. B., Edit.: Physiology of Plant Growth and Development, *review* by Daphne J. Osborne, 480
Williams, E. D.: *see under* Collins, G. G. S., 817
Williams, J. C. C.: Simple Photogrammetry, *review* by P. F. Dale, 769
Williams, R. T.: Essays in Toxicology, *review*, 669
Williams, W. P.: Spatial Organization and Interaction of the Two Photosystems in Photosynthesis, 1214
Willmouth, F. M.: *see under* Keller, A., 538
Wills, R. B. H., and Patterson, B. D.: Ethylene, a Plant Hormone from Fluorescent Lighting, 199
Willshaw, D. J., Longuet-Higgins, H. C., and Buneman, O. P.: reply to Models for the Brain (P. J. van Heerden), 177
Willson, R. L.: *see under* Fielden, E. M., 632
Wilson, A. D., Kent, B. E., Mesley, R. J., Miller, R. P., Clinton, D., and Fletcher, K. E.: Formation of Dental Silicate Cement, 272
Wilson, Don E., and Findley, James S.: Reproductive Cycle of a Neotropical Insectivorous Bat, *Myotis nigricans*, 1155
Wilson, H. R.: *see under* Rahman, A., 64
Wilson, J. E.: *see under* Glassman, E., 967
Wilson, J. G.: Measurements on Cloud Chamber Tracks, 1238
Wilson, John T.: Alteration of Normal Development of Drug Metabolism by Injection of Growth Hormone, 861
Wilson, H. R.: Changes in Nucleoside Conformation, 545
Wilson, T. L.: *see under* Altenhoff, W. J., 245
Williams, W. J.: *see under* Goldman, A., 443
Williamson, Ethel: *see under* Burstein, Sumner H., 87
Willis, J. H., and Lawrence, P. A.: Deferred Action of Juvenile Hormone, 81
Winshell, Elaine P.: *see under* Neu, Harold C., 763
Wolfe, Raymond, Edit.: Applied Solid State Science, *review* by G. T. Wright, 771
Wolfers, D.: Problems of Expanding Populations, 593
Wolfgang, Richard: Carbon Monoxide as a Basis for Primitive Life on Other Planets, 876
Wolstencroft, R. D.: *see under* Nandy, K., 1068
Wood, Clive: Birth Control, *review* by D. M. Potts, 299
Wood, D. L., and Silverstein, R. M.: Bark Beetle Pheromones, 557
Wood, R.: *see under* Brück, M. T., 531
Wood, R. K. S.: Diseases of Higher Plants, *review*, 107
Wood, T. E.: *see under* Halsey, M. J., 1151
Woodhall, Maureen: *see under* Blaug, Mark, 297
Woodruff, Michael, and Smith, Lindsay Hamilton: Cytotoxic Efficiency and Effect on Tumour Growth of Heterospecific Antilymphocytic and Antitumour Sera, 377
Woodruff, M. F. A.: *see under* Dalton, R. G., 1140
Woore, J.: *see under* Cooper, J. D., 721
Worley, J. F.: *see under* Mitchell, J. W., 1065
Worsley, T., and Martini, E.: Late Maastrichtian Nannoplankton Provinces, 1242
  see under Martini, E., 289
Wright, Francis W.: *see under* Hodge, Paul W., 717
  see under Usher, P. D., 365
Wright, G. T.: Applied Solid State Science, *review*, 771
Wright, V.: *see under* Walker, P. S., 956
Wuensch, B. J.: *see under* Cho, Seung-Am, 444
Wyatt, A. F. G.: Tunnelling in Solids, *review*, 667
Wyllie, J. H.: *see under* Vane, J. R., 600

Y

Yamamoto, S.: *see under* Yoshinaga, M., 1138
Yen, J. L.: *see under* Galt, J. A., 530
Yoshinaga, M., Mayumi, M., Yamamoto, S., and Hayashi, H.: Immunoglobulin G as Possible Precursor of Chemotactic Factor, 1138
Youdim, M. B. H.: *see under* Collins, G. G. S., 817
Young, D. A.: *see under* Cooper, J. D., 721
Young, Elizabeth: Mild Thaw on Disarmament, *correspondence*, 482
Yourno, Joseph, and Tanemura, Shigegi: Restoration of In-phase Translation by an Unlinked Suppressor of a Frameshift Mutation in *Salmonella typhimurium*, 422
Yu, Paul: *see under* Lu, Benjamin C.-Y., 1128
Yudkin, John: *see under* Szanto, Stephen, 467

Z

Zahler, Stanley A.: *see under* Eisner, Thomas, 661
Zaikin, A. N., and Zhabotinsky, A. M.: Concentration Wave Propagation in Two-dimensional Liquid-phase Self-oscillating System, 535
Zakay-Rones, Zichriya: *see under* Becker, Yechiel, 454
Zavada, Jan, and Macpherson, Ian: Transformation of Hamster Cell Lines *in vitro* by a Hamster Sarcoma Virus, 24
Zeh, H. D.: Evidence for the Existence of Heavy Nuclei Prior to the s-Process, 361
Zeier, H.: Lack of Eye to Eye Transfer of an Early Response Modification in Birds, 708
Zeitman, Benjamin B.: *see under* McDonald, J. Ken, 1048
Zhabotinsky, A. M.: *see under* Zaikin, A. N., 535
Zukas, E. G.: *see under* Green, W. V., 628
Zienau, S.: Quantum Mechanics, *review*, 109
Ziolkowski, A. M.: *see under* Roberts, D. G., 170
Zuill, E.: *see under* Crossley, S., 1064
Zurita, Victoria E.: *see under* De-Angeli, Lavinia Carta, 549

SUBJECT INDEX

A

Abortion. Aberrant Karyotypes and Spontaneous — in a Japanese Family (Tetsuji Kadotani, Koso Ohama, Toshio Sofuni and Howard B. Hamilton), 735
Abortion by Prostaglandin Injection, *news*, 415
Abortion Law, Amendment, *news*, 676
Abortion Statistics, *news*, 579
Abortion Test, *news*, 691
Abscission. Ethylene, the Natural Regulator of Leaf — (Michael B. Jackson and Daphne J. Osborne), 1019
Academic Village, USSR, *news*, 782
Acanthaster Disaster (Lowell P. Thomas), *correspondence*, 1269
Accelerator, Bubble Chamber, Mirabelle, *news*, 127
Accelerator, CERN, *news*, 491
Acetylcholine. Complexes between — and Catecholamines and their Tolerance to Mental Illness (Lauro Galzigna), 1058
  Interaction of — with Different Cholinergic Nerve Receptors (Cyrus Chothia), 36
Acetylcholinesterase. Localization of — by Immunofluorescence in Eel Electroplax (Philippe Benda, Shigeru Tsuji, Jean Daussant and Jean-Pierre Changeux), 1149
Achene. — Spacing of Strawberries as an Aid to calculating Potential Yield (A. J. Abbott and R. W. Webb), 663
Acoustics. — Basis for Individual Recognition by Voice in the Gannet (Sheila J. White, R. E. C. White and W. H. Thorpe), 1156
Actinomycin *D*: —: Effects on Memory at Different Times after Training (Larry R. Squire and Samuel H. Barondes), 649
  Effects of — and Cycloheximide on RNA and Protein Synthesis in Cleavage Stage Mouse Embryos (Richard J. Tasca and Nina Hillman), 1022
  Effect of Cycloheximide and — on the Behaviour of the Headless Cockroach (E. Glassman, A. Henderson, M. Cordle, H. M. Moon and J. E. Wilson), 967
Activity Periodicity. Cyclical "On Demand" Oral Intake by Adults (Ian Oswald, Judith Merrington and Heather Lewis), 959
Adenosine Phosphates. Non-enzymatic Hydrolysis of — (H. R. Hulett), 1248
Adenosine Triphosphate. Three Dimensional Structure of — (Olga Kennard, N. W. Isaacs, J. C. Coppola, A. J. Kirby, Stuart Warren, W. D. S. Motherwell, D. G. Watson, D. L. Wampler, D. H. Chenery, A. C. Larson, K. Ann Kerr and L. Riva di Sanseberine), 333
Adenoviruses. Effects of — on the Chromosomes of Normal Human Cells and Cells Trisomic for an *E* Chromosome (J. K. McDougall), 456
Adjuvants. Effect of — on the Immunosuppressive Properties of Antilymphocytic Globulin (Randall A. Allardyce, Robert D. Hunter and Keith James), 1141
Adrenal. Effect of Foetal Hypophysectomy on Catecholamine Levels in Lamb — during Prolonged Gestation (R. S. Comline, Marian Silver and I. A. Silver), 739
Adrenaline. Changes in Enzymes involved in the Biosynthesis and Metabolism of Noradrenaline and — after Psychosocial Stimulation (Julius Axelrod, Robert A. Mueller, James P. Henry and Patricia M. Stephens), 1059
AEC and Nuclear Research, *news*, 684
AEC Finance, *news*, 582
Aflatoxin B_1. Acute Toxicity of — in Wethers (Bernard H. Armbrecht, William T. Shalkop, Larry D. Rollins, Albert E. Pohland and Leonard Stoloff), 1062
  Mutagenic Action of — on Transforming DNA and Inhibition of DNA Template Activity *in vitro* (Veronica M. Maher and William C. Summers), 68
Age. — of Cosmic Rays and Abundance of Antimatter in the Galaxy (L. Marshall Libby), 166
  — of Pulsar *PSR* 0833-45 (I. S. Shklovsky), 252
  Chromosome Loss compared with Chromosome Size, Age and Sex of Subjects (P. Neurath, K. DeRemer, B. Bell, L. Jarvick and T. Kato), 281
  Neurosecretion-like Material in the Hindbrain of Ageing Sheep and Sheep Affected with Natural Scrapie (A. Bignami, Elizabeth Beck and H. B. Parry), 194
Ageing. Senescent Changes in the Heart of *Drosophila repleta* Wollaston (G. E. Burch, R. S. Sohal and L. D. Fairbanks), 266
Aggression. Intensity of — in the Crayfish, *Orconectes virilis* (Hagen) (Donald B. Heckenlively), 180
Agricultural Land Services, *news*, 901
Agriculture Developments versus Population, 1
Agriculture Finance, *news*, 582
Air Pollution, *news*, 681
Air Shower. Frequency Spectrum of — Radio Pulses (H. R. Allan, R. W. Clay and J. K. Jones), 253
"Airborne Organism". — Identified (A. C. Neville), 198; (David S. Smith), 199
Airborne Particles. Deposition and Uptake by Cattle of Particles (A. C. Chamberlain), 99
Aircraft Smoke Pollution, *parliamentary news*, 680
Aircraft, VTOL, *news*, 577
Airflow Receptor. Antennae: Another Wind-sensitive Receptor in Locusts (M. Gewecke), 1263
Airport, Third London *news*, 466; (W. R. P. Bourne), *correspondence*, 773
Alcohol. Decomposition of Isopropyl — photosensitized by Zinc Oxide (Heinrich Dieter Muller and Friedrich Steinbach), 729
Alcohol in Ireland, *miscellaneous intelligence*, 991
Alcohol Dehydrogenase. Differences in E and S Chains from Isoenzymes of Liver — (Hans Jörnvall), 1133
Aldabra. Sublittoral Reef Phenomena of — (J. Barnes, D. J. Bellamy, D. J. Jones, B. A. Whitton, E. Drew and J. Lythgoe), 268
A-Levels. — and University Performance, (D. G. Bagg), 1105
Alga. Gliding in a Blue-Green —: a Possible Mechanism (Lawrence N. Halfen and Richard W. Castenholz), 1163
  Soluble Nitrogenase from Vegetative Cells of the Blue-Green — *Anabaena cylindrica* (R. V. Smith and M. C. W. Evans), 1253
Algae Conference in India, *news*, 320
Algae: "Redsnow", *news*, 587
Algae. Eustigmatophyceae — a New Algal Glass with Unique Organization of the Motile Cell (David J. Hibberd and Gordon F. Leedale), 759
Alginic Acids. Crystalline Structure of — (E. D. T. Atkins, W. Mackie and E. E. Smolko), 626
Alkali Metal Thiocyanate. Crystal Structure of a Cyclic Polyether Complex of — (D. Bright and Mary R. Truter), 176
Alkaloids. — of an *Ipomoea* Seed known as *Kaladana* in Pakistan (Charles I. Abou-Chaar), 663
Allelopathy. Root Exudation of Herbicides by Woody Plants: Allelopathic Implications (C. P. P. Reid and W. Hurtt), 291
Allergic Encephalomyelitis. Increases in Serum Lactate Dehydrogenase in Experimental — (A. E. Kaplan, L. V. Brown and J. Salk), 384
Allostery. Allosteric Interactions in $(Na^+ + K^+)$-ATPase (T. Tobin, S. P. Banerjee and A. K. Sen), 745
Allostery in Enzyme Regulation, *news*, 319
ALS Globulin. Effect of — on Amino-acid Incorporation by Human Peripheral Lymphocytes (H. Bétuel, G. B. Richard, L. Colobert and L. Revol), 549
Altitude Effects. — on the Biological Half Life of Caesium in Man (J. Rundo and C. R. Richmond), 83
α-Amanitin. Specific Action of — on Mammalian RNA Polymerase Protein (S. T. Jacob, E. M. Sajdel and H. N. Munro), 60
Ameliotes malgassicus, *news*, 224
Amines. Charge Transfer of Tropylium and *N*-Heterocyclic Cations with Aromatic — (T. G. Beaumont and K. N. C. Davis), 632
  Secondary — in Cooking (A. Courts), *correspondence*, 302
Amino-acids. Effect of ALS Globulin on — Incorporation by Human Peripheral Lymphocytes (H. Bétuel, G. B. Richard, L. Colobert and L. Revol), 549
  Effects of Lithium on — in the Mouse Brain *in vivo* (F. V. DeFeudis and J. M. R. Delgado), 749
  Piezoelectric Resonances in — (D. Vasilescu, R. Cornillon and G. Mallet), 635
L-Amino-acid Oxidase. —, a Bactericidal System (Robert C. Skarnes), 1072
Amino-acid Sequence making the Longest English Word, *miscellaneous intelligence*, 8
γ-Aminobutyric Acid. Distribution of ^{14}C-Thiosemicarbazide in the Rat Brain: an Attempt to localize Sites of — Production (Bertalan Csillik and Elizabeth Knyhar), 562
Ammonia Ice in Saturn's Rings, *news*, 687
Ammonite Evolution, *news*, 1101
Amphetamine. Effects of 6-Hydroxydopamine on CNS Catecholamines, Spontaneous Motor Activity and — induced Hyperactivity in Rats (K. D. Evetts, N. J. Uretsky, L. L. Iversen and S. D. Iversen), 961
  Investigation of *p*-Methoxyamphetamine Excretion in — Induced Psychosis (B. M. Angrist, J. W. Schweitzer, A. J. Friedhoff and S. Gershon), 651
Analysis. Absolute, Unambiguous Ultramicroanalysis of Metabolites present in Complex Biological Extracts (J. R. Majer and Alan A. Boulton), 658
Analytical Group of the Society of Analytical Chemistry, *news*, 1104
Angara Reservoirs, Geological Effects, *news*, 7
Angiotensin Converting Enzyme. Some Properties of — in the Lung *in vivo* (K. K. F. Ng and J. R. Vane), 1142
Amphibian. Stimulation of Mitosis in — Kidney by Organ Specific Antiserum (D. P. Chopra and J. D. Simnet), 657
Amphibian: Ancestry, *news*, 1101; Evolution, *news*, 904
Amsterdam Excavated, *news*, 1090
Anaesthetics. Effects of General — on Na^+ Transport in Human Red Cells (M. J. Halsey, E. B. Smith and T. E. Wood), 1151
Angiotensin I Converting Enzyme. Activity of Various Fractions of Bradykinin Potentiating Factor against — (S. H. Ferreira, L. J. Greene, V. A. Alabaster, Y. S. Bakhle and J. R. Vane), 379
Anglerfish Islets. Biosynthesis of Insulin in — (Anthony C. Trakatellis and Gerald P. Schwartz), 548
Animal Experiments, *parliamentary news*, 495
Animal Foodstuffs with Antibiotics, *news*, 311
Anisoles. Microinvasion of Epidermis caused by Substituted — (P. Grasso and Christine C. Rostron), 188
Anisotropic Media. Stoneley Waves in — (T. C. Lim and M. J. P. Musgrave), 372
Annual Production. — and Respiration in Animal Populations (S. McNeill and J. H. Lawton), 472
Anopheles annulipes. Transmission of *Plasmodium berghei* (NK 65 Strain) by *Anopheles annulipes* Walker (Elizabeth C. Kalucy and B. McMillan), 97
Antarctic and Arctic Russian Expeditions, *news*, 118
Antarctic Building Techniques, *news*, 221
Antarctic Research, US, news, 121
Antennae. —: Another Wind-sensitive Receptor in Locusts (M. Gewecke), 1263
Anthropology, *miscellaneous intelligence*, 495
Antibiotics. Discreteness of Conductance Change in Bimolecular Lipid Membranes in the Presence of Certain — (S. B. Hladky and D. A. Haydon), 451
Antibiotics Action, *news*, 901
Antibiotics in Animal Foodstuffs, *news*, 311
Antibiotics Mechanisms, *news*, 795
Antibody. Demonstration in Sarcoma Patients of Anti-tumour — which Fix Only Human Complement (F. R. Eilber and D. L. Morton), 1137
  Effect of DNA Tumour Virus on — Forming Cells in Neonatally Infected Hamsters (Herman Friedman and Herman Goldner), 455
  Fractionation of — against Staphylococcal Nuclease on 'Sepharose' Immunoadsorbents (Gilbert S. Omenn, David A. Ontjes and Christian B. Anfisen), 189
  Inhibition of Primary — Response by *E. coli* Asparaginase (Morton D. Prager and Ina Derr), 952
  Lymphocyte Cytotoxic — in Systemic Lupus Erythematosus (Kamal K. Mittal, Roger D. Rosen, John T. Sharp, Martin D. Lidsky and William T. Butler), 1255
  Properties of Protective Malaria — (S. Cohen and G. A. Butcher), 732
Antigen. Antigenic Expression of a Murine Lymphoma during Growth *in vitro* (Matko Cikes), 645
  Antigenic Site(s) of Synthetic Human Calcitonin *M* (Felix M. Dietrich and Werner Rittel), 75
  Antigenic Properties of a Homogeneous Tobacco Mosaic Virus-Hapten Conjugate (L. I. Slobin), 698
  Evidence against the Presence of H2 Histocompatibility — in Ehrlich Ascites Tumour Cells (L. Chen and J. F. Watkins), 734
  Failure to detect an — associated with Hepatitis in a Community Epidemic (James W. Mosley, Lewellys F. Barker, N. Raphael Shulman and Milford H. Hatch), 953

  - Localization of — associated with the Pollen Grain Wall by Immunofluorescence (R. B. Knox, J. Heslop-Harrison and C. Reed), 1066
  - Radioimmune Assay for — (Gabriel V. Foschi and Lionel A. Manson), 853
  - Surface Leukaemic — and Malignancy of Cells in Mice infected with Mazurenko Virus (V. E. Gurtsevich, N. P. Mazurenko, E. I. Jarova, N. A. Probatova and G. N. Stepanova), 1047
- 'Anti-helium' nuclei, *news*, 678
- Anti-lymphocyte Serum. Effect of — on the Electrophoretic Mobility of the Lymph Node Cells of the Rat (G. P. Phondyke, K. Sundaram and P. Sundaresan), 79
- **Antilymphocytic Globulin.** Effect of Adjuvants on the Immunosuppressive Properties of — (Randall A. Allardyce, Robert D. Hunter and Keith James), 1141
  - Effect of — on the Appearance of Reticular Neoplasms in SJL/J Mice (N. A. Burstein and A. C. Allison), 1139
  - Effect of Route of Administration of — on Skin Allograft Survival in Rats (R. G. Dalton, J. L. Touraine, N. F. Anderson and M. F. A. Woodruff), 1140
- Antimacrophage Serum. Transformation of Hamster Macrophages into Giant Cells with — (W. Ptak, Z. Porwit-Bobr and Z. Chlap), 655
- Antimatter. Age of Cosmic Rays and Abundance of — in the Galaxy (L. Marshall Libby), 166
- Antimicrobials. Lack of Synergy of EDTA with — in Resistant Enterobacteriaceae (Harold C. Neu and Elaine B. Winshell), 763
- Antimicrobial Substances. Absence of — in the Egg Capsules of Millipedes (Thomas Eisner, Stanley A. Zahler, James E. Carrel, Dorothy J. Brown and George W. Lones), 661
- Aphid. Suspected Insecticide Resistance Mechanism in the Peach–Potato — (V. F. Eastop and C. J. Banks), 970
- **Apollo 11.** Carbon Compounds in — Lunar Samples (Bartholomew Nagy, Ward M. Scott, Vincent Modzeleski, Lois Anne Nagy, Charles M. Drew, William S. McEwan, Joseph E. Thomas, Paul B. Hamilton and Harold C. Urey), 1028
  - Gaseous Species in Equilibrium with the — Holocrystalline Rocks during their Crystallization (T. R. Wellman), 716
- Apollo 11: Data, *news*, 15; Lunar Science Conference, *news*, 321
- Apollo 12: Moon Rocks Distribution, *news*, 782; Report, *news*, 1194
- Apple Scab Disease. Antagonism between the Effects of Kinetin and Extracellular Metabolites of the Pathogen in — (D. S. Kirkham and R. C. Hignett), 388
- Aphrodite Research by Dr Iris Love, *miscellaneous intelligence*, 120
- ARC, and Plant Breeding, *news*, 779
- Archaeology Mathematics, Conference, *news*, 1182
- Archeology Underwater and the Law, *news*, 1090
- Architecture Civic Trust Awards, *news*, 493
- Architecture School Merger Cancelled, *news*, 575
- Arctic and Antarctic Russian Expeditions, *news*, 118
- Arctic Norway Geology, *news*, 313
- Argentina. Palaeomagnetism of some Middle Jurassic Lavas from South-east — (D. A. Valencio and J. F. Vilas), 262
- Ariel Vertebrate. Flying Speed of the Largest — (Cherrie D. Bramwell and G. R. Whitfield), 660
- Artery Growth. Post-natal Growth of the Arterial Net in the Human Cerebral Pia Mater (Deowattee Harnarine-Singh and John Baskerville Hyde), 86
- Ascites Sarcoma. Effect of L-Asparaginase from *Aspergillus terreus* on — in the Rat (Lavinia Carta de-Angeli, F. Pocchiari, Serena Russi. A. Tonolo, Victoria E. Zurita, E. Ciaranfi and A. Perin), 549
- Asiatic Flu Conference, *miscellaneous intelligence*, 217
- Asparaginase. Inhibition of Primary Antibody Response by *E. coli* — (Morton D. Prager and Ina Derr), 952
- L-**Asparaginase.** Effect of — from *Aspergillus terreus* on Ascites Sarcoma in the Rat (Lavinia Carta De-Angeli, F. Pocchiari, Serena Russi, A. Tonolo, Victoria E. Zurita, E. Ciaranfi and A. Perin), 549
  - Immunosuppression by — (M. C. Berenbaum), 550
  - Inhibition of ^{3}H-Thymidine Incorporation into Rat Liver Nuclei by *E. coli* — (Siegfried Seeber and Ulrich Wesker), 652
- L-Aspartate Esters. Orientation of Polypeptide Side Chains in Solution (E. M. Bradbury, B. G. Carpenter, C. Crane-Robinson and H. Goldman), 65
- Association of Teachers in Technical Institutions, Report, *news*, 782
- Astronomers, Chinese, *miscellaneous intelligence*, 894
- Astronomer. Lockyer as — (A. J. Meadows), 230
- Astronomical Society Presidential Address, *news*, 686
- Astronomy. High Energy Gamma-ray — (G. G. Fazio), 905
- Astronomy: Galaxy Machine, *news*, 308; Society, *news*, 497
- Astronaut Death, *news*, 216
- Atlantic. Umbellulidae Distribution extended in the — (W. Jahn), 1068
- Atlantic Salmon Fishing Battle, *news*, 887
- Atlas of Disease, *news*, 1091
- **Atmosphere.** Detection and Location of an Atmospheric Nuclear Explosion by Microbarograph Arrays (T. G. Varghese and Vijai Kumar), 258
  - Equatorial Jets in Planetary — (R. Hide), 254
  - Interstellar and Atmospheric Clouds (G. R. Evans and M. V. Penston), 357
  - Observations of Atmospheric Turbulence with a Radio Telescope at 5 GHz (R. A. Hinder), 614
  - Origin and Dynamical Behaviour of Thermal Protons in the Venusian Ionosphere (P. M. Banks and W. I. Axford), 924
  - Tungsten-181 in Fallout in Finland (L. Blomqvist and M. Asikainen), 58
  - Vertical Distribution of CO near the Tropopause (Eigil Hesstvedt), 50
  - Water Uptake from Subsaturated —: its Site in Insects (J. Noble-Nesbitt), 754
  - Windborne Dispersal of Foot and Mouth Virus (K. P. Norris and G. N. Harper), 98
- Atmosphere of Venus, *news*, 689
- Atmosphere Pollution by Petrol Lead, *news*, 990
- Atmosphere Probing, *news*, 7
- Atmospheric Agency, *news*, 897
- Atomic Beam Velocity, *news*, 591
- Atomic Energy Commission, Finance, *news*, 582
- ATP Synthesis. Net Synthesis of ATP by Reversal of the Sodium Pump (V. L. Lew, I. M. Glynn and J. C. Ellory), 865
- **ATPase.** Allosteric Interactions in $(Na^+ + K^+)$— (T. Tobin, S. P. Banerjee and A. K. Sen), 745
  - —Activity of the Sodium Pump needs Phosphatodylserine (K. P. Wheeler and R. Whittam), 449
- ATTI Report, *news*, 782
- Auditory Illusions, *news*, 901
- Auroral Zone. Satellite Observations of Soft Particle Fluxes in the — (W. J. Heikkila), 369
- Australian Ancient Human Bones, *news*, 216
- Australian Biochemical Society Presidential Address: Communication in Biochemistry (E. C. Webb), 132
- Australian Grape Research, *news*, 228
- Australopithecine Site. New Finds at the Swartkrans — (C. K. Brain), 1112
- Australopithecines and Early Man, *news*, 1188
- Avalanches, *news*, 678
- Aviation, Blur Zone (T. C. D. Whiteside and G. D. Samuel), 94

B

- BAC 3-11, *parliamentary news*, 894
- **Bacteria.** Bacterial Mechanism of Gley Formation in Artificially Submerged Soil (J. C. G. Ottow), 103
  - Complete Mutagenesis in a — Population induced by Thymine Starvation on Solid Media (S. Bresler, M. Mosevitsky and L. Vyacheslavov), 764
- Bacteria Cell Walls. Teichoic Acids and Membrane Function in Bacteria (S. Hepinstall, A. R. Archibald and J. Baddiley), 519
- Bacterial Cell Walls, *news*, 587
- Bacterial Cell Wall Synthesis, Role of Mesosomes, *news*, 130
- Bacterial Reproduction. Effects of Cell Wall Polysaccharide on the Mating Ability of *Salmonella typhimurium* (Tsutomu Watanabe, Toshihiko Arai and Takanori Hattori), 70
- Bactericide. L-Amino-acid Oxidase, a Bactericidal System (Robert C. Skarnes), 1072
- Bacteriophage R 17 RNA. Nucleotide Sequence from the Polypeptide Chain Termination Region of the Coat Protein Cistron in — (J. L. Nichols) 147
- **Bacteriophage T4.** Involvement of a — σ Factor and Anti-terminator Protein in the Transcription of Early T4 Genes *in vivo* (D. A. Schmidt, A. J. Mazaitis, T. Kasai and E. K. F. Bautz), 1012
  - Positive Control of Transcription by a — Sigma Factor (Andrew A. Travers), 1009
  - RNA Polymerase and the Shut-off Host RNA and Protein Synthesis in — Infection (Shirley Su, Fanyela Weinberg, Antero G. So and Earl W, Davie), 62
- Bahamian Platform. Conceptual Model of the — for the Last 135 Million Years (George W. Lynts), 1226
- Baird's Invention and the P.O., *miscellaneous intelligence*, 405
- Banana. Possible Side Effects of Fungicides on — and Coffee Diseases (I. D. Firman), 1161
- Band Structure. Hückel-level Calculations on the — of Poly-AT (R. L. Flurry, jun., and Donna Breen), 729
- Band Structure of Copper, *news*, 901
- Bark Beetle. — Pheromones (D. L. Wood and R. M. Silverstein), 557
- Barley. High Frequency Haploid Production in — (*Hordeum vulgare* L.), (K. J. Kasha and K. N. Kao), 874
- Barnard's Book, *miscellaneous intelligence*, 581
- Bat. Reproductive Cycle of a Neotropical Insectivorous —, *Myotis nigricans*, 1153
- Battelle Memorial Institute, Research Spending Estimates, *news*, 7
- Beagle Aircraft Company, *parliamentary news*, 784
- Beech Bark Disease, *news*, 691
- Beetle: *Rhyparus sculpturatus*, *news*, 131
- **Behaviour.** — Patterns in Chromatin-positive Klinefelter's Syndrome of Sheep (R. Kilgour and A. N. Bruere), 71
  - Changes in Enzymes involved in the Biosynthesis and Metabolism of Noradrenaline and Adrenaline after Psychosocial Stimulation (Julius Axelrod, Robert A. Mueller, James P. Henry and Patricia M. Stephens), 1590
  - Courtship — of some *Drosophila melanogaster* Mutants (S. Crossley and E. Zuill), 1064
  - Effect of Cycloheximide and Actinomycin *D* on the Behaviour of the Headless Cockroach (E. Glassman, A. Henderson, M. Cordle, H. M. Moon and J. E. Wilson), 967
  - Effects of Hallucinogenic and other Drugs on the Nest-building Behaviour of Mice (C. W. Schneider and M. B. Chenoweth), 1262
  - Effect of Early Malnutrition on the Reaction of Adult Rats to Aversive Stimuli (David Levitsky and Richard H. Barnes), 468
  - Intensity of Aggression in the Crayfish, *Orconectes virilis* (Hagen), (Donald B. Heckenlively), 180
  - Stereoscopic Vision in the Macaque Monkey: a Behavioural Demonstration (Edward W. Bough), 42
- Bell Biology European Organization, *news*, 677
- Benioff Zones. Symmetry of Crustal Earthquakes above — (Trevor Hatherton), 844
- Benn, at Manchester Technology Association Meeting, 890
- Benn's Story, *miscellaneous intelligence*, 894
- Beyaev's Death, *news*, 216
- Bile. Inhibition by — Salts of the Jejunal Transport of 3-O-Methyl-Glucose (Claude C. Roy, Reuben S. Dubois and Fernand Philippon), 1055
- **Binary Stars.** 57 Cygmi, *news*, 222; WZ Sagittae. Origin of the Ultra-short Period — (Samuel C. Vila), 1229
- Biochemical and Chemical Societies Meeting in Oxford, *news*, 224
- Biochemical Society Brain Function Symposium, *news*, 317
- Biochemical Thermodynamics of Enzyme Reactions, *news*, 690
- Biochemistry. Communications in — (E. C. Webb), 132
- Biochemistry Symposium at Miami, *news*, 498
- Biological Control. Introduction of a New Virus Disease of the Coconut Rhinoceros Beetle in Western Samoa (K. J. Marschall), 288
- Biological Molecules Crystal Studies, *news*, 1000
- Biologists and ESR, *news*, 1102
- Biology Information Experiment, *news*, 989
- Biomedicine Computing Journals, *miscellaneous intelligence*, 581
- Biopolymer Structure Conference, Problems, *miscellaneous intelligence*, 11
- Bird Netting in Italy, *news*, 494; 677
- Birds. Lack of Eye to Eye Transfer of an Early Response Modification in — (H. Zeier), 708
- Bisalbuminaemia. Eight Types of — (A. L. Tárnoky, B. Dowding and A. L. Lakin), 742
- Black Arrow Success, *news*, 987
- Black Velvet (J. W. Warren), *correspondence*, 206
- Black Widow Spider Venom. Effects of — on the Frog Neuromuscular Junction (Herbert E. Longenecker, jun., William P. Hurlbut, Alexander Mauro and Allen W. Clarke), 701
- Blastogenesis. Origin of Small Lymphocytes following — induced by Short-term PHA Stimulation (Peter R. Polgar and Sidney Kibrick), 857
- Blind Viewing of Slides, *correspondence* (F. J. C. Roe, R. L. Carter, E. Cotchin and G. M. Bonser), 1081; (P. Grasso), 1269
- Blindness. Genetic Curing of — in *Phycomyces blakesleeanus*: a Quantitative Assessment of Dominance (R. H. Villet), 453
- Blood. Serum Lipids and the Death of Spawning Pacific Salmon (Stuart Patton, G. F. Crozier and A. A. Benson), 754
- Blood-Brain Barrier. Absence of a — in a Crustacean, *Carinus maenas* L. (Joan Abbott), 291
- **Blood Platelets.** — Adhesion in Rabbit Arteries observed by Scanning Electron Microscopy (B. L. Sheppard and J. E. French), 1054
  - Dietary Sucrose and Platelet Behaviour (Stephen Szanto and John Yudkin), 467
  - Magnetic Fields and the Number of — (Madelaine F. Barnothy and Jeno M. Barnothy). 1146
- **Blood Red Cells.** Effects of Drugs on — Fragility (W. Mikikits, A. Mortara and R. G. Spector), 1150
  - Effects of General Anaesthetics on Na^+ Transport in Human — (M. J. Halsey, E. B. Smith and T. E. Wood), 1151
- Blood Vessels. Platelet Adhesion in Rabbit Arteries observed by Scanning Electron Microscopy (B. L. Sheppard and J. E. French), 1054
- Blue-green Algae, Madras Conference, *news*, 320
- Blur Zone (T. C. D. Whiteside and G. D. Samuel), 94
- *BMJ* Christmas Quiz, *miscellanoeus intelligence*, 8
- Bode's Law. Dynamic Relaxation of Planetary Systems and — (J. G. Hills), 840
- Boeing Dismissals, *news*, 312
- Bohr Effect. Negative — in Newt Haemolysates and its Regulation (G. Morpurgo, P. A. Battaglia and T. Leggio), 76
- Boll Weevil Pheromones Synthesized, *news*, 1100

INDEX

Bone. Scanning Electron Microscope Studies of Human Trabecular — (E. D. Dyson, C. K. Jackson and W. H. Whitehouse), 957
Bone Marrow Grafting. Growth of Haemopoietic Spleen Colonies after Grafting of Human Bone Marrow in Mice (A. C. Louwagie and R. L. Verwilghen), 383

Book Reviews

- ABC of Medical Genetics, An (C. O. Carter), *review* by E. H. R. Ford, 430
- Aflatoxin (Edit. by Leo A. Goldblatt), *review* by M. F. Dutton, 1171
- Anatomy of Monocotyledons, Vol. 3 (P. B. Tomlinson); Vol. 4 (D. F. Cutler), *review* by D. H. Valentine, 1078
- Applied Solid State Science (Edit. by Raymond Wolfe), *review* by G. T. Wright, 771
- Atmospheric Circulation Systems (E. Palmén and C. W. Newton), *review* by F. H. Ludham, 479
- Atmospheric Emissions (Edit. by Billy M. McCormac and Anders Omholt), *review* by David W. Hughes, 392
- Atomic Shield (US Atomic Energy Commission) *review* by J. F. Jackson, 1167
- Autoimmunization and the Autoimmune Haemolytic Anaemias (Bernard Pirofsky), *review* by M. M. Pickles, 566
- Axenic Mammalian Cell Reactions (Edit. by George L. Tritsch), *review* by J. A. Sharp, 772
- Basic Radiation Protection (C. W. Easley), *review* by M. Ebert, 880
- Behavioural Repertoire of the Stumptail Macaque, The (Mireille Bertrand), *review* by Y. Spencer-Booth, 1080
- Biological Aspects of Water Pollution, The (Charles G. Wilber), *review* by A. L. Downing, 106
- Birth Control (Clive Wood), *review* by D. M. Potts, 299
- Can Primitive Farming be Modernised? (F. Jurion and J. Henry), *review* by A. N. Duckham, 298
- Causes of Graduate Unemployment in India, The (Mark Braug, Richard Layard and Maureen Woodhall), *review* by John Vaizey, 297
- Celestial Mechanics (Marquis de la Place), *review* by L. Rosenfeld, 880
- Chemical and Botanical Guide to Lichen Products (Chicita F. Culberson), *review* by D. C. Smith, 977
- Chemistry and Biology of Viruses, The (Heinz Fraenkel-Conrat), *review* by D. H. Roscoe, 565
- Chemistry of the Isoquinolone Alkaloids, The (Tetsuji Kametani), *review* by J. A. Joule, 479
- Climate of the Free Atmosphere (Edit. by D. F. Rex), *review* by James Paton, 769
- Communication (Nobel Conference), *review* by J. D. Carthy, 481
- Comparative Endocrinology of the Invertebrates, The (Kenneth C. Highnam and Leonard Hill), *review* by V. B. Wigglesworth, 1265
- Complexometric Titrations (Gerold Schwarzenbach and Hermann Flaschka), *review* by T. S. West, 202
- Conception, Birth and Contraception (Robert J. Demarest and John J. Sciarra), *review* by D. M. Potts, 299
- Coordination Chemistry (Edit. by Stanley Kirschner), *review* by D. W. A. Sharp, 202
- Critical Path Method, The (A. Kaufman and G. Desbazeille), *review* by P. L. Cloot, 668
- Cyclopentanoid Terpene Derivatives (Edit. by W. I. Taylor and A. R. Battersby), *review* by W. B. Whalley, 1266
- Decision Models for Education Planning (Peter Armitage, Cyril Smith and Paul Alper), *review* by John Vaisey, 1075
- Deserts of the World (Edit. by William G. McGinnies, Bram J. Goldman and Patricia Paylore), *review* by J. L. Cloudsley-Thompson, 768
- Die Werke von Jakob Bernoulli, *review* by J. R. Ravetz, 1167
- Dyscrasias of Immunoglobulin Production (Benjamin L. Gordon), *review* by David S. Rowe, 204
- Economics of Computers, The (William F. Sharpe), *review* by J. Howlett, 1168
- Education for Democracy (Edit. by David Rubinstein and Colin Stoneman), *review* by Colin Norman, 1076
- Elasticity, Fracture and Flow with Engineering and Geological Applications (J. C. Jaeger), *review* by J. G. Oldroyd, 568
- Electrophoresis of Proteins in Polyacrylamide and Starch Gels (A. H. Gordon), *review* by N. Shaw, 976
- Electro-slag Refining (W. E. Duckworth and G. Hoyle), 478
- Elementary Molecular Bondings (Peter G. Perkins), *review* by A. D. Buckingham, 478
- Engineering Geology and Rock Mechanics (Neil Duncan), *review* by John R. V. Brooks, 667
- Environmental Revolution, The (Max Nicholson), *review* by A. R. Clapham, 389
- Enzyme Handbook (Thomas E. Barman), *review* by K. F. Tipton, 108
- Essays in Toxicology (Edit. by Frank R. Blood), *review* by R. T. Williams, 669
- Essentials of Meteorology (D. H. McIntosh and A. S.Thom), *review* by R. S. Harwood, 974
- Factors in the Transfer of Technology (Edit. by William H. Gruber and Donald G. Marquis), *review* by John Langrish, 201
- Fluorescent Antibody Techniques and their Applications (Edit. by Akiyoski Kawamura, jun.), *review* by E. J. Holborow, 878
- Flow Through Porous Media (Edit. by Roger J. M. De Wiest), *review* by M. J. Hall, 1267
- Flowers of Europe (Oleg Polunin), *review* by A. R. Perry, 566
- Formal and Transcendental Logic (Edmund Husserl), *review* by R. A. Crowson, 477
- Geological History of Southern States (S. H. Haughton), *review* by R. M. Shackleton, 975
- Geometric Inequalities (O. Bottema, R. Z. Djordjević, R. R. Janić, D. S. Mitrinović and P. M. Vasić), *review* by James Hirschfeld, 1268
- Grooks (Piet Hein), *review* by Nicholas Wade, 390
- Guide to Superconductivity, A (Edit. by David Fishlock), *review* by David Dew-Hughes, 108
- Halides of the First Row Transition Metals (R. Colton and J. H. Canterford), *review* by D. J. Machin, 203
- Halides of the Second and Third Row Transition Metals (J. H. Canterford and R. Colton), *review* by D. J. Machin, 203
- Helium-3 and Helium-4 (William E. Keller), *review* by D. F. Brewer, 770
- History of Coal Mining in Great Britain, A (Robert L. Galloway), *review* by R. F. Roxborough, 665
- Hormonal Conception (Jürgen Haller), *review* by M. H. Briggs, 299
- Human Growth after Birth (David Sinclair), *review* by W. A. Marshall, 108
- Hydrodynamics (Daniel Bernoulli), *review* by J. R. Ravetz, 1167
- Immunochemical Techniques for the Identification and Estimation of Macromolecules (J. Clausen), *review* by N. Shaw, 976
- Immunological Theory of Ageing, The (Roy L. Walford), *review* by Alex Comfort, 1265
- Induced Radioactivity (Marcel Barbier), *review* by R. W. P. Dreever, 1170
- Induction of Flowering, The (Edit. by L. T. Evans), *review* by P. F. Wareing, 567
- Introduction to Gel Chromatography, An (L. Fischer), *review* by N. Shaw, 976
- Introduction to Ionospheric Physics (Henry Rishbeth and Owen K. Garriott), *review* by W. J. G. Beynon, 568
- Last of the Lands, The (Edit. by J. L. Webb, D. Whitelock and J. Le Gay Brereton), *review* by Richard Fitter, 768
- Lectures on Analysis (Gustave Choquet), *review* by D. A. Edwards, 880
- Lectures on Lie Groups (J. Frank Adams), *review* by R. W. Carter, 976
- Life of Mammals, The (Harrison Matthews), *review* by R. J. Harrison, 107
- Life of the Honourable Robert Boyles, F.R.S., The (R. E. W. Maddison), *review* by A. J. Turner, 1076
- Lysosomes in Biology and Pathology (Edit. by J. T. Dingle and Honor B. Fell), *review* by Lucille Bitensky, 877
- Marine Algae (Tore Levring, Heinz A. Hoppe and Otto J. Schmid), *review* by E. M. Burrows, 106
- Marine Chemistry (R. A. Horne), *review* by J. P. Riley, 567
- Mathematical Geodesy (Martin Hotine), *review* by A. R. Robbins, 666
- Mathematical Logic and Hilbert's ε-Symbol (A. C. Leisenring), *review* by R. M. Dicker, 1077
- Mechanical Properties of Matter, The (M. T. Sprackling), *review* by A. Kelly, 1267
- Metaphysics and Common Sense (A. J. Ayer), *review* by John Watling, 767
- Methods for the Study of Sedimentary Structures (Arnold H. Bouma), *review* by J. R. L. Allen, 666
- Microwaves (A. J. Baden Fuller), *review* by A. L. Cullen, 392
- Models in Regression and Related Topics (Peter Sprent), *review* by D. V. Lindley, 770
- Modular Forms and Dirichlet Series (Andrew Ogg), *review* by A. O. L. Atkin, 1077
- More Grooks (Piet Hein), *review* by Nicholas Wade, 390
- Named Organic Reactions (Ronald C. Denney), *review* by E. J. Forbes, 108
- Neurobiology of Invertebrates (Edit. by J. Salanki), *review* by R. de G. Weevers, 391
- Neurological Basis of Motivation, The (Edit. by Stephen E. Glickman and Peter M. Milner), *review* by J. L. Malcolm, 205
- New Lives, New Landscapes (Nan Fairbrother), *review* by R. J. S. Hookway, 1075
- Non-Aqueous Solvents (T. C. Waddington), *review* by S. R. C. Hughes, 1077
- Nuclear Fusion (H. R. Hulme and A. McB. Collieu), *review* by Daphne F. Jackson), 479
- Nuclear Magnetic Resonance Spectroscopy (Ruth M. Lynden-Bell and Robin K. Harris), *review* by D. E. Webster, 1169
- Organic Charge-Transfer Complexes (R. Foster), *review* by A. S. Bailey, 299
- Organization and Development of the Embryo (Rass Granville Harrison), *review* by D. R. Newth, 480
- Parasitic Protozoa (J. R. Baker), *review* by D. Walliker, 205
- Periodic System of Chemical Elements, The (J. W. von Spronsen), *review* by Frank Greenaway), 477
- Photometric Methods of Analysis (A. B. Calder), *review* by T. S. West, 202
- Photographic Action of Ionizing Radiations in Dosimetry and Medical, Industrial, Neutron, Auto- and Microradiography (R. H. Herz), *review* by J. E. Roberts, 1266
- Physics (William A. Blanpied), *review* by E. J. Wenham, 392
- Physiology of Plant Growth and Development (Edit. by M. B. Wilkins), *review* by Daphne J. Osborne, 480
- Plant Cells (Roger Buvat), *review* by D. H. Northcote, 391
- Plant Pathology (George N. Agrios), *review* by R. K. S. Wood, 107
- Plasma Physics and Controlled Nuclear Fusion Research (HMSO), *review* by George Rowlands, 478
- Plasma Waves in Space and in the Laboratory (Edit. by J. O. Thomas and P. J. Landmark), *review* by M. K. Wallis, 975
- Politics of Social Research (Ralph L. Beals), *review* by Leslie Sklair, 297
- Population and Food Supply (Edit. by Joseph Hutchinson), *review* by G. W. Cooke, 105
- Population Dynamics (Maurice Dynamics), *review* by F. B. O'Connor, 1266
- Précis de Biologie Végétale (A. Nougarède), *review* by Peter Barlow, 878
- Principles of Comparative Anatomy of Invertebrates (W. N. Beklemishev), *review* by R. P. Dales, 1079
- Probability Theory (Yu. V. Prohorov and Yu. A. Rozanov), *review* by J. F. C. Kingman, 1169
- Psychiatry in a Changing Society (S. H. Foulkes and G. Stewart Prince), *review* by G. M. Carstairs, 565
- Quantum Mechanics (A. Rubinowicz), *review* by S. Zienau, 109
- Radiotelescopes (W. N. Christiansen and J. A. Hogbom), *review* by R. C. Jennison, 974
- Rotational Structure in the Spectra of Diatomic Molecules (Istvan Kovacs), *review* by W. G. Richards, 569
- Rubidomycin (Edit. by J. Bernard, R. Paul, M. Boiron, Cl. Jacquillat and R. Maral), *review* by F. G. J. Hayhoe, 879
- Rutherford and Boltwood (Edit. by Lawrence Badash), *review* by A. S. Russell, 201
- Scattering of Light and Other Electromagnetic Radiation, The (Milton Kerner), *review* by B. R. Jennings, 300
- Scattering Theory (Edit. by A. O. Barut), *review* by M. H. Choudhury, 668
- Seismicity of the Earth, The (J. P. Rothé), *review* by D. Davies, 1268
- Short History of Botany in the United States, A (Edit. by Joseph Ewan), *review* by Phyllis I. Edwards, 1078
- Simple Photogrammetry (J. C. C. Williams), *review* by P. F. Dale, 769
- Social Status and Psychological Disorder (Bruce P. Dohrenwend and Barbara Snell Dohrenwend), *review* by Peter Kelvin, 879
- Solute–Solvent Interaction (Edit. by J. F. Coetzee and Calvin D. Ritchie), *review* by J. A. Salthouse, 1170
- Statistics and Experimental Design (Geoffrey M. Clarks), *review* by K. L. Q. Read, 770
- Stellar Astronomy (Edit. by Hong-Yee Chiu, Robert L. Warasila and John L. Remo), *review* by B. E. J. Pagel, 202
- Steroid Hormones and Metabolism (Kenneth W. McKerns), *review* by May Reed, 204
- Stratigraphy and Palaeontology (Edit. by K. S. W. Campbell), *review* by J. Shirley, 1078
- Structural and Functional Aspects of Lipoproteins in Living Systems (Edit. by E. Tria and A. M. Scanu), *review* by J. B. Finean, 877
- Sundials (Frank W. Cousins), *review* by F. A. B. Ward, 665
- Superconductivity (Edit. by R. D. Parks), *review* by H. Fröhlich, 300
- Synthesis of Penicillin, Cephalosporin C and Analogs (Maghar S. Manhas and Ajay K. Bose), *review* by G. Lowe, 976
- Textile Industry, The (W. English), *review* by C. S. Whewell, 973
- Theoretical Biochemistry (Hans Netter), *review* by H. Gutfreund, 1267
- Theory of Economic History, A (J. Hicks), *review* by John Vaizey, 973
- Thermodynamique et Physique Statistique, Premier Cycle (Bernard Jancivici), *review* by G. L. Sewell, 568
- Think (William Rodgers), *review* by Edward Phillips, 1168
- Times Atlas of the Moon, The (Edit. by H. A. G. Lewis), *review* by Edward Phillips, 770
- Tissue Cultures (O. J. Pollack), *review* by G. A. Gresham, 977
- Tongues of Conscience (R. W. Reid), *review* by C. F. Barnaby, 105
- Toxic Properties of Inorganic Fluorine Compounds (R. Y. Eagers), *review* by L. Magos, 1171
- Triumph of the Darwinian Method, The (Michael T. Ghiselin), *review* by Sydney Smith, 390

  Tunneling in Solids (C. B. Duke), *review* by A. F. G. Wyatt, 667
  Understanding Chemical Thermodynamics (George C. Pimentel and Richard D. Spratley), *review* by D. D. Eley, 203
  Urban Decay (Franklin Medhurst and Parry Lewis), *review* by Barry J. Garner, 974
  Urban Planning Aspects of Water Pollution Control (Sigurd Grava), *review* by G. E. Eden, 767
  Yeasts, The (Edit. by Anthony H. Rose and J. S. Harrison), *review* by J. S. Hough, 1171

Bowyer and Field. Intensity of the Soft X-ray Background: Reply to — with comment by the same (R. C. Henry, G. Fritz, J. F. Meekins, H. Friedman and E. T. Byram), 362
Bradykinin. Correlation of — Structure with Ability to increase Vascular Permeability (G. J. H. Melrose, H. K. Muller and W. J. Vagg), 547
Bradykinin Potentiating Factor. Activity of Various Fractions of — against Angiotensin Converting Enzyme (S. H. Ferreira, L. J. Greene, V. A. Alabaster, Y. S. Bakhle and J. R. Vane), 379
Brain. Actin-like Properties of Colchicine Binding Protein isolated from — (S. Puszkkin and S. Berl), 558
  — Mechanisms and Motivation (Keith Oatley), 797
  Distribution of ^{14}C-Thiosemicarbazide in the Rat —: an Attempt to localize Sites of γ-Aminobutyric Acid Production (Bertalan Csillik and Elizabeth Knyihar), 562
  Effect of an Imprinting Procedure on Regional Incorporation of Tritiated Uracil into Chick — RNA (S. P. R. Rose, P. P. G. Bateson, Ann L. D. Horn and G. Horn), 650
  Effects of Lithium on Amino-acids in Mouse — *in vivo* (F. V. DeFeudis and J. M. R. Delgado), 749
  Eye Dominance in the Visual Cortex (Colin Blakemore and John D. Pettigrew), 426
  Interaction between Papova-like Virus and Paramyxovirus in Human — Cells: a Hypothesis (Hilary Koprowski, Giuseppe Barbanti-Brodano and Michael Katz), 1045
  Learning of Leg Position in Chronic Spinal Rats (A. A. Buerger and A. Fennessy), 751
  Models for the — (P. J. van Heerden), reply by D. J. Willshaw, H. C. Longuet-Higgins and O. P. Buneman, 177
  Multiple Forms of Human — Mitochondrial Monoamine Oxidase (G. G. S. Collins, M. Sandler, E. D. Williams and M. B. H. Youdim), 817
  Nervous Activity and RNA Metabolism in the Visual Cortex of the Rat — (A. J. Dewar and H. W. Reading), 869
  Neurosecretion-like Material in the Hindbrain of Ageing Sheep and Sheep Affected with Natural Scrapie (A. Bignami, Elizabeth Beck and H. B. Parry), 194
  Possible Mechanism of Action of Morphine on — (E. Eidelberg and A. S. Schwartz), 1152
  Post-natal Growth of the Arterial Net in the Human Cerebral Pia Mater (Deowattee Harnarine-Singh and John Baskerville Hyde), 86
Brain Drain: from Scotland, *news*, 116; *miscellaneous intelligence*, 1184; *news*, 213; 312; 488
Brain Function Symposium, Biochemical Society, *news*, 317
Brain Lipid Catabolism, *news*, 12
Brain Membrane. Stimulation by Cyclic AMP of Intrinsic Protein Kinase Activity in Ox — Preparations (M. Weller and R. Rodnight), 187
Brassins. — a New Family of Plant Hormones from Rape Pollen (J. W. Mitchell, N. Mandava, J. F. Worley, J. R. Plimmer and M. V. Smith), 1065
BRDC, *news*, 673
Bremsstrahlung Radiation. — in an Intense Magnetic Field and Emission from Pulsars (Hong-Yee Chiu and Vittorio Canuto), 1230
Breton Island Bird Sanctuary Polluted, *news*, 1096
Britain's Population, *news*, 214
British Computer Industry Development, *news*, 679
British Computer Society before the Select Committee, *news*, 1088
British Deterrent, *news*, 306
British Museum (Natural History), Open Day, *news* 892
British Nuclear Fuels Ltd., *news*, 1089
British Nuclear Power Industry, *news*, 892
British Rail. — Advanced Passenger Train (S. Jones), 417
British Research and Development Corporation, *news*, 209; 673
British Space Attempt, *news*, 987
British Society for History of Science Catalogue, *news*, 311
Brochosomes, *news*, 126
Brown, Boveri and Company Research, *news*, 404
Browne, R. C., Professor, *news*, 406
Bubble Chamber, Mirabelle, *news*, 127
Buffers. Effect of Phosphate and Non-Phosphate — on Thermolability of Unstable Haemoglobins (Lawrence J. Schneiderman, Irene G. Junga and Dorothea F. Fawley), 1041
Building Techniques in the Antarctic, *news*, 221
Burnham Committee Machinery, *news*, 113
Butt Rot, *news*, 691
Butterfly. Inheritance of Sex Ratio in the — *Acraea encedon* (D. P. Owen), 662
  Plant Pigments identified in the Common Blue — (John Feltwell and L. R. G. Valadon), 969

C

Cable Television, *news*, 488
Caesium. Altitude Effects on the Biological Half Life of — (J. Rundo and C. R. Richmond), 83
Calcification. — and the Ground Substance: Precipitation of Calcium Phosphate Crystals from a Nutrient Gel (Bernard Rubin and Arthur Saffir), 78
Calcitonin *M*. Antigenic Site(s) of Synthetic Human — (Felix M. Dietrich and Werner Bittel), 75
Calcium Ions. Parathyroid Hormone Secretion *in vitro*: Regulation by — and Magnesium Ions (Louis M. Sherwood, Ingeborg Herrman and C. Andrew Bassett), 1056
  Relation of Intracellular — to retention of K^+ by Liver Slices (G. D. V. van Rossum), 638
  The Shape and Shift of the Resonance Lines of Ca^+ produced by Electron Collisions (K. S. Barnes and G. Peach), 374
Calcium Phosphate. Calcification and the Ground Substance: Precipitation of — Crystals from a Nutrient Gel (Bernard Rubin and Arthur Saffir), 78
Caledonian Geology of Arctic Norway, *news*, 313
Calories, in Biochemical Thermodynamics, *news*, 690
Cambridge Microorganisms, *news*, 680
Canadian Research Policy, *news*, 10
Canary Islands Origin, *news*, 128
Cancer. A New Approach to — (P. R. J. Burch), 512
  Anti-Nucleic Acid Specificities of Mouse Myeloma Immunoglobulins (David Schubert, Ann Roman and Melvin Cohn), 154
  Antitumour Polysaccharides derived Chemically from Natural Glucan (Pachyman) (Goro Chihara, Junji Hamuro, Yukiko Maeda, Yoshiko Arai and Fumiko Fukuoka), 943
  Control of the Reversion of Properties in Transformed Cells (Zelig Rabinowitz and Leo Sachs), 136
  Mammalian Cell Fusion: Studies on the Regulation of DNA Synthesis and Mitosis (Potu N. Rao and Robert T. Johnson), 159
  Microinvasion of Epidermis caused by Substituted Anisoles (P. Grasso and Christine C. Rostron), 188
  Surface Leukaemic Antigen and Malignancy of Cells in Mice infected with Mazurenko Virus (V. E. Gurtsevich, N. P. Mazurenko, E. I. Jarova, N. A. Probatova and G. N. Stepanova), 1047
  Cancer: and Viruses, *news*, 226; Lung, *parliamentary news*, 680; Metastasis and Heparin, *news*, 1192; Neutron Therapy, *news*, 1182; Research Book, *miscellaneous intelligence*, 991; Research Coordination, *news*, 116; Symposium, Miami, *news*, 588
Cannabis: effects, *miscellaneous intelligence*, 991; Use (F. Bergel), *correspondence*, 979
Car: Collisions, *news*, 228; Exhaust Pollution, *news*, 681; 990
Carbon. Interstellar Dust and Aromatic — (G. P. Vdovykin), 254
  Isotopic Evidence for Diminishing Supply of Available — during Diatom Bloom in the Black Sea (W. G. Deuser), 1069
Carbon Compounds. — in Apollo 11 Lunar Samples (Bartholomew Nagy, Ward M. Scott, Vincent Modzeleski, Lois Anne Nagy, Charles M. Drew, William S. McEwan, Joseph E. Thomas, Paul B. Hamilton and Harold C. Urey), 1028
Carbon Fibres. Strength-Modulus Relation for Carbonized Acrylic Fibres (D. J. Thorne), 1040
Carbon Fibres: and Lasers, *parliamentary news*, 581; Structure, *news*, 1002
Carbon Films. Catalysis Oxidation of SO_2 on — by Electrical Resistance Measurements (I. S. McLintock and J. C. Orr), 629
Carbon Monoxide. — as a Basis for Primitive Life on Other Planets (Richard Wolfgang), 876
Carcinogens. Nitrosamines as Environmental — (William Lijinsky and Samuel S. Epstein), 21
Carcinoma. Positive α_1-Foetoprotein Tests in Pyridoxine deprived Baboons: Relevance to Liver — in Africa (Henry Foy, Athena Kondi, C. A. Linsell, Alison M. Parker and P. Sizaret), 952
Cariostasis. Possible Basis for the Cariostatic Effect of Inorganic Phosphates (K. M. Pruitt, A. D. Jamieson and R. C. Caldwell), 1249
Carnegie Observatories, *news*, 403
Carotid Body. Simultaneous Measurement of Cytochrome a_3 Reduction and Chemoreceptor Afferent Activity in the — (E. Mills and F. F. Jobsis), 1147
Careers. Career Choices by Science Studies Students (D. O. Edge), 506
Cartilage. Morphogenetic Substratum for Differentiation of — in Tissue Culture (Marshall R. Urist and Hiroshi Nogami), 1051
Cassiterite. Mössbauer Study to find the Origin of Weak Ferromagnetism in — (Subir K. Banerjee, C. E. Johnson and Miroslav Krs), 173
Casts. Hollow — of Lungs for Experimental Purposes (V. Timbrell, N. E. Bevan, A S. Davies and D. E. Munday), 97
Catalogue. — of Work in Progress; the British Society for History of Science, *news*, 311
Catalysis. — of Polynucleotides in Ionic Reactions (Norio Ise), 66
  Catalytic Inhibition by Adsorbed Oxygen (R. P. H. Gasser and P. R. Vaight), 933
  Catalytic Oxidation of SO_2 on Carbon Films by Electrical Resistance Measurements (I. S. McLintock and J. C. Orr), 629
Catecholamines. Complexes between Acetylcholine and — and their Tolerance to Mental Illness (Lauro Galzigna), 1058
  Effect of Foetal Hypophysectomy on — Levels in the Lamb Adrenal during Prolonged Gestation (R. S. Comline, Marian Silver and I. A. Silver), 739
  Effects of 6-Hydroxydopamine on CNS —, Spontaneous Motor Activity and Amphetamine induced Hyperactivity in Rats (K. D. Evetts, N. J. Uretsky, L. L. Iversen and S. D. Iversen), 961
Cathepsins B and B′. Leucine Naphthylamide: an Appropriate Substrate for the Histochemical Detection of — (J. Ken McDonald, Benjamin B. Zeitman and Stanley Ellis), 1048
Cattle. Probable Occurrence of Infectious Bovine Rhinotracheatic Virus in Tanzania in Wildlife and — (M. M. Rweyemamu), 738
CBW Report (WHO), *news*, 400
CEGB: and Select Committee, *news*, 497; Generating Capacity, *parliamentary news*, 494
Celestial Mechanics. Poor Man's Guide to — (Hannes Alfvén), 229
Cell Culture. Cell Movement and Cell Spreading: a Passive or an Active Process? (S. B. Carter), 858
  Evidence for the Multiplication of Scrapie Agent in — (M. C. Clarke and D. A. Haig), 100
Cell Differentiation, *news*, 1188
Cell Division Inhibition, *news*, 413
Cell Fusion. Mammalian —: Studies on the Regulation of DNA Synthesis and Mitosis (Potu N. Rao and Robert T. Johnson), 159
Cell Movement. — and Cell Spreading: a Passive or an Active Process? (S. B. Carter), 858
Cell Walls. Altered — of *Staphylococcus aureus* resistant to Methicillin (L. D. Sabath, C. D. Leaf, D. A. Gerstein and M. Finland), 1074
  Effects of — Polysaccharide on the Mating Ability of *Salmonella typhimurium* (Tsutomu Watanabe, Toshihoko Arai and Takanori Hattori), 70
  Teichoic Acids and Membrane Function in Bacteria (S. Hepinstall, A. R. Archibald and J. Baddiley), 519
Cell Wall Protein. Proline in the Vesicles and Sporelings of *Valonia ventricosa* and the Concept of — (F. C. Steward, R. L. Mott, H. W. Israel and P. M. Ludford), 760
Cell Wall Synthesis. Involvement of Mesosomes in, *news*, 130
Cell Walls, Bacterial, *news*, 587
Cellular Deficit. — in Thymectomized Mice (D. E. Mosier, F. W. Fitch, D. A. Rowley and A. J. S. Davies), 276
Cellulose. Crystallite Size in Natural — (I. Nieduszynski and R. D. Preston), 273
Centipede in Guildford, *miscellaneous intelligence*, 120
Centenary. American Museum of Natural History at 100 (James A. Oliver), 1199
Central Nervous System. Effects of 6-Hydroxydopamine on — Catecholamines, Spontaneous Motor Activity and Amphetamine induced Hyperactivity in Rats (K. D. Evetts, N. J. Uretsky, L. L. Iversen and S. D. Iversen), 961
  Transplantation Antigens in the—(A. G. Gervais), 647
Central Nervous System Cholinergic Mechanisms, Effects of Drugs on, Symposium, *news*, 1103
Centrifuge Agreement, *news*, 490
Cerebral Cortex. Spread of Responses in the — to Meaningful Stimuli (B. Delisle Burns and A. C. Webb), 469
Cerebrospinal Fluid. 5-Hydroxyindoleacetic Acid Levels in the — of Depressive Patients treated with Probenecid (H. M. van Praag, J. Korf and J. Puite), 1259
Čerenkov Process. VLF Wave Generation by the — in the Inner Magnetosphere (R. P. Singh and R. N. Singh), 49
Čerenkov Pulses. Observation of Structure in — from Extensive Air Showers using Fast Techniques (G. Bosia, G. Castagnoli, M. Dardo and G. Marangoni), 532
CERN Accelerator: *news*, 4; 491; Site, *news*, 310
Cerviniella, Two New Species, *news*, 224
Channel Tunnel: *100 years ago*, 212; Merger, *news*, 310
Charge Transfer. — Complexes of Tropylium and *N*-Heterocyclic Cations with Aromatic Amines (T. G. Beaumont and K. M. C. Davis), 632
Chemical and Biochemical Societies Meeting in Oxford, *news*, 224
Chemical Industry Growth, *news*, 891
Chemical Synthesis, New Cyclization Reaction, *news*, 1001

Chemoreceptor. Simultaneous Measurement of Cytochrome a_3 Reduction and — Afferent Activity in the Carotid Body (E. Mills and F. F. Jobsis), 1147
Chemotactic Factor. Immunoglobulin G as Possible Precursor of — (M. Yoshinaga, M. Mayumi, S. Yamamoto and H. Hayashi), 1139
Chimaeras. Production of Chick — (G. Marzullo), 72
Chinese Astronomers, *miscellaneous intelligence*, 894
Chitinase. Lytic Enzyme produced *in vitro* by Insect Cells: Lysosome or —? (J. C. Landureau and P. Jollès), 968
Chloramphenicol. Aberrant mRNA in Parkinsonism: Support for Hypothesis from Studies with — (C. N. Stefanis and M. Issidorides), 962
Chlorotic Dwarf. — of Eastern White Pine caused by an Ozone and Sulphur Dioxide Interaction (L. S. Dochinger, F. W. Bender, F. L. Fox and W. W. Heck), 476
Cholesterol. Differential Inhibition of Rat Liver — Biosynthesis by Plant Growth Retardants (L. G. Paleg), 1252
  Pituitary Growth Hormone Essential for Regulation of Serum — (Meyer Friedman, Sanford O. Byers and Stephen R. Elek), 464
Cholinergic Agent. Induction of Hepatic Tyrosine Aminotransferase mediated by a — (Ira B. Black), 648
Cholinergic Nerve Receptors. Interaction of Acetylcholine with Different — (Cyrus Chothia), 36
Cholinergic Neurones, *news*, 1103
Chromatophores. Fast Spectrophotometric Measurement of H^+ Changes in *Chromatium* — activated by a Liquid Dye Laser (B. Chance, J. A. McCray and J. Bunkenburg), 705
Chromosomes. Aberrant Karyotypes and Spontaneous Abortion in a Japanese Family (Tetsuji Kadotani, Koso Ohama, Toshio Sofuni and Howard B. Hamilton), 735
  — and Nuclear Location of Mouse Satellite DNA in Individual Cells (K. W. Jones), 912
  Effects of Adenovirus on the — of Normal Human Cells and Cells Trisomic for an E Chromosome (J. K. McDougall), 456
  High Frequency Haploid Production in Barley (*Hordeum vulgare* L.), (K. J. Kasha and K. N. Kao), 874
  Prenatal Chromosome Analysis (Fredrica Lisgar, Melvin Gertner, Sheldon Cherry, Lillian Y. Hsu and Kurt Hirschhorn), 280
  Presence of an Additional Metacentric — in AKR/TIALD Leukaemic Cells (E. Legrand and J. F. Duplan), 737
B Chromosomes. Isolation of DNA from — in Grasshoppers (Ian Gibson and Godfrey Hewitt), 67
Chromosome Arrangement on Genes, *news*, 129
Chromosome Loss. — compared with Chromosome Size, Age and Sex of Subjects (P. Neurath, K. DeRemer, B. Bell, L. Jarvik and T. Kato), 281
Chromosomes Manipulated, Grasshopper, *news*, 1191
Chromosome Puffs. Dosage Compensation and Effect for RNA Synthesis in — of *Drosophila melanogaster* (K. Groge), 386
Chromosome Replication, *news*, 499
Ciliate Structure, *news*, 13
Circles. Osculatory Packing of Finite Areas with — (H. H. Kausch-Blecken, von Schmeling and N. W. Tschoegl), 1119
Circular Dichroism. Comparison of Soluble and Native Elastin Conformations by Far-ultraviolet — (M. Mammi, L. Gotte and G. Pezzin), 380
Cistron. Nucleotide Sequence from the Polypeptide Chain Termination Region of the Coat Protein — in Bacteriophage R17 RNA (J. L. Nichols), 147
Civic Trust Awards for Architecture, *news*, 493
Civilization and the Atmosphere, *miscellaneous intelligence*, 1184
Clingfishes, *news*, 416
Clouds. Interstellar and Atmospheric — (G. R. Evans and M. V. Penston), 357
Cloud Chamber Tracks. Measurements on — (J. G. Wilson), 1239
Clouds of Venus, *news*, 689
Coast: Conservation, *news*, 891; Differences in, North America, *news*, 314
Cochlea. Travelling Waves as Frequency Analysers in the — (Georg von Békésy), 1207
Cocking's Work, *news*, 999
Coconut Rhinoceros Beetle. Introduction of a New Virus Disease of the — in Western Samoa (K. J. Marschall), 288
Codon and Nodoc, *news*, 497
Coffee. Possible Side Effects of Fungicides on Banana and — Diseases (I. D. Firman), 1161
Colchicine. Actin-like Properties of — Binding Protein isolated from Brain (S. Puszkin and S. Berl), 558
Cold Spring Harbor, Watson at, *news*, 896
Collaboration Chronology, US and Soviet Union Space, *news*, 1095
Collagen. NMR Investigation of Deuterated — (G. E. Chapman, I. D. Campbell and K. A. McLauchlan), 639
Collagen. Size-specific Metal Ions Complexing Sites in Native — (Joseph A. Spadaro, Robert O. Becker and Charles H. Backman), 1134
College Administration Appointment of Scientists, *news*, 998
Colloid Chemistry. Solid State Chemistry and — (E. P. Honig), 537
Comet in the Ultraviolet, *news*, 413
Comet Mellish 1917 I. The 11 Canis Minorids—a New Meteor Stream probably associated with — (Keith B. Hindley and Michael A. Houlden), 1232
Communication. — in Biochemistry (E. C. Webb), 132
Communications Satellite Conference: *news*, 789; Disagreement, *news*, 1095; Soviet Union, *news*, 1092; UK, *news*, 309
Companion Objects. Ejection of — by Supernovae (Stirling A. Colgate), 247
Complement. Demonstration in Sarcoma Patients of Anti-tumour Antibodies which Fix Only Human — (F. R. Eilber and D. L. Morton), 1137
Complementary Oligonucleotide Binding. — to the Anticodon Loop of fMet-tRNA (Olke C. Uhlenbeck, Julie Baller and Paul Doty), 508
Composite Materials, Strong Fibrous Solids, *news*, 592
Computer Industry: *news*, 308; Development, *news*, 679; in UK, *news*, 575; Policy, 986; 1088; 1180; Survey, OECD, *news*, 576
Computer Society, British, before the Select Committee, *news*, 1088
Computer-aided Design, *news*, 118
Computers: *parliamentary news*, 581; and Select Committee, *news* 496; at the Physics Exhibition, *news*, 891; IBM, before the Select Committee, *news*, 889
Concealed Randomized Objects. Identification of — (I. Stevenson and J. G. Pratt), *correspondence*, 394
Concorde, *parliamentary news*, 784
Conditioning. Alarm Pheromone and Avoidance Conditioning in Goldfish, *Carassius auratus* (G. McA. Kimbrell, Mark R. Weinrott, Edward K. Morris, jun., Jennifer Scheid and Deborah Sangston), 754
Conductance, Ion Dependent. —: Switching in Lipid Bilayers (M. C. Goodall), 1257
Congress and Research, *news*, 1177
Conservation: Attitudes (Thomas H. Jukes), *correspondence*, 1173, and (Eliot Slater), *correspondence*, 773; Endowment (Peter Scott), *correspondence*, 1174; Film, *news*, 579; of Coasts, *news*, 891; of Italian Birds, *news*, 677; on the Farm, *news*, 902; Postgraduate Course, *news*, 570
Continental Drift. Imperfections of Elasticity and — (Harold Jeffreys), 1007
Continental Drift of North America, *news*, 314
Continental Fit. Fit of the Southern Continents (A. Gilbert Smith and A. Hallam), 139
Continental Growth. Plate Tectonics, Orogeny and — (J. F. Dewey and Brenda Horsfield), 521
Continental Shelf. Deep Sedimentary Basins proved in the Shetland–Hebridean — and Margin (M. H. P. Bott and A. B. Watts), 265
Contraceptive for Pigeons, *miscellaneous intelligence*, 894
Contraceptives. Oral — and Copper Metabolism (Michael Briggs, J. Austin and Maxine Staniford), 81
Cooking. Secondary Amines in — (A. Courts), *correspondence*, 302
Copepod, new species, *news*, 224
Copolymer. Macro-lattice from Segregated Amorphous Phases of a Three Block — (A. Keller, E. Pedemonte and F. M. Willmouth), 538
Copper *d* Band Structure, *news*, 901
Copper Metabolism. Oral Contraceptives and — (Michael Briggs, J. Austin and Maxine Staniford), 81
Coronary Infarction and Procreation, Miscellaneous Intelligence (A. E. M. McLean and E. K. McLean), *correspondence*, 206
Cortex. Cells sensitive to Binocular Depth in Area 18 of the Macaque Monkey — (D. H. Hubel and T. N. Wiesel), 41
Corticosteroid. Development (Schizogony) of *Eimeria tenella* in the Liver of Chickens treated with — (P. L. Long), 290
Cosmetic Chemist's Buoyancy, *miscellaneous intelligence*, 581
Cosmic Rays. Age of — and Abundance of Antimatter in the Galaxy (L. Marshall Libby), 166
Cosmology. Cosmological Theory, of Gravitation (S. J. Prokhovnik), 359
Council for Scientific Policy, *news*, 679
Council of Europe's Strasbourg Conference on European Environment, *news*, 888
Council for Biology in Human Affairs, *news*, 788
Countryside Commission Second Annual Report, *news*, 118
Courtaulds/ICI Mergers, *news*, 114
Crab Nebula. Coherent Synchrotron Emission in the — (Lawrence J. Caroff and Jeffrey D. Scargle), 169
  Low-frequency Spectrum of the — (A. H. Bridle), 1035
Crack. Measurements of Electrical Potential for detecting — Extension in COD Tests (J. Davies, D. F. Cannon and R. J. Allen), 1240
Crack Opening Displacement Tests. Mesaurements of Electrical Potential for Detecting Crack Extensions in — (J. Davies, D. F. Cannon and R. J. Allen), 1240
Craters. Impact — and the Relative Ages of Earth and Moon (C. S. Beals), 368
  Meteoritic Spherules in the Soil surrounding Terrestrial Impact — (Paul W. Hodge and Frances W. Wright), 717
  Twin Terrestrial Impact — (W. D. Ehmann and J. W. Morgan), 255
Crayfish. Intensity of Aggression in the —, *Orconectes virilis* (Hagen) (Donald B. Heckenlively), 180
Creep, Magnesium in Oxygen, *news*, 16
Cromwell Current, *news*, 119
Crustacea. Absence of Blood-Brain Barrier in a Crustacean, *Carinus maenus* L. (Joan Abbott), 291
Crystals. — Chemistry of the Plagionite Group (Seung-Am Cho and B. J. Wuensch), 444
  Crystallite Size in Natural Cellulose (I. Nieduszynski and R. D. Preston), 273
  Crystalline Structures of Alginic Acids (E. D. T. Atkins, W. Mackie and E. E. Smolko), 626
  Enzymes in — (Hans Neurath, Kenneth A. Walsh and Ralph A. Bradshaw), *correspondence*, 881
Crystal Structure. — of a Cyclic Polyether Complex of Alkali Metal Thiocyanate (D. Bright and Mary R. Truter), 176
Crystallography: Internation Comparison, *news*, 1193; Nuclear Magnetic Resonance Techniques in the Study of, *news*, 1000
CS Gas, *parliamentary news*, 894
CSIRO Research in Horticulture, *news*, 228
Cultures for Cambridge, *news*, 680
Cuticle Formation and Structure, Insect, *news*, 1192
Cyanide. Resonance Fluorescence of the CN Free Radical (J. A. Myer and R. W. Nicholls), 928
Cyclamate Affair, Prevention of Recurrence, *news*, 676
Cyclamates, Saccharin and Tumours, *miscellaneous intelligence*, 1184
Cyclic AMP. Stimulation by — of Intrinsic Protein Kinase Activity in Ox Brain Membrane Preparations (M. Weller and R. Rodnight), 187
Cyclic 3′,5′-AMP and Uterine Contractility (Lubos Triner, N. I. A. Overweg and Gabriel G. Nahas), 282
Cyclic Polyether Complex. Crystal Structure of a — of Alkali Metal Thiocyanate (D. Bright and Mary R. Truter), 176
Cyclization Reactions, New, *news*, 1001
Cycloheximide. Effects of Actinomycin D and — on RNA and Protein Synthesis in Cleavage Stage Mouse Embryos (Richard J. Tasca and Nina Hillman), 1022
  Effect of — and Actinomycin *D* on the Behaviour of the Headless Cockroach (E. Glassman, A. Henderson, M. Cordle, H. M. Moon and J. E. Wilson), 967
Cyclotron Resonances. Harmonic Ion — observed by the OGO-4 Satellite (H. Kikuchi), 257
Cystamine. Electron Reactions in γ-irradiated Thymidine and Cystamine (A. D. Lenherr and M. G. Ormerod), 546
Cytochrome a_3. Simultaneous Measurements of — Reduction and Chemoreceptor Afferent Activity in the Carotid Body (E. Mills and F. F. Jobsis), 1147
Czechoslovak Medical Literature Annual, *miscellaneous intelligence*, 581
Czechoslovakian Scientists' Troubles, *miscellaneous intelligence*, 120

D

Dainton on Science Policy, *news*, 679
Darwin. Non-Darwinian Evolution: A Critique (Rollin C. Richmond), 1025
Darwin Rise Hypothesis. The — of the Biogeographical Dispersal of Marsupials (P. G. Martin), 197
DDT in Human Milk (Thomas H. Jukes), *correspondence*, 301
DDT in Milk (Goran Lofroth), *correspondence*, 881
Decametric Variability. — of Cas A and 3C 84 (A. H. Bridle and J. L. Caswell), 356
Decimalization. Numerical Notation: — (F. W. Derwent), 483
Decomposition. — of Isopropyl Alcohol photosensitized by Zinc Oxide (Heinrich Dieter Müller and Friedrich Steinbach), 728
Deep Sea Diving Experiments, *news*, 1091
Deep Space Tracking, *news*, 124
Defence: *parliamentary news*, 991; Department Research Budget, *news*, 777; Finance, US, *news*, 582; Research Establishments, *news*, 673; US and Soviet Union, *news*, 1178
Dehydroascorbic. — in Cell Division Inhibition, *news*, 413
Demography. Problems of Expanding Populations (D. Wolfers), 593
Demography: *news*, 214; of Disease, *news*, 1091
Density Stratification. Density Stratified Lakes in Northern Ellesmere Island (G. Hattersley-Smith, J. E. Keys, H. Serson and James E. Mielke), 55
Dental Silicate Cement. Formation of — (A. D. Wilson, B. E. Kent, R. J. Mesley, R. P. Miller, D. Clinton and K. E. Fletcher), 272
Depressives. 5-Hydroxyindoleacetic Acid Levels in the Cerebrospinal Fluid of — treated with Probenecid (H. M. van Praag, J. Korf and J. Puite), 1259

Desert. — Stream Channels resembling Lunar Rilles (J. D. Burke, R. G. Brereton and P. M. Muller), 1234
Desert Research Finance, *news*, 583
Detergents, Enzyme, *parliamentary news*, 405
Detonation. Wall Precursor Effects in Gaseous — (M. C. Cavenor), 718
Diatom Bloom. Isotopic Evidence for Diminishing Supply of Available Carbon during — in the Black Sea (W. G. Deuser), 1069
Diatomic Molecules. Observations of Vibration–Vibration Energy Pumping between Diatomic Molecules (J. D. Teare, R. L. Taylor and C. W. von Rosenberg, jun.), 241
Dielectric Effects. — and Anelastic Effects in Inorganic Glasses (D. K. Hale), 930
Diet. Dietary Sucrose and Platelet Behaviour (Stephen Szanto and John Yudkin), 457
  Early Assessment of Malnutrition (R. B. Bradfield and E. F. P. Jelliffe), 283
  Positive α_1-Foetoprotein Tests in Pyridoxine Deprived Baboons: Relevance to Liver Carcinoma in Africans (Henry Foy, Athena Kondi, C. A. Linsell, Allison M. Parker and P. Sizaret), 952
Differential Equation Research, *news*, 989
Diffusion. — in Embryogenesis (Francis Crick), 420
  Ionic — in Quartz (S. White), 375
Diffusion Research, *news*, 900
Diffusion-creep, *news*, 16
Disarmament (Elizabeth Young), *correspondence*, 481
Disarmament Talks: *news*, 211; Prospects, *news*, 1178
Discharge Tubes. Production of Oxygen Atoms in Quartz and Polytetrafluoroethylene — (A. M. Mearns and A. J. Morris), 59
Disease Atlas, UK, *news*, 1091
Disease Resistance Assay. Host Response of Tobacco to *Verticillium dahlia* (Tob.) Kleb.—a Cytological Method of assaying Resistance (H. K. Mahanty), 755
Dispersal. Deposition and Uptake by Cattle of Airborne Particles (A. C. Chamberlain), 99
  The Darwin Hypothesis of the Biogeographical — of Marsupials (P. G. Martin), 197
  Windborne — of Foot and Mouth Virus (K. P. Norris and G. N. Harper), 98
Dispersion. Assignment of Absolute Configuration from Anomalous — by Oxygen Atoms (S. Neidle and D. Rogers), 376
  Self-induced Transparency and — Delays in Potassium Vapour (D. J. Bradley, G. M. Gale and P. D. Smith), 719
Diving. Possible Relationships between Orienting and — Reflexes (David A. Goodman and Norman M. Weinberger), 1153
Diving Experiment, Deep Sea, *news*, 1091
DNA. Anti-Nucleic Acid Specificities of Mouse Myeloma Immunoglobulins (David Schubert, Ann Roman and Melvin Cohn), 154
  Autoradiographic Detection of Molecular Hybrids between rRNA and — in Tissue Culture (M. Buongiorno-Nardelli and F. Amaldi), 946
  Bonding of Small Molecules to — following Ionizing Radiation (John E. Byfield, Young C. Lee and Leslie R. Bennett), 859
  Exchange of Bound Tritium between — and Water at Different Temperatures (Robert E. Bird, Karl G. Lark, Basil Curnutte and John E. Maxfield), 1043
  Isolation of — from *B* Chromosomes in Grasshoppers (Ian Gibson and Godfrey Hewitt), 67
  Rare Bases in Animal — (B. F. Vanyushin, S. G. Tkacheva and A. N. Belozersky), 948
  Variations in the — from Two Rodent Families (Cricetidae and Muridae), (W. Hennig and P. M. B. Walker), 915
DNA Complexity, *news*, 1189
DNA Replication. Evidence for "the Wedge", Initiator of — (Semih Erhan, Sam Reisher, Elizabeth A. Franko, S. A. Kamath and R. J. Rutman), 340
  Ribosomal Genes and — in Germinating Wheat Embryos (David Chen and Daphne J. Osborne), 336
DNA, Satellite, *news*, 899
DNA Synthesis. Mammalian Cell Fusion: Studies on the Regulation of — and Mitosis (Potu N. Rao and Robert T. Johnson), 159
DNA Template Activity. Mutagenic Action of Aflatoxin B_1 on Transforming DNA and Inhibition of — *in vitro* (Veronica M. Maher and William C. Summers), 68
DNA Transformation. Mutagenic Action of Aflatoxin B_1 on Transforming DNA and Inhibition of DNA Template Activity *in vitro* (Veronica M. Maher and William C. Summers), 68
DNA Tumour Virus. Effect of — on Antibody Forming Cells in Neonatally Infected Hamsters (Herman Friedman and Herman Goldner), 455
Dogs, in Tokyo, *miscellaneous intelligence*, 784
Dominance. Genetic Curing of Blindness in *Phycomyces blakesleeanus*: a Quantitative Assessment of — (R. H. Villet), 453
L-Dopa: Availability, *news*, 490; *parliamentary news*, 680; Preparations to Marketing, *news*, 675
Dopamine. — Excretion in Affective States and following Li_2CO_3 Therapy (F. S. Messiha, D. Agallianos and C. Clower), 868
Drag. Effect of — Reducing Additives on Pressure Loss across Transitions (V. G. Pisolkar), 936
Dragon Project Report, *news*, 200
Dreadnought Repair Delay, *news*, 306
Drug Addiction and the Law, *news*, 1087; 1089
Drug: Effects on Cholinergic Mechanisms, Symposium, *news*, 1103; Legislation, UK, *news*, 485; Pushers, *news*, 1089; Safety Committee: *parliamentary news*, 680; on L-Dopa, 675; Use (F. Bergel), *correspondence*, 979
Drug Metabolism. Alteration of Normal Development of — by Injection of Growth Hormone (John T. Wilson), 861
Drugs. Effects of — on Red Cell Fragility (W. Mikikits, A. Mortara and R. G. Spector), 1150
Drugs Bill, *news*, 1087
Duodenal Ulcers. Production of — and Hypertrophic Pyloric Stenosis by Administration of Pentagastrin to Pregnant and Newborn Dogs (J. A. Dodge), 284
Dust Grains. Retention of — near Galactic Nuclei (N. C. Wickramasinghe), 145
Dwarf Stars, *news*, 901

E

Ear. — Movements in a Hipposiderid Bat (J. D. Pye and L. H. Roberts), 285
  Travelling Waves as Frequency Analysers in the Cochlea (Georg von Békésy), 1207
Early Receptor Potential. Action Spectrum of the Human — (Ronald E. Carr and Irwin M. Siegel), 89
Earth. Impact Craters and the Relative Ages of — and Moon (C. S. Beals), 368
  Imperfections of Elasticity and Continental Drift (Harold Jeffreys), 1007
  Lunar Rilles and a Possible Terrestrial Analogue (G. H. J. McCall), 714
  Meteoritic Spherules in the Soil Surrounding Terrestrial Impact Craters (Paul W. Hodge and Frances W. Wright), 717
  Novel Correlations between Global Features of the Earth's Gravitational and Magnetic Fields (R. Hide and S. R. C. Malin), 605
  Possibility of a Terrestrial Component in the Doppler Shifted Zodiacal Light (V. Vanysek and M. Harwit), 1231
  Pulsar Periods and Rapid Changes in the Terrestrial Rotation Rate: a Reply (Jörg Pfleiderer), 437
  Seismicity of the — (C. F. Richter), 170
Earth: and Ocean Conference, *news*, 591; Core, *news*, 793; Mantle, *news*, 793; Transition Zone, *news*, 1003
Earthquakes. — and Explosions: Comment on Molnar *et al.* (H. I. S. Thirlaway), *reply* by Peter Molnar, John Savino, Lynn R. Sykes, Robert C. Liebermann, George Hade and Paul W. Pomeroy), 1238
  Some Aspects of the Structure of Southern India based on Recent Bhadrachalam — (S. K. Arora, T. G. Varghese and C. A. Krishnan), 261
  Symmetry of Crustal — above Benioff Zones (Trevor Hatherton), 844
Earthquakes: and Nuclear Tests, *news*, 1099; in US, *news*, 583
East African Rift Valley, *news*, 314
ECBO, *news*, 677
Echolocation. Ear Movements in a Hipposiderid Bat (J. D. Pye and L. H. Roberts), 285
Eclipse: Observation, Finance, *news*, 886; Path, *news*, 584
Ecology. — of Fouling Organisms in a Polluted Area (Lydia Ignatiades and Th. Becacos-Kontos), 293
  Transition Probabilities for a Forest (Paul E. Waggoner and George R. Stephens), 1160
  Trends in Montane Grasslands in Snowdonia, expressed in Terms of "Relative Entropy" (R. Elfyn Hughes and J. Dale), 756
Ecotypes. Characterization of the Germination Response to Temperature of Species and — (P. A. Thompson), 827
Edinburgh Ichthyosaur Auction, *news*, 781
Edinburgh University Industrial Consultancy, *news*, 308
EDTA. Lack of Synergy of — with Antimicrobials in Resistant Enterobacteriaceae (Harold C. Neu and Elaine B. Winshell), 763
Education: and Science Appointment, *news*, 573; Committee for the Royal Society, *news*, 579; Expenditure, *parliamentary news*, 405; Institute Set Up for US, *news* 997; Money Value *news*, 1187; Pamphlet (Fabian Society), *news*, 491; Policy, US, *news*, 985; Priorities, *news*, 491; Quality, American Schools, *news*, 997; Statistics (UK), *news*, 115
Education for the Future (ATT), *news*, 782
EEC, Biology Research, *news*, 6
Egg. Absence of Antimicrobial Substances in the — Capsules of Millipedes (Thomas Eisner, Stanley A. Zahler, James E. Carrel, Dorothy J. Brown and George W. Lones), 661
Eidetic Images. The Detailed Texture of — (C. F. Stromeyer II and J. Psotka), 346
Eidetic Imagery, *news*, 227
Elastase. Amino-acid Sequence of Porcine Pancreatic — and its Homologies with other Serine Proteinases (D. M. Shotton and B. S. Hartley) 802
  Three-dimensional Fourier Synthesis of Tosyl-elastase at 3.5 Å Resolution (H. C. Watson, D. M. Shotton, Joyce M. Cox and Hilary Muirhead), 806
  Three-dimensional Structure of Tosyl-elastase (D. M. Shotton and H. C. Watson), 811
Elastase Research, *news*, 791
Elastic Effects. Dielectric Effects and — in Inorganic Glasses (D. K. Hale), 930
Elasticity. Imperfections of — and Continental Drift (Harold Jeffreys), 1007
Elastin. Comparison of Soluble and Native — Conformations by Far-ultraviolet Circular Dichroism (M. Mammi, L. Gotte and G. Pezzi), 380
  Thermoelasticity of — (D. Volpin and A. Cifferi), 382
Electric Field. Effect of — on Phase Separation of Glass (R. C. de Vekey and A. J. Majumdar), 172
  Oriented Grain Growth of Chlorinated Naphthalene induced by an — (Keiji Miwa and Takeo Fukutomi), 271
Electrodynamics. Frequency Mixing in the Scattering of Light by Harmonically Bound Electrons (D. Andreou and L. M. Bali), 1239
  Hoyle–Narlikar Quantization of Wheeler–Feynman — (Amnon Katz, Ezuz Ornan and Moshe Schwartz), 534
  Quantization of Wheeler–Feynman — (F. Hoyle and J. V. Narlikar), 1233
Electroluminescent Devices, *news*, 1004
Electrolysis of Water (Sydney Gill), *correspondence*, 775
Electromagnetism. Magnets in Electromagnetic Theory (W. F. Lovering), 1127
Electron Beams, *news*, 15; 502
Electron Channelling Patterns. — from Small (10 μm) Selected Areas in the Scanning Electron Microscope (C. G. van Essen, E. M. Schulson and R. H. Donaghay), 847
Electron Collisions. The Shape and Shift of the Resonance Lines of Ca^+ produced by — (K. S. Barnes and G. Peach), 374
Electron Densities, Planetary Nebulae, *news*, 1099
Electron Microscope Microanalyser, *news*, 493
Electron Microscope. Scanning — Studies of Human Trabecular Bone (E. D. Dyson, C. K. Jackson and W. H. Whitehouse), 957
Electron Microscopy of Non-metals, *news*, 689
Electron Microscopy. Platelet Adhesion in Rabbit Arteries observed by Scanning — (B. L. Sheppard and J. E. French), 1054
Electron Microscopy Symposium in Calcutta, *news*, 226
Electron Modulation by Light (Helmut Schwarz), *correspondence*, 1173
Electron Spin Resonance. —, of Hydrogen Atoms in a Free-burning Flame at Atmospheric Pressure (J. E. Bennett, B. Mile and R. Summers), 932
Electron Trap. Molecular Oxygen as an — in the Thermoluminescence of Saturated Hydrocarbons (I. Boustead), 846
Electrons. Source of — in a Selected Energy Range (SESER), (J. F. Andrews and R. H. Varey), 270
Electrophoresis. Effect of Anti-lymphocyte Serum on the Electrophoretic Mobility of the Lymph Node Cells of the Rat (G. P. Phondyke, K. Sundaram and P. Sundaresan), 79
Electroplax. Localization of Acetylcholinesterase by Immunofluorescence in Eel — (Philippe Benda, Shigeru Tsuji, Jean Daussant and Jean-Pierre Changeux), 1149
Elements. Heavy — in 73 Draconis (Mercedes Jaschek and Stella Malaroda), 246
Elephantidae. Early — of Africa and a Tentative Correlation of African Plio-Pleistocene Deposits (Vincent J. Maglio), 328
Embryos. Culture of Rabbit — in Circulating Medium (Joseph C. Daniel, jun.), 193
  Prenatal Chromosome Analysis (Fredrica Lisgar, Melvin Gertner, Sheldon Cherry, Lillian Y. Hsu and Kurt Hirschhorn), 280
  Ribosomal Genes and DNA Replication in Germinating Wheat — (David Chen and Daphne J. Osborne), 336
Embryo Culture. Culture of Rabbit Embryos in Circulating Medium (Joseph C. Daniels, jun.), 193
Embryogenesis. Diffusion in — (Francis Crick), 420; *correspondence* (Elizabeth M. Denehard), 671
Embryology. Enzyme Patterns on Tetraparental Mouse Liver (Thomas G. Wegmann), 462
EMMA, *news*, 493
Encephalomyocarditis Virus. Translation of RNA from — in a Mammalian Cell-free System (A. E. Smith, K. A. Marcker and M. B. Mathews), 184
ENEA Report for 1969, *news*, 1092
Engine, Howdens, *news*, 494
Engineering: and the SRC, *news*, 577; Award, *news*, 119; Nuclear Explosives in, *news*, 218
Enterobacteriaceae. Lack of Synergy of EDTA with Antimicrobials in Resistant — (Harold C. Neu and Elaine B. Winshell), 763
Entropy. — of Microbial Growth (W. W. Forrest), 1165
  Probability, Information and — (J. S. Rowlinson), 1196
  Trends in Montane Grasslands in Snowdonia, expressed in Terms of "Relative —" (R. Elfyn Hughes and J. Dale), 756

Environment: and Family Planning, *news*, 788; Research at NSF, *news*, 785; Royal Commission, *news*, 780
Environmental Conservation, Postgraduate Course, *news*, 580
Environmental Pollution: *Cleaning Our Environment. The Chemical Basis for Action* (American Chemical Society), *news*, 122; Control, *news*, 885
Enzyme. — Patterns in Tetraparental Mouse Liver (Thomas G. Wegmann), 462
Enzyme: Detergents, *parliamentary news*, 405; Function, *news*, 224; Reactions, *news*, 690; Regulation, Allostery in, *news*, 319
Enzymes in Crystals (Hans Neurath, Kenneth A. Walsh and Ralph A. Bradshaw), *correspondence*, 881
Epidemic. Failure to detect an Antigen associated with Hepatitis in a Community — (James W. Mosley, Lewellys F. Barker, N. Raphael Shulman and Milford H. Hatch), 953
Epidermis. Uniquely Oriented — Lipid (Lowell A. Goldsmith and Howard P. Baden), 1052
Epithelial Basement Membrane. Formation of — is restricted by Scurvy *in vitro* and is stimulated by Vitamin C (Robert E. Priest), 744
Equatorial Jets. — in Planetary Atmosphere (R. Hide), 254
Equatorial Undercurrent, *news*, 119
Erosion. Linear Erosional Furrows in Southampton Water (K. R. Dyer), 56
E SR Spectroscopy Symposium, *news*, 1102
Estuaries. Linear Erosional Furrows in Southampton Water (K. R. Dyer), 56
Ethylene. —, a Plant Hormone from Fluorescent Lighting (R. B. H. Wills and B. D. Patterson), 189
  —, the Natural Regulator of Leaf Abscission (Michael B. Jackson and Daphne P. Osborne), 1019
Eukaryotes. Model for Quantitative and Qualitative Control of mRNA Translation in — (Maurice Sussman), 1245
Euratom, *news*, 490
Euratom Thriller, *miscellaneous intelligence*, 217
European: Cell Biology Organization, *news*, 677; Conservation Year, Film, *news*, 579; Fund, *news*, 783; Enriched Uranium, *news*, 490; Environment Pollution, *news*, 888; Nuclear Power Report, 1969, *news*, 1092
Euro-Spectra, December, 1969, 6
Eustigmatophyceae. — —a New Algal Class with Unique Organization of the Motile Cell (David J. Hibberd and Gordon F. Leedale), 759
Evening Standard Headlines, *miscellaneous intelligence*, 894
Evolution. Early Elephantidae of Africa and a Tentative Correlation of African Plio-Pleistocene Deposits (Vincent J. Maglio), 328
  Natural Selection and the Concept of a Protein Space (John Maynard Smith), 563
  Non-Darwinian —: A Critique (Rollin C. Richmond), 1025
  Protein Sequences or Anatomy for Solving, *miscellaneous intelligence*, 8
  Rates of Evolutionary Change in the Hominid Canine Teeth (W. G. Kinzey), 296
Evolution of: Ammonites, *news*, 1101; Amphibians, *news*, 1100; Frog, *news*, 904; Reptiles, *news*, 1101
Exchange Diffusion (H. G. Britton), 748
Experimental Techniques. Hollow Casts of Lungs for Experimental Purposes (V. Timbrell, N. E. Bevan, A. S. Davies and D. E. Munday), 97
Explosions. Earthquakes and —: Comment on Molnar *et al.* (H. I. S. Thirlaway), reply by Peter Molnar, John Savino, Lynn R. Sykes, Robert C. Liebermann, George Hade and Paul W. Pomeroy), 1238
Extensive Air Showers. Observation of Structure in Čerenkov Pulses from — using Fast Techniques (C. Bosia, G. Castagnoli, M. Dardo and G. Marangoni), 532
Extinction Law. The — in the Magellanic Clouds (M. T. Brück, L. C. Lawrence, K. N. Nandy, A. D. Thackeray and R. Wood), 531
Extra-sensory Perception. Identification of Concealed Randomized Objects (I. Stevenson and J. G. Pratt), *correspondence*, 394
Eye. — Dominance in the Visual Cortex (Colin Blakemore and John D. Pettigrew), 426
  Interocular Transfer of Suppressive Effects of Retinal Image Displacement (D. M. MacKay), 872
  Lack of — to Eye Transfer of an Early Response Modification in Birds (H. Zeier), 708

F

Fabian Society Pamphlet on Education, *news*, 491
Fallout. Tungsten-181 in — in Finland (L. Blomqvist and M. Asikainen), 58
Family Planning and Environment, *news*, 788
Fauna. — Provinces in the Ordovician of North Atlantic Areas (M. Linström), 1158
Feeding. Cyclical "On Demand" Oral Intake by Adults (Ian Oswald, Judith Merrington and Heather Lewis), 959
Feodor Lyden Lecture, *news*, 498
Ferromagnetism. Mössbauer Study to find the Origin of Weak — in Cassiterite (Subir K. Banerjee, C. E. Johnson and Miroslav Krs), 173
Fertilization *in vitro*, *news*, 886; a Misunderstanding (R. A. Beatty), *correspondence*, 1082
Fibrinopeptides. Identity of Chimpanzee with Human — (R. F. Doolittle and G. A. Cross), 643
Field Emission. — from Silicon Carbide Whiskers (Francis S. Baker), 539
Film: *Environment in the Balance*, 579
Fingerprint Classification (A. Ganson), *correspondence*, 205
Fireball. — and Meteorite of April 25, 1969 (Keith B. Hindley and Howard G. Miles), 255
Flame Retardant Finish. New Durable — for Polypropylene Textiles (A. Adler and W. Brenner), 60
Flame. Electron Spin Resonance of Hydrogen Atoms in a Free-burning — at Atmospheric Pressure (J. E. Bennett, B. Mile and R. Summers), 932
Flies. Courtship Behaviour of some *Drosophila melanogaster* Mutants (S. Crossley and E. Zuill), 1064
  Sperm Transfer by Spermatophores in *Glossinia austeni* Newstead (J. N. Pollock), 1063
Flooding of the Thames, *news*, 403
Flora Europea Project, *news*, 780
Flow. Effect of Drag Reducing Additives on Pressure Loss across Transitions (V. G. Pisolkar), 936
  Friction Reduction Characteristics of Fibrous and Colloidal Substances (A. B. Arranaga), 447
  Karman Vortices in the — of Drag-reducing Polymer Solutions (V. N. Kalashnikov and A. M. Kudin), 445
  Migration of a Neutrally Buoyant Particle in Poiseuille —: a Possible Explanation (Keishi Gotoh), 848
  Visualization of Spiral Motion in Curved Open Channels of Large Width (J. R. D. Francis and A. F. Asfari), 725
Fluorescence. Resonance — of the CN Free Radical (J. A. Myer and R. W. Nicholls), 928
Fluorescent Lighting. Ethylene, a Plant Hormone from — (R. B. H. Wills and B. D. Patterson), 199
Fluoridation, *parliamentary news*, 680; 991
Flux Standard Error, *news*, 684
Flying. — Speed of the Largest Ariel Vertebrate (Cherrie D. Bramwell and G. R. Whitfield), 660
Foetus. Effect of Foetal Hypophysectomy on Catecholamine Levels in the Lamb Adrenal during Prolonged Gestation (R. S. Comline, Marian Silver and I. A. Silver), 739
  Levels of Lysozyme in Human — and Newborns (A. A. Glynn, W. Martin and M. Adinolfi), 77
  Teratogenic Effect of Lithium Carbonate in the Foetal Mouse (K. T. Szabo), 73
Food and Agriculture: Organization, 1; UN Publication, *news*, 9
Food Production, *news*, 9
Foot and Mouth Virus. Deposition and Uptake by Cattle of Airborne Particles (A. C. Chamberlain), 99
  Windborne Dispersal of — (K. P. Norris and G. J. Haroer), 98
Forest. Transition Probabilities for a — (Paul E. Waggoner and George R. Stephens), 1160
Forestry Research, *news*, 691
Foulness and Third London Airport, *news*, 1180
Fouling Organisms. Ecology of — in a Polluted Area (Lydia Ignatiades and Th. Becacos-Kontos), 293
Fourier Synthesis. Three-dimensional — of Tosylelastase at 3·5 Å Resolution (H. C. Watson, D. M. Shotton, Joyce M. Cox and Hilary Muirhead), 806
Fowler's Appointment, *news*, 573
Fracture Mechanics and Thermal Shock, *news*, 688
Fracture Zone, Emperor —: a Newly Discovered Feature in the Central North Pacific (Barrett H. Erickson, Frederic P. Naugler and William H. Lucas), 53
France: French Lasers on Moon, *news*, 117; French–Soviet Space Collaboration, *news*, 117; Research Budget, *news*, 778
Freeman Appointment, *news*, 487
Frequency Unit. Proposed New Unit of Frequency (Lawrence Stark), *correspondence*, 394
Friction. Effect of Rotational Phase Transformations on Dry (Coulomb) — (Thomas E. Simkins), 445
  — Reduction Characteristics of Fibrous and Colloidal Substances (A. B. Arranaga), 447
Frog Evolution, *news*, 904
Fruit Yield. Achene Spacing of Strawberries as an Aid to calculating Potential Yield (A. J. Abbott and R. A. Webb), 663
Fungi. Kinetics of Growth of Filamentous — with Particular Reference to Wood Decay (T. Gillespie and M. A. Hulme), 102
Fungicides. Possible Side Effects of — on Banana and Coffee Diseases (I. D. Firman), 1161
Fungistasis. Volatile Factor in Soil — (T. S. Hora and Ralph Baker), 1071
Fur, Gina Lollobrigida's, *news*, 215
Further Education, ATTI Report, *news*, 782
Fusion Catalysis. Difficulties with — by Quarks (E. E. Saltpeter), 165

G

GABA and Glutamate, *news*, 317
Galactic Nuclei. Retention of Dust Grains near — (N. C. Wickramasinghe), 145
Galaxies: Andromeda Nebula, *news*, 1194; Nuclei, *news*, 1000
Galaxy. Age of Cosmic Rays and Abundance of Antimatter in the — (L. Marshall Libby), 166
  Extra-galactic Distance Scale (Sidney van den Bergh), 503
  Percentage number of Nonthermal Sources in the Northern and Southern Halves of the Galaxy (W. J. Altenhoff and T. L. Wilson), 245
  Primordial Synthesis of Superheavy Nuclei in the — (B. Kichowics), 440
  Redshifts of Companion — (Halton Arp), 1033
  The Galactic Continuum Spurs and Neutral Hydrogen (Elly M. Berkhuijsen, C. G. T. Haslam and C. J. Salter), 364
  The Galactic Halo (J. W. Truran and A. G. W. Cameron), 710
Galaxy Machine, *news*, 308
Galaxy NGC 1068. Origin of the Optical Polarization in the Nucleus of — (K. Nandy and R. D. Wolstencroft), 621
Galaxy PK 0048–09. —: a Possible Radio Variable Galaxy (Mark A. Stull), 832
Galton–Watson Process. Population Growth and the Multi-type — (E. Seneta), 766
Galvanic Oxygen Electrode, *news*, 1104
Gamma Rays. Search for High Energy — from Four Pulsars (B. K. Chatterjee, G. T. Murthy, P. V. Ramana Murthy, B. V. Sreekantan and S. C. Tonwar), 839
Gamma Ray Astronomy Accelerators, *news*, 127
Gamma-ray Astronomy. High-energy — (G. G. Fazio), 905
Gas Centrifuge: Agreement, *parliamentary news*, 991; Plant, *parliamentary news*, 784
Gastrin. Production of Duodenal Ulcers and Hypertrophic Pyloric Stenosis by Administration of Pentagastrin to Pregnant Newborn Dogs (J. A. Dodge), 284
Gastrointestinal Tract. Effect of Serum from Irradiated Rats on Gastrointestinal Function (Charles K. Levy, Frank R. Irwin and John Garcia), 463
  Potent Peripheral and Splanchnic Vasodilator Peptide from Normal Gut (Sami I. Said and Viktor Mutt), 863
Genes. Acute Childhood Leukaemia in Relation to the HL-A Human Transplantation — (Roy L. Walford, Simon Finkelstein, Robert Neerhout, Par Konrad and Edward Shanbrom), 461
  Ribosomal — and DNA Replication in Germinating Wheat Embryos (David Chen and Daphne J. Osborne), 336
Genes: Chromosome Arrangement on —, *news*, 129; Expression and Control, *news*, 795
Genealogical Conundrum, *miscellaneous intelligence*, 991
Genetic Background. Is there a Viral Component in the —? (Hans Grüneberg), 39
Genetic Control. — of Deficiencies in γG Subclass observed among Families with Hypogammaglobulinaemia (Liliane Rivat, C. Ropartz, P. Burtin and A. Cruchard), 1136
Genetic Engineering, *news*, 999
Genetic Morphism. Schizophrenia as a — (William H. James), 1153
Genetic Transcription, *news*, 999
Genetics. Aberrant Karyotype and Spontaneous Abortion in a Japanese Family (Tetsuji Kadotani, Koso Ohama, Toshio Sofuni and Howard B. Hamilton), 735
  Allotype Polymorphism of Low Density β-Lipoproteins in Pig Serum (LDLpp 1, LDLpp 2) (J. Rapacz, R. H. Grummer, Judith Hasler and R. M. Shackelford), 941
  Chromosome Loss Compared with Chromosome Size, Age and Sex of Subjects (P. Neurath, K. DeRemer, B. Bell, L. Jatvik and T. Kato), 281
  Complete Mutagenesis in a Bacterial Population induced by Thymine Starvation on Solid Media (S. Bresler, M. Mosevitsky and L. Vyacheslavov), 764
  Effect of Adenoviruses on the Chromosomes of Normal Human Cells and Cells Trisonic for an E Chromosome (J. K. McDougall), 456
  Genetic Curing of Blindness in *Phycomyces blakesleeanus*: a Quantitative Assessment of Dominance (R. H. Villet), 453
  — of NADP Isocitrate Dehydrogenase in *Paramecium aurelia* (A. Tait), 181
  Inheritance of Sex Ratio in the Butterfly *Acraea encedon* (D. F. Owen), 662
  Prenatal Chromosome Analysis (Fredrica Lisgar, Melvin Gertner, Sheldon Cherry, Lillian Y. Hsu and Kurt Hirschhorn), 280
  Probable Haploid Expression of *HL-A* Antigens on Human Spermatozoon (M. Fellous and J. Dausset), 191
  Production of Chick Chimaeras (G. Marzullo), 72
  Restoration of In-phase Translation by an Unlinked Suppressor of a Frameshift Mutation in *Salmonella typhimurium* (Joseph Yourno and Shigegi Tanemura), 422
Geological Museum Exhibition, *news*, 900
Geological Survey of Canada. Laurentide Ice Sheet Map, *news*, 224

INDEX

Geology. Conceptual Model of the Bahamian Platform for the Last 135 Million Years (George W. Lynts), 1226
Geology: Institute Report, *news*, 402: of Moon, *news*, 127
Geomagnetic Field: Models, *news*, 501; Reversals, *news*, 222
Geophysics. Pyrolysis of Early Pre-Cambrian Onverwacht Organic Matter ($>3\times10^9$ yr old) (Ward M. Scott, Vincent E. Modzeleski and Bartholomew Nagy), 1129
Geophysics: Conference, Sydney, *news*, 591; Progress US, *news*, 121
Germination. Characterization of the — Response to Temperature of Species and Ecotypes (P. A. Thompson), 827
  Ribosomal Genes and DNA Replication in Germinating Wheat Embryos (David Chen and Daphne J. Osborne), 336
Gestation. Effect of Foetal Hypophysectomy on Catecholamine Levels in Lamb Adrenal during Prolonged — (R. S. Comline, Marian Silver and I. A. Silver), 739
Gigantopithecus. — and the Origins of Hominidae (David Pilbeam), 516
Gina Lollobrigida's Fur, *news*, 215
Glaciation, Saharan, *news*, 792
Glass. Effect of Electric Field on Phase Separation of — (R. C. de Vekey and A. J. Majumdar), 172
Glasses. Dielectric and Anelastic Effects in Inorganic — (D. K. Hale), 930
Glasses, Micrographs, *news*, 689
Gley Soils. Bacterial Mechanism of Gley Formation in Artificially Submerged Soil (J. C. G. Ottow), 103
Glomar Challenger, *miscellaneous intelligence*, 581
Glucan. Antitumour Polysaccharide derived Chemically from Natural — (Pachyman), (Goro Chihara, Junji Hamuro, Yukiko Maeda, Yoshiko Arai and Fumiko Fukuoka), 943
Glucose. Inhibition by Bile Salts of the Jejunal Transport of 3-O-Methyl — (Claude C. Roy, Reuben S. Dubois and Fernand Philippon), 1055
Glutamate and GABA, Brain, *news*, 317
Glycine. Strychnine Antagonism and —, a Reply (S. Roper and J. Diamond), 1259
  Strychnine, — and Vertebrate Postsynaptic Inhibition (D. R. Curtis and G. A. R. Johnston), 1258
Gobius cruentatus, *news*, 499
Goby, in British Waters, *news*, 499
Goldfish. Alarm Pheromone and Avoidance Conditioning in —, *Carassius auratus* (G. McA. Kimbrell, Mark R. Weinrott, Edward K. Morris, jun., Jennifer Scheid and Deborah Sangston), 754
Gollcher Foundation Publication, *news*, 404
Gonadotrophin: — Inhibition by the Synergistic Action of Vasopressin and Oxytocin (L. J. Hipkin), 740
Gondwanaland. Fit of the Southern Continents (A. Gilbert Smith and A. Hallam), 139
Government: Help for Industry Research, *news*, 783; Laboratory Reorganization, *news*, 209; R and D Savings, *parliamentary news*, 405; Research Establishments, *parliamentary news*, 894
Graduate: Education, US, *news*, 985; Employment, *news*, 488; Recruitment to Industry, *news*, 118
Grafting. Radiation — of Polysulphides to Wool (C. Samways and G. W. Hastings), 634
Granite. Gravity Measurements over the Nigerian Younger — Province (Deborah E. Ajakaiye), 50
Grants from SRC Altered, *news*, 781
Grape Research, Australia, *news*, 228
Graphite. Electronic Properties of Well Oriented — (J. D. Cooper, J. Woore and D. A. Young), 721
  Interaction of Atomic Hydrogen and Nitrogen with — Surfaces (Bruce McCarroll and D. W. McKee), 722
  Stored Energy in Creep Deformed — (W. V. Green and E. G. Zukas), 628
Graphite Growth. Ribbed Growth of — in Ni–C Solutions containing Boron (I. Minkoff and B. Lux), 540
Grass. Lead Accumulation in Roadside Soil and — (Tsaihwa J. Chow), 295
Grasshoppers. Isolation of DNA from B Chromosomes in — (Ian Gibson and Godfrey Hewitt), 67
Grasslands. Trends in Montane — in Snowdonia expressed in Terms of "Relative Entropy" (R. Elfyn Hughes and J. Dale), 756
Graves on Science, *miscellaneous intelligence*, 405
Gravitational Radiation, *news*, 687
Gravity. An Oscillating State as an Alternative to Gravitational Collapse (Clement Leibovitz), 711
  Cosmological Theory of Gravitation (S. J. Prokhovnik), 359
Gravitation Measurements over the Nigerian Younger Granite Province (Deborah E. Ajakaiye), 50
  Novel Correlations between Global Features of the Earth's Gravitational and Magnetic Fields (R. Hide and S. R. C. Malin), 605
Gravity Waves. Microbarograph Observation of Acoustic — (R. H. Clarke), 535
Gregarines. —: their Effect on the Growth of the Desert Locust (*Schistocerca gregaria*), (Owen G. Harry), 964
Ground-based Observations of Solar-terrestrial Phenomena, Development, *news*, 431
Grove, Sir William (Sydney Gill), *correspondence*, 775
Growth. Gregarines: their Effect on the — of the Desert Locust (*Schistocerca gregaria*), (Owen G. Harry), 694
Growth Hormone. Alteration of Normal Development of Drug Metabolism by Injection of — (John T. Wilson), 861
Guildford Centipede, *miscellaneous intelligence*, 120

H

Haem Synthesis in Nodules, *news*, 413
Haemodialysis Facilities, *news*, 1183
Haemoglobin. Effect of Phosphate and Non-phosphate Buffers on Thermolability of Unstable — (Lawrence J. Schneiderman, Irene G. Junga and Dorothea F. Fawley), 1041
  High Resolution Nuclear Magnetic Resonance Studies of — J. Capetown (S. Ogawa, R. G. Shulman, P. A. M. Kynoch and H. Lehmann), 1042
  In vitro Synthesis of Hb Hammersmith (CD1 Phe → Ser), (J. M. White and J. V. Dacie), 860
Haemoglobin Shepherds Bush. Mild "Unstable Haemoglobin Haemolytic Anaemia" caused by — (B74 (E18)Gly→Asp) (J. M. White, M. C. Brain, P. A. Lorkin, H. Lehmann and M. Smith), 939
Haemolysates. Negative Bohr Effect in Newt — and its Regulation (G. Morpurgo, P. A. Battaglia and T. Leggio), 76
Haemolytic Anaemia. Mild "Unstable Haemoglobin Haemolytic Anaemia" caused by Haemoglobin Shepherds Bush (B74 (E18)Gly→Asp) (J. M. White, M. C. Brain, P. A. Lorkin, H. Lehmann and M. Smith), 939
Haemopoiesis. Growth of Haemopoietic Spleen Colonies after Grafting on Human Bone Marrow in Mice (A. C. Louwagie and R. L. Verwilghen), 363
Hale Observatories, *news*, 403
Hallucinogenic Drugs. Effects of — and other Drugs on the Nest-building Behaviour of Mice (C. W. Schneider and M. B. Chenoweth), 1262
Halo Star, ν Indi: a Weak Velocity — with Excess Deficiency of Nirtogen (D. L. Harmer and B. E. J. Pagel), 349
Halothane. Changes underlying —-induced Malignant Hyperpyrexia in Landrace Pigs (M. C. Berman, G. G. Harrison, A. B. Bull and J. E. Kench), 653
Handley Page Recruitment, *news*, 312
Haploid Cell Lines, *news*, 1190
Haploidy. High Frequency Haploid Production in Barley (*Hordeum vulgare* L.), (K. J. Kasha and K. N. Kao), 874
Hapten. Antigenic Properties of a Homogeneous Tobacco Mosaic Virus– — Conjugate (L. I. Slobin), 698
Haptoglobin β Chain. Amino-acid Sequence of the Carboxyl Terminal Octapeptide of Human — (Don R. Barnett, Tong-Ho Lee and Barbara H. Bowman), 938
Harmonic Forces, *news*, 688
Harvard: Medical Directory, *miscellaneous intelligence*, 120; Program on Society and Technology, *news*, 219
Harwell's UFOs, *news*, 126
Headlines from the *Evening Standard*, *miscellaneous intelligence*, 894
Health Education and Welfare Department (US), Finance, *news*, 582
Health Service, Survival, *news*, 676
Hearing. Travelling Waves as Frequency Analysers in the Cochlea (Georg von Békésy), 1207
Hearing Illusions, *news*, 901
Heart. Senescent Changes in the — of *Drosophila repleta* Wollaston (G. E. Burch, R. S. Sohal and L. D. Fairbanks), 266
Heart Disease Research (WHO), *news*, 217
Heart Transplant. Adding a Ventricle instead of transplanting a Heart (Kenneth Bloor), 1142
Heat Flow. — Measurements on the Reykjanes Ridge (Ki-iti Horai, Mary Chessman and Gene Simmons), 264
Heavy Nuclei. Evidence for the Existence of — prior to the s-Process (H. D. Zeh), 361
Helsinki SALT Talks, *news*, 211
Heparin. Effect of — on the Detection of Human Isoantigens by the Cytotoxicity Technique (Mark E. Oren, Ronald B. Herberman and G. Nicholas Rogentine, jun.), 951
Heparin and Metastasis, *news*, 1192
Hepatitis. Failure to detect an Antigen associated with — in a Community Epidemic (James W. Mosley, Lewellys F. Barker, N. Raphael Shulman and Milford H. Hatch), 953
Herbicide 2,4,5-T, *parliamentary news*, 1093
Herbicides. Root Exudation of — by Woody Plants: Allelopathic Implications (C. P. P. Reid and W. Hurtt), 291
High Energy Physics Finance, *news*, 898
Higher Education Policy, *news*, 398
Hill on Allostery in Enzyme Regulation, *news*, 219
Hipposiderid Bat. Ear Movements in a — (J. D. Pye and L. H. Roberts), 285
Histamine. Effects of — on Toad Spinal Cor[illegible] (A. K. Tēbcis), 196
  Extract of *Ipomoea pes-caprae* (Convolvulaceae) antagonistic to — and Jellyfish Poison (S. Wasuwat), 758
Histocompatibility. — and Human Placentation (Markku Seppälä and Matti Tolonen), 950
Histocompatibility Antigens. Evidence against the Presence of H2 — in Ehrlich Ascites Tumour Cells (L. Chen and J. F. Watkins), 734
  Radioimmune Assay for — (Gabriel V. Foschi and Lionel A. Manson), 853
Histocompatibility Testing. Radioimmune Assay for Histocompatibility Antigens (Gabriel V. Foschi and Lionel A. Manson), 853
Historical Science Mathematics Conference, *news*, 1182
History of Science Society Catalogue of Work, *news*, 311
HL-A Antigens. Probable Haploid Expression of — on Human Spermatozoon (M. Fellous and J. Dausset), 191
HL-A System. Acute Childhood Leukaemia in Relation to the — Human Transplantation Genes (Roy L. Walford, Simon Finkelstein, Robert Neerhout, Pat Konrad and Edward Shanbrom), 451
HMSO
  Annual Report of the Institute of Geological Science, 402
  Annual Report of the National Physical Laboratory at Teddington, 5
  Annual Survey (1968–69) of the University Grants Committee, 439
  Atomic Energy Authority Bill, 1089
  First Employment of University Graduates, 1967–68, 498
  Forestry Research, 691
  Industrial Report by Chemicals EDC on Economic Assessment to 1972, 891
  Industrial Research and Development in Government Laboratories, 209
  Nature Conservation of the Coast, 891
  OECD Survey of Computer Industry in OECD Countries, 576
  Ordnance Survey, Annual Report for 1968–69, 6
  Second Annual Report of the Country Side Commission, 118
  Statistics of Education: Finance and Awards, 115
  The Future of the National Health Service, 674
  The Misuse of Drugs Bill, 1089
  The State of Food and Agriculture (UN), 9
  Third London Airport (Papers and Proceedings Volume VII), 466; 486
Hoff and Americzn Scientists, *miscellaneous intelligence*, 1184
Holography. Models for the Brain (P. J. van Heerden), *reply* by D. J. Willshaw, H. C. Longuet-Higgins and O. P. Buneman, 177
Hominid. *Gigantopithecus* and the Origins of — (David Pilbeam), 516
  Rates of Evolutionary Change in the — Canine Teeth (W. G. Kinzey), 296
  More Evidence of an Advanced — at Swartkrans (R. J. Clarke, F. Clark Howell and C. K. Brain), 1219
  Two New — Vertebrae from Swartkrans (J. T. Robinson), 1217
Homografts. Comparison of the Fates of Ag-B Locus Compatible — of Skin and Hearts in Inbred Rats (Clyde F. Barker and R. E. Billingham), 851
Honeywell before the Select Committee, *news*, 986
Hong Kong Wigs, *news*, 217
Hormones. Brassins — a New Family of Plant — from Rape Pollen (J. W. Mitchell, N. Mandeva, J. F. Worley, J. R. Plimmer, and M. V. Smith), 1065
  Ethylene, a Plant Hormone from Fluorescent Lighting (R. B. H. Wills and B. D. Patterson), 199
Houston Moon Conference, *news*, 127; 321
Hovertrains for Japan, *news*, 214
Howden's Engine, *news*, 494
Hubble Constant, Extra-galactic Distance Scale (Sidney van den Bergh), 503
Hückel-level Calculations. — on the Band Structure of Poly-AT (R. L. Flurry, jun., and Donna Breen), 729
Human Affairs Biology Council, *news*, 788
Human Placental Lactogen, *news*, 691
Human Remains in Australia, *news*, 216
100 Years Ago
  Athenaeum, on early CBW, 1079
  Channel Tunnel, 212
  "Correlation". Use of the word — (W. R. Grove), 307
  Education (*letter* from A. R. Wallace), 114
  Equatorial Telescope, 574
  French Observatory 'Situation', 488
  Huxley, on Miocene Mammalian Fauna of Europe, 675
  Intellectual Status of Man Today, from a *review* by Alfred Wallace, 986
  Society of Natural History on remains of *Tarandus rangijer*, 399
  Solar Disc Shadows (Alexander Herschel), 1088
  Sylvester on motions of a free rotating Body, 887
Hybrids. Hybrid Vigour in Seedlings of Recreated London Plane (Frank S. Santamour, jun.), 1159

Hybrid Plant Cells. Fusion of Isolated Plant Protoplasts (J. B. Power, S. E. Cummins and E. C. Cocking), 1016
Hybrid Plant Cells, *news*, 999
Hybridity. Vegetative — in *Phytophthora infestans* (Jean F. Malcolmson), 971
Hydra graysoni sp. nov. *Hydramoeba hydroxena* (Entz) discovered on — (Thomas R. A. Maxwell), 1068
Hydramoeba hydroxena. (Entz). — discovered on *Hydra graysoni* sp. nov. (Thomas R. A. Maxwell), 1068
Hydrocarbons. Molecular Oxygen as an Electron Trap in the Thermoluminescence of Saturated Hydrocarbons (I. Boustead), 846
Hydrogen. Anisotropic Atomic — Distribution in Interplanetary Space (W. H. Chambers, P. E. Fehlau, J. C. Fuller and W. E. Kunz), 713
  Electron Spin Resonance of — Atoms in a Free-burning Flame at Atmospheric Pressure (J. E. Bennett, B. Mile and R. Summers), 932
  Interaction of Atomic — and Nitrogen with Graphite Surfaces (Bruce McCarroll and D. W. McKee), 722
  Interstellar Solid —: How Much and Where? (V. C. Reddish), 367
  Level Populations of Excited States of — in Gaseous Nebulae (M. Brocklehurst), 618
  The Galactic Continuum Spurs and Neutral — (Elly M. Berkhuijsen, C. G. T. Haslam and C. J. Salter), 364
Hydrogen Ion. Fast Spectrophotometric Measurement of H^+ Changes in *Chromatium* Chromatophores activated by a Liquid Dye Laser (B. Chance, J. A. McCray and J. Bunkenburg), 705
Hydrolysis. — of Phospholipid Esters localized in Lysosomes and Related Structures of Rat Muscle Cells in Culture (Grace Norris and Minocher Reporter), 1246
  Non-enzymatic — of Adenosine Phophates (H. R. Hulett), 1248
5-Hydroxyindoleacetic Acid. — Levels in the Cerebrospinal Fluid of Depressive Patients treated with Probenecid (H. M. van Praag, J. Korf and J. Puite), 1259
Hyperactivity. Effects of 6-Hydroxydopamine on CNS Catecholamines, Spontaneous Motor Activity and Amphetamine Induced — in Rats (K. D. Evetts, N. J. Uretsky, L. L. Iversen and S. D. Iversen), 961
Hypersensitivity. Delayed — in *Trypanosoma cruzi* Infection (Stanely Seah), 1256
Hypogammaglobulinaemia. Genetic Control of Deficiencies in γG Subclass observed among Families with — (Liliane Rivat, C. Ropartz, P. Burtin and A. Cruchard), 1136
Hypotheses. Generation of — and Theories (Bernard Meltzer), 972

I

IBM: and Select Committee, *news*, 575; 889; Shares Fall, *news*, 308
Ice. Vapour Pressure of $H_2{}^{18}O$—(I) (− 17° C to 0° C and $H_2{}^{18}O$ Water (0°C to 16°C) (Gabor Jancso, Jovan Pupezin and W. Alexander Van Hook), 723
Ice: Pollution, *parliamentary news*, 581; Worms, *news*, 587
Ichthyology, *news*, 416; 589
Ichthyosaur Auction, *news*, 781
ICI/Courtaulds Merger, *news*, 114
ICL: and Computer-aided Design, *news*, 118; and Select Committee, *news*, 575
Idex, *news*, 309
Immune Response. *In vitro* Reconstitution of the — to Thymus-deprived Mice to Sheep Red Blood Cells (Alân Munro and Philippa Hunter), 277
Immunity. Induction of — and Tolerance *in vitro* in the Absence of Phagocytic Cells (Erwin Diener, Ken Shortman and Pamela Russell), 731
Immunoadsorbents. Fractionation of Antibodies against Staphylococcal Nucleae on 'Sepharose' — (Gilbert C. Omenn, David A. Ontjes and Christian B. Anfisen), 189
Immunofluorescence. Localization of Acetylcholinesterase by — in Eel Electroplax (Philippe Benda, Shigeru Tsuji, Jean Daussant and Jean-Pierre Changeux), 1149
  Localization of Antigens associated with the Pollen Grain Wall by — (R. B. Knox, J. Heslop-Harrison and C. Reed), 1066
Immunoglobulin. Anti-Nucleic Acid Specificities of Mouse Myeloma Immunoglobulins (David Schubert, Ann Rowan and Melvin Cohn), 154
  Anti-Rh Activity of Human γG4 Proteins (Marion Frame, P. L. Mollison and William D. Terry), 641
  Genetic Control of Deficiencies in γG Subclasses observed among Families with Hypogammaglobulinaemia (Liliane Rivat, C. Ropartz, P. Burtin and A. Cruchard), 1136
  — Determinants on the Surface of Mouse Lymphoid Cells (M. C. Raff, M. Sternberg and R. B. Taylor), 553
  IgG as Possible Precursor of Chemotactic Factor (M. Yoshinaga, M. Mayumi, S. Yamamoto and H. Hayashi), 1138
  Intrauterine Isoimmunization caused by Maternal IgG crossing the Placenta (G. N. Vyas, A. S. Levin and H. H. Fudenberg), 275
  Rheumatoid Anti Gm Factors with Specificity for the pFc′ Subfragment of Human — G (J. B. Natvig and M. W. Turner), 855
  Simple Method of Subtyping Human G-Myeloma Proteins based on Sensitivity to Pepsin Digestion (M. W. Turner, H. H. Bennich and J. B. Natvig), 853
Immunology. Cellular Deficit in Thymectomized Mice (D. E. Mosier, F. W. Fitch, D. A. Rowley and A. J. S. Davies), 276
Immunosuppression. Cytotoxic Efficiency and Effect on Tumour Growth of Heterospecific Antilymphocytic and Antitumour Sera (Michael Woodruff and Lindsay Hamilton Smith), 377
  Effect of Adjuvants on the Immunosuppressive Properties of Anti-lymphocytic Globulin (Randall A. Allardyce Robert D. Hunter and Keith James), 1141
  — by L-Asparaginase (M. C. Berenbaum), 550
Imperial College Infrared Telescope, *news*, 492
Imperial College Merger Cancelled, *news*, 575
Impotence and Pesticides, *news*, 796
Imprinting. Effects of an — Procedure on Regional Incorporation of Tritiated Uracil into Chick Brain RNA (S. P. R. Rose, P. P. G. Bateson, Ann D. Horn and G. Horn), 650
Independent University Costs, *news*, 988
ν Indi: A Weak-lined Halo Star with Excess Deficiency of Nitrogen (D. L. Harmer and B. E. J. Pagel), 349
India. Some Aspects of the Structure of Southern India based on Recent Bhadrachalam Earthquakes (S. K. Arora, T. G. Varghese and C. A. Krishnan), 261
Indian Scientists. Restrictions on —, *news*, 116
Indium. Occurrence — in Seawater and some Marine Sediments (A. D. Matthews, and J. P. Riley), 1242
Industrial: Consultancy, *news*, 308; Health Department at Newcastle University, *news*, 406; Mergers, *news*, 114; Recruitment of Graduates, *news*, 118;
Infant Mortality, Nuclear Testing, and Sternglass, *news*, 397; 779
Information. Probability, — and Entropy (J. S. Rowlinson), 1196
Information Explosion, *correspondence*, (F. Bruce Sanford), 979; (A. Johnson), 1173
Information Retrieval. Communication in Biochemistry (E. C. Webb), 132
Information System, for Nuclear Science, *news*, 791
Information Taping Experiment, *news*, 989
Influenza Virus. Virus of the 1918 Influenza Pandemic (C. H. Stuart-Harris), 850
Infrared Astronomy, *news*, 14
Infrared IRC + 10216, *news*, 502
Infrared Stars, *news*, 502
Infrared Telescope Transfer, *news*, 492
Inheritance. The Chemical Basis of — (P. Fleischman), 30
INIS, *news*, 791
Initiation. Reinitiation of RNA Chain Synthesis *in vitro* (John P. Richardson), 1109
Initiation Complex. Formation of the — using Muscle Messenger RNAs (Stuart M. Heywood), 694
Initiation Factors in Protein Synthesis, *news*, 903
Initiator. Position of the — and Peptidyl Sites in the *E. coli* Ribosome (Ernst Kuechler and Alexander Rich), 920
Innovations. Case Histories of — (R. W. Cahn), 693
Insect. Lytic Enzyme produced *in vitro* by — Cells: Lysozyme or Chitinase? (J. C. Landureau and P. Jolles), 968
  Water Uptake from Subsaturated Atmospheres: its Site in — (J. Noble-Nesbit), 754
Insect: Control, *news*, 1100; Cuticle Formation and Structure, *news*, 1192
Insecticide. Suspected — Resistance Mechanism in the Peach-Potato Aphid (V. F. Eastop and C. J. Banks), 970
Insulin. Biosynthesis of — in Anglerfish Islets (Anthony C. Trakatellis and Gerlad P. Schwartz), 548
Intelligence Quotient of Scientists, *miscellaneous intelligence*, 784
Intelsat Conference, *news*, 789; 1095; 1187
Interdisciplinary Research Relevant to Problems of Our Society NSF, *news*, 1234
Interkosmos Satellites, Launching, *news*, 117
International Scientific Communication (O. Wichterle), *correspondence*, 773
International Handbook of Universities, *miscellaneous intelligence*, 312
Interocular Transfer. Lack of Eye to Eye Transfer of an Early Response Modification in Birds (H. Zeier), 708
Interplanetary Space. Anisotropic Atomic Hydrogen Distribution in — (W. H. Chambers, P. E. Fehlau, J. C. Fuller and W. E. Kunz), 713
Interstellar Clouds. — and Atmospheric Clouds (G. R. Evans and M. V. Penston), 357
Interstellar Dust. — and Aromatic Carbon (G. P. Vdovykin), 254
Interstellar Grains. Scattering of X-rays from Pulsar NP 0532 by — (S. Naranan and G. A. Shah), 834
Interstellar Scintillations. Size and Motion of the — Pattern from Observations of CP 1133 (Kenneth B. Lang and B. J. Rickett), 529
Interstellar Solid Hydrogen. —: How Much and Where? (V. C. Reddish), 367
Intestine. Effects on Smooth Muscle Preparations of Unidentified Vasoactive Peptides from — and Lung (Priscilla J. Piper, S. I. Said and J. R. Vane), 1144
Iodination. Binding Activity of Oestrogen Receptors destroyed by — (G. A. Puca and F. Bresciani), 1251
Iodine. — as a Boundary Lubricant: A Contrary View (Michael J. Furey), 630
Iodine-Lecithin Interaction. Spin-Label Study of — (Gordon L. Jendrasiak and Robert Hayes), 278
5-Iodouridine. Molecular Conformations of — (A. Rahman and H. R. Wilson), 64
Ion Erosion in Electron Microscopy, *news*, 689
Ion Implantation Grant, SRC, *news*, 889
Ionic Transport. Active — across Biological Membrane: Possible Role of Electrons and Protons (Lazaro J. Mandel), 450
Ionosphere. Harmonic Ion Cyclotron Resonances observed by OGO-4 Satellite (K. Kikuchi), 257
  New Type of Ionospheric Disturbance (R. G. Rastogi), 259
  Origin and Dynamical Behaviour of Thermal Protons in the Venusian — (P. M. Banks and W. I. Axford), 924
  Penetration of Solar Particles to Ionospheric Height at Low Latitudes (S. Ganguly and Manoranjam Rao), 169
  Topside — of Venus and its Interaction with the Solar Wind (S. J. Bauer, R. E. Hartle and J. R. Herman), 533
Ionosphere: Investigation, Interkosmos Satellites, *news*, 117; Research, *news*, 579
Ipomoae per-caprae. Extract of — (Convolvulaceae) antagonistic to Histamine and Jellyfish Poison (S. Wasuwat), 758
Ipomoea. Alkaloids of an — Seed know and *Kaladana* in Pakistan (Charles I. Abou-Chaar), 663
Ireland. Research and Development in —, *news*, 114
Irish: Drunkenness, *miscellaneous intelligence*, 991; Research and Development Report, *news*, 114
Iron. Abundance of — in the Solar Photosphere (John E. Ross), 610
  Influence of Shock Deformation in the Transverse Magnetoresistivity of Polycrystalline — (A. Cristou), 372
Irradiation Effects. Localization of Irradiation Damage in Rat Seminiferous Tubules (Elizabeth R. Hall and E. W. Hupp), 85
IRRPOS, *news*, 124
Isoantigens. Effect of Heparin on the Detection of Human — by the Cytotoxicity Technique (Mark E. Oren, Ronald B. Herberman and G. Nicholas Rogentine, jun.), 951
Isoenzymes. Differences in E and S Chains from — of Horse Liver Alcohol Dehydrogenase (Hans Jörnvall), 1133
  Separation of — by Zonal Centrifugation (Colin H. Self and P. D. J. Weitzman), 644
Isoimmunization. Intrauterine — caused by Maternal IgA crossing the Placenta (G. N. Vyas, A. S. Levin and H. H. Fudenberg), 275
Italy: Bird Netting, *news*, 494; Bird Conservation, *news*, 677; Brain Drain, *news*, 132

J

"Jack-in-the-Box" Effect. Physical Entrapment of Polyelectrolytes within Microporous Solids: the— (G. G. Allan, K. Akagane, A. N. Neogi, W. M. Reif and T. Mattila), 175
Japan, Hovertrains for, *news*, 214
Japanese Space Research, *news*, 678
Jejunum. Inhibition by Bile Salts of the — Transport of 3-O-Methyl Glucose (Claude C. Roy, Reuben S. Dubois and Fernard Philippon), 1055
Jelly-fish Poison. Extract of *Ipomoea per-caprae* (Convolvulaceae) antagonistic to Histamine and — (S. Wasuwat), 758
Jet Propulsion Laboratory Deep Space Network, *news*, 124
JOIDES, *miscellaneous intelligence*, 581
Joint. Features of the Synovial Fluid Film in Human — Lubrication (P. S. Walker, J. Sikorski, D. Dowson, M. D. Longfield and V. Wright), 956
Journal of the British Society of Dowsers, *miscellaneous intelligence*, 8
Journals of Biomedicine Computing, *news*, 581
Jurassic. Gravity Measurements over the Nigerian Younger Granite Province (Deborah E. Ajakaiye), 50
  Palaeomagnetism of some Middle — Lavas from South-east Argentina (D. A. Valencio and J. F. Vilas), 262
Juvenile Hormone. Deferred Action of — (J. H. Willis and P. A. Lawrence), 81

K

Karman Vortices. — in the Flow of Drag-reducing Polymer Solutions (V. N. Kalashnikov and A. M Kudim), 445
Kew and Reading, Academic Cooperation, *news*, 780
Key Words. "Evolution"—"Development"—Anatomical and Cerebral Features and the Pathological Consequences (D. Davies, D. P. McKenzie and J. S. Turner), 636

Kidney. Stimulation of Mitosis in Amphibian — by Organ Specific Antiserum (D. P. Chopra and J. D. Simnet), 657
Kidney Machines, *parliamentary news*, 1184
Kidney Patients Demand for Renal Dialysis Units, *news*, 1183
Killifish. Osmoregulation by the — *Fundulus heteroclitus* in Fresh Water at Temperatures near Freezing (Bruce L. Umminger), 294
Kinetin. Antagonism between the Effects of — and Extracellular Metabolites of the Pathogen in Apple Scab Disease (D. S. Kirkham and R. C. Hignett), 388
Klinefelter's Syndrome. Behaviour Patterns in Chromatin-positive — of Sheep (R. Kilgour and A. N. Bruere), 71
Knee-whistlers. Longitudinal Structure in the Plasmapause: VLF Goniometer Observations of — (K. Bullough and J. L. Sagredo), 1038

L

Laboratory Primate Diseases, *news*, 319
Lactic Dehydrogenase. Increases in Serum — in Experimental Allergic Encephalomyelitis (A. E. Kaplan, L. V. Brown and J. Salk), 384
Lactose-operon DNA Isolation (S. E. Luria), *correspondence*, 301
Lakes. Density Stratified — in Northern Ellesmere Island (G. Hattersley-Smith, J. E. Keys, H. Serson and James E. Mielke), 55
Lasers. Dye — in the Ultraviolet (James A. Myer, Irving Itzkan and E. Kierstead), 544
  Fast Spectrophotometric Measurement of H$^+$ Changes in *Chromatium* Chromatophores activated by a Liquid Dye — (B. Chance, J. A. McCray and J. Bunkenburg), 705
Lasers: *news*, 15; and Carbon Fibres, *parliamentary news*, 581; French, on the Moon, *news*, 117
Laurentide Ice Sheet Map, *news*, 224
Lavas. Palaeomagnetism of some Middle Jurassic — from South-east Argentina (D. A. Valencio and J. F. Vilas), 262
Law: and Drug Addiction, *news*, 1087; and Underwater Archaeology, *news*, 1090; on Drug Misuse, *news*, 1089
Lead. — Accumulation in Roadside Soil and Grass (Tsaihwa J. Chow), 295
Lead in Petrol, and Atmospheric Pollution, *news*, 990
Leaf Abscission, Ethylene, the Natural Regulator of — (Michael B. Jackson and Daphne J. Osborn), 1019
Learning. Effects of Presenting Novel Verbal Material during Slow-wave Sleep (D. J. Bruce, C. R. Evans, P. B. C. Fenwick and Valerie Spencer), 873
  — of Leg Position in Chronic Spinal Rats (A. A. Buerger and A. Fennessy), 751
  Survival in Adult Mealworm Beetles (*Tenebrio molitur*) of Learning acquired at the Larval Stage (Antonio Borsellino, Ruggero Pierantonio and Barbara Schieti-Cavazza), 963
Leghaemoglobin Synthesis, *news*, 415
Legislation on Drugs, UK, *news*, 485
Leucine Naphthylamide. —: an Appropriate Substrate for the Histochemical Detection of Cathepsins B and B′ (J. Ken McDonald, Benjamin B. Zeitman and Stanley Ellis), 1048
Leukaemia. Acute Childhood — in Relation to the HL-A Human Transplantation Genes (Roy L. Walford, Simon Finkelstein, Robert Neerhout, Pat Konrad and Edward Shanbrom), 461
  Presence of an Additional Metacentric Chromosome in AKR/TIALD Leukaemic Cells (E. Legrand and J. F. Duplan), 737
  Surface Leukaemic Antigen and Malignancy of Cells in Mice infected with Mazurenko Virus (V. E. Gurtsevich, N. P. Mazurenko, E. I. Jarova, N. A. Probatova and G. N. Štepanova), 1047
Libel in Science: *news*, 1079; *correspondence*, (John R. Lewis, John S. Gray, Leland W. Pollock and P. Geoffrey Moore), 1081
Liberian Oil, *news*, 781
Life. Carbon monoxide as a Basis for Primitive — on Other Planets (Richard Wolfgang), 876
Light. Charge Moving with the Speed of — (W. B. Bonnor), 932
  Frequency Mixing in the Scattering of — by Harmonically Bound Electrons (D. Anreou and L. M. Bali), 1239
Lightning the Killer, *miscellaneous intelligence*, 1184
Linnean Society Funds, *news*, 402
Lipid. Uniquely Oriented Epidermal — (Lowell A. Goldsmith and Howard P. Baden), 1052
Lipid Bilayers. Ion Dependent Conductance: Switching in — (M. C. Goodall), 1257
Lipid Catabolism, Brain, *news*, 12
Lipid Membranes. Discreteness of Conductance Change in Biomolecular — in the Presence of Certain Antibiotics (S. B. Hladky and D. A. Haydon), 451
β-Lipoproteins. Allotype Polymorphism of Low Density — in Pig Serum (LDLpp 1, LDLpp2), (J. Rapacz, R. H. Grummer, Judith Hasler and R. M. Shackleford), 942
Liquid Crystals. Mossbauer Effect in —, *news*, 1001
Liquid Phase Inversion (Benjamin C. -Y. Lu, Paul Yu and David P. L. Poon), 1128
Liquori Publications, *miscellaneous intelligence*, 991
Lithium. Dopamine Excretion in Affective States and following Li_2CO_3 Therapy (F. S. Messiha, D. Agallianos and C. Clower), 868
  Effects of — on Amino-acids in Mouse Brain *in vivo* (F. V. DeFeudis and J. M. R. Delgado), 749
  Teratogenic Effect of — Carbonate in the Foetal Mouse (K. T. Szabo), 73
Littlewood Report, *parliamentary news*, 494
Liver. Differential Inhibition of Rat — Cholesterol Biosynthesis by Plant Growth Retardants (L. G. Paleg), 1252
  Development (Schizogony) of *Eimeria tenella* in the — of Chickens treated with Corticosteroid (P. L. Long), 290
  Enzyme Patterns in Tetraparental Mouse — (Thomas G. Wegman), 463
  Inhibition of ^{3}H-Thymidine Incorporation into Rat — Nuclei by *E. coli* L-Asparaginase (Siegfried Seeber and Ulrich Wesker), 652
  Positive α_1-Foetoprotein Tests in Pyridoxine Deprived Baboons: Relevance to — Carcinoma in Africans (Henry Foy, Athena Kondi, C. A. Linsell, Allison M. Parker and P. Sizaret), 952
  Production of "Sulphaton Factor" by the Perfused — (Paddy McConaghey and Clement B. Sledge), 1249
Liver, and Proline Hydroxylase, *news*, 500
Lockyer. — as Astronomer (A. J. Meadows), 230
Locust. Gregarines: their Effect on the Growth of the Desert — (*Schistocerca gregaria*), (Owen G. Harry), 964
  Antennae: Another Wind-sensitive Receptor in — (M. Gewecke), 1263
Lomonosov: Gold Medals, *news*, 213; Stream, *news*, 118
London Plane. Hybrid Vigour in Seedlings of Recreated — (Frank S. Santamour, jun.), 1159
London University Enquiry, *news*, 491
Longest English Word, *miscellaneous intelligence*, 8
Louse, *news*, 690
Love. Dr Iris, Aphrodite Research, *miscellaneous intelligence*, 120
Lovell at Royal Astronomical Society, *news*, 686
Lubricant. Iodine as a Boundary —: Contrary View (Michael J. Furey), 630
Lung. Effects on Smooth Muscle Preparations of Unidentified Vasoactive Peptides from Intestine and — (Priscilla J. Piper, S. I. Said and J. R. Vane), 1144
  Hollow Casts of — for Experimental Purposes (V. Timbrel, N. E. Bevan, A. S. Davies and D.E. Munday), 97
  Inactivation of Prostaglandins by the — (Priscilla J. Piper, J. R. Vane and J. H. Wyllie), 600
  Some Properties of Angiotensin Converting Enzyme in the — *in vivo* (K. K. F. Ng and J. R. Vane), 1142
Lyme Regis Icthyosaur Auction, *news*, 781
Lymph Node Cells. Effect of Anti-Lymphocyte Serum on the Electrophoretic Mobility of the — of the Rat (G. P. Phondyke, K. Sundaram and P. Sundaresan), 79
Lymphocytes. Effect of ALS Globulins on Amino-acid Incorporation by Human Peripheral — (H. Betuel, G. B. Richard, L. Colobert and L. Revol), 543
  — Cytotoxic Antibodies in Systemic Lupus Erythematosus (Kamal K. Mittal Roger D. Rossen, John T. Sharp, Martin D. Lidsky and William T. Butler), 1255
  Origin of Small — following Blastogenesis induced by Short-term PHA Stimulation (Peter R. Polgar and Sidney Kibrick), 857
  Potentiation of *in vitro* — Reactivity (Martin Janus and Fritz H. Bach), 238
  Release of Skin Reactive Factor in Guinea-pig — by Mitogens (E. Pick, J. Krejčí and J. L. Turk), 236
Lymphoid Cells. Immunoglobulin Determinants on the Surface of Mouse Lymphoid Cells (M. C. Raff, M. Sternberg and R. B. Taylor), 553
Lymphoma. Association of Murine Leukaemia Virus from a Mouse — (2731/L) with Reovirus Type 3 Infection (Jay A. Levy and Robert J. Huebner), 949
Lysosomes. Effect of Pregnancy Sera on Isolated — (Karl H. Hempel, Linda A. Fernandez and Robert H. Persellin), 955
  Histochemistry of some — "Proteolytic" Enzymes of Human Rheumatoid Synovia (J. Chayen, Lucille Bitensky and L. W. Poulter), 1050
  Hydrolysis of Phospholipid Esters localized in — and Related Structures of Rat Muscle Cells in Culture (Grace Norris and Minocher Reporter), 1245
T4 Lysosomes, Synthesis *in vitro*, *news*, 12
Lysozyme. Levels of — in Human Foetuses and Newborns (A. A. Glynn, W. Martin and M. Adinolfi), 77
  Lytic Enzyme produced *in vitro* by Insect Cells: — or Chitinase$_1$ (J. C. Landureau and P. Jollès), 968
Lytic Enzyme. — produced *in vitro* by Insect Cells: Lysozyme or Chitinase? (J. C. Landureau and P. Jollès), 968

M

Maastrichtian Fauna. Late Maastrichtian Nannoplankton Provinces (T. Worsley and E. Martini), 1242
MacKay, Donald: Geographical Mobility and the Brain Drain, *news*, 116
MacRobert Award, First, *news*, 119
Macro-lattice. — from Segregated Amorphous Phases of a Three Block Copolymer (A. Keller, E. Pedemonte and F. M. Willmouth), 538
Macrophages. Transformation of Hamster — into Giant Cells with Antimacrophage Serum (W. Ptak, Z. Porwit-Bobr and Z. Chlap), 655
Madras, Blue Green Algae Conference, *news*, 320
Magellanic Clouds. The Extinction Law in the — (M. T. Brück, L. C. Lawrence, K. N. Nandy, A. D. Thackeray and R. Wood), 531
Magnesium. Nature of Peristaltic Block Produced by — (D. B. Beleslin), 383
Magnesium Ions. Parathyroid Hormone Secretion *in vitro:* Regulation by Calcium and — (Louis M. Sherwood, Ingeborg Herrman and C. Andrew Basset), 1056
Magnets. — in Electromagnetic Theory (W. F. Lovering), 1127
Magnetic Evidence. Direct — of Sea Floor Spreading, *news*, 223
Magnetic Field. Bremsstrahlung Radiation in an Intense — and Emission from Pulsars (Hong-Yee Chiu and Vittorio Canuto), 1230
  — and the Number of Blood Platelets (Madelaine F. Barnothy and Jeno M. Barnothy), 1146
  New Source of Intense — in Neutron Stars. (V. Canuto, H. Y. Chiu, C. Chiuderi and H. J. Lee), 47
  Novel Correlations between Global Features of the Earth's Gravitational and — (R. Hide and S. R. C. Malin), 605
Magnetic Minerals Oxidation, *news*, 1100
Magnetic Relaxation. — in Ordered Systems (J. R. Hansen and K. D. Lawson), 542
Magnetic Poles Abundance, *news*, 411
Magnetoresistivity. Influence of Shock Deformation on the Transverse — of Polycrystalline Iron (A. Cristou), 372
Magnetosphere. VLF Wave Generation by the Čerenkov Process in the Inner — (R. P. Singh and R. N. Singh), 49
Magnox Power Station, *news*, 892
Malaria. Properties of Protective — Antibody (S. Cohen and G. A. Butcher), 732
  Transmission of *Plasmodium berghei* (NK 65 Strain) by *Anopheles annulipes* Walker (Elizabeth C. Kalucy and B. McMillan), 97
Malignant Hyperpyrexia. Changes underlying Halothane-induced — in Landrace Pigs (M. C. Berman, G. G. Harrison, A. B. Bull and J. E. Kench), 653
Malnutrition. Early Assessment of — (R. B. Bradfield and E. F. P. Jelliffe), 283
  Effect of Early — on the Reaction of Adult rats to Aversive Stimuli (David A. Levitsky and Richard H. Barnes), 468
Mammal Teeth. New Approach to Zahnreihen (J. W. Osborn), 343
Mammary Gland. Synthesis of Progesterone by the — of the Goat (Cecilia A. Slotin, R. B. Heap, J. M. Christiansen and J. L. Linzell), 385
Man: Early, *news*, 590; *miscellaneous intelligence*, 405
Management Selection Limited, *news*, 312
Manchester Solid State Physics Conference, *news*, 315
Manchester Technology Association Meeting, *news*, 890
Mandible. Newly Recognized — of *Ramapithecus* (L. S. B. Leakey), 199
Mansfield and Research Budget, *news*, 1177; 1185
Many-body Problem, *news*, 688
Marihuana. Metabolism of $\Delta^{1(6)}$(-Tetrahydrocannabinol, an Active Marihuana Constituent (Sumner H. Burstein, Franco Menezes, Ethel Williamson and Raphael Mechoulam), 88
Marine Octopods, *news*, 1191
Marine Pollution: *news*, 1096; and Sea Bird Deaths, *parliamentary news*, 784; Studies (J. R. Lewis), *correspondence*, 774
Mariner Spacecraft, *news*, 995
Mars: NASA Experiments, *news*, 124; Progress Report, *news*, 995
Marsupials. The Darwin Hypothesis of the Biogeographical Dispersal of — (P. G. Martin), 197
Mascons Structure, *news*, 412
Mathematics: Committee Report, SRC, *news*, 989; in Archaeology and History, Sciences, Conference, *news*, 1182
Mathematicians Longevity, *100 Years ago*, 3
Maxwell's Equations. Charge Moving with the Speed of Light (W. B. Bonnor), 932
Mazurenko Virus. Surface Leukaemic Antigen and Malignancy of Cells in Mice infected with — (V. E. Gurtsevich, N. P. Mazurenko, E. I. Jarova, N. A. Probatova and G. N. Stepanova), 1047
Mealworm Beetles. Survival in Adult — (*Tenebrio molitur*) of Learning acquired at the Larval Stage (Antonio Borsellino, Ruggero Pierantoni and Barbara Schieti-Cavazza), 963
Measles Virus. Adaptation to Hamsters of Neurotropic — from Subacute Sclerosing Panancephalitis (D. P. Byington, A. E. Castro and T. Burnstein), 554

- Mechanics. Poor Man's Guide to Celestial — (Hannes Aflevén), 229
- Medical Faculty at Harvard, *miscellaneous intelligence*, 120
- Medical Literature Annual, Czechoslovak, *miscellaneous intelligence*, 581
- *Medicine in the 1990's*, *miscellaneous intelligence*, 405
- **Membranes**. Effect of X-rays on the Electrical Conductance of Phospholipid Bilayer —: a Retraction (A. M. Sutton and D. Rosen), 274
  - Nuclear Relaxation in Phospholipids and Biological — (Stanley Kaufman, Joseph M. Steim, and Julian H. Gibbs), 743
- Membrane Transport. Active Ionic Transport across Biological Membranes: Possible Role of Electrons and Protons (Lazaro J. Mandel), 450
- **Memory.** Actinomycin-D: Effects on — at Different Times after Training (Larry R. Squire and Samuel H. Barondes), 649
  - Chemospecific Synaptic Repression as a Possible — Store (R. F. Mark), 178
- Meningococcal Disease Vaccine, *news*, 1193
- Mental Efficiency. Traffic Pollution and — (J. Lewis, A. D. Baddeley, K. G. Bonham and D. Lovett), 95
- Mental Illness. Complexes between Acetylcholine and Catecholamines and their Tolerance of — (Lauro Galzigna), 1058
- Mercury Structure, *news*, 411
- Mesosomes, Involvement in Cell Wall Synthesis, *news*, 130
- Metal Ions. Size-specific — Complexing Sites in Native Collagen (Joseph A. Spadaro, Robert O. Becker and Charles H. Backman), 1134
- Metastasis and Heparin, *news*, 1192
- Meteors. Meteoritic Spherules in the Soil surrounding Terrestrial Impact Craters (Paul W. Hodge and Frances W. Wright), 717
- Meteor Stream. The II Canis Minorids — a New — probably associated with Comet Mellish 1917 I (Keith B. Hindley and Michael A. Houlden), 1232
- **Meteorite.** Fireball and — of April 25, 1969 (K. B. Hindey and Howard G. Miles), 255
  - 81 Kr Radiation Ages of Stone — (M. W. Rowe), 368
- Meteorites Radio Sources. Excess Surface Density of — at 8 GHz (G. W. Brandie), 352
- Meteorology: *news*, 7; Forecasting, *news*, 683
- Methicillin. Altered Cell Walls of *Staphylococcus aureus* resistant to — (L. D. Sabath, C. D. Leaf, D. A. Gerstein and M. Finland), 1074
- *p*-Methoxyamphetamine. Investigation of — Excretion in Amphetamine Induced Psychosis (B. M. Angrist, J. W. Schweitzer, A. J. Friedhoff and S. Gershon), 651
- Microanalyser, *news*, 493
- Microbarograph. — Observation of Acoustic Gravity Waves (R. H. Clarke), 535
- Microbarograph Arrays. Detection and Location of an Atmospheric Nuclear Explosion by — (T. G. Varghese and Vijai Kumar), 258
- Microbial Growth. Entropy of — (W. W. Forrest), 1165
- Microbiology Society Symposium on Virology, *news*, 318
- Microinvasion. — of Epidermis caused by Substituted Anisoles (P. Grasso and Christine C. Rostron), 188
- Microporous Solids. Physical Entrapment of Polyelectrolytes within —: The "Jack-in-the-Box" Effect (G. G. Allan, K. Akagane, A. N. Neogi, W. M. Reif and T. Mattila), 175
- Microwave Astronomy, *news*, 1002
- Microwave Bursts. High Resolution Observations of Solar — (Haruo Tanaka and Shinzo Énomé), 435
- **Milk.** DDT Content (Goran Lofroth), *correspondence*, 881
  - DDT in Human — (Thomas H. Jukes), *correspondence*, 301
- Milky Way. Cassiopeia A Absorption Spectrum and Interarm Medium (G. de Jaegar), 622
- Milky Way Clouds, *news*, 1000
- Millipedes. Absence of Antimicrobial Substances in the Egg Capsules of — (Thomas Eisner, Stanley A. Zahler, James E. Carrel, Dorothy J. Brown and George W. Lones), 661
- Mineral Extraction Exhibition, *news*, 900
- Mirabelle and Serpukhov, *news*, 127
- Miró on Science, *miscellaneous intelligence*, 405
- Mitochondria. Multiple Forms of Human Brain — Monoamine Oxidase (G. S. S. Collins, M. Sandler, E. D. Williams and M. B. H. Youdim), 817
- Mitogens. Release of Skin Reactive Factor (E. Pick, J. Krejči and J. L. Turk), 236
- **Mitosis.** Mammalian Cell Fusion: Studies on the Regulation of DNA Synthesis and — (Potu N. Rao and Robert T. Johnson), 158
  - Stimulation of — in Amphibian Kidney by Organ Specific Antiserum (D. P. Chopra and J. D. Simnet), 657
- Mole. Black Velvet (J. W. Warren), *correspondence*, 206
- Molecular Biology Language, *news*, 999
- Molniya-I Soviet Satellite, *news*, 1092
- Money Value of Education, *news*, 1187
- Monoamine Oxidase. Multiple Forms of Human Brain Mitochondrial — (G. G. S. Collins, M. Sandler, E. D. Williams, and M. B. H. Houdim), 817
- Monopoles on the Sea Bed, *news*, 411
- **Moon.** Desert Stream Channels resembling Lunar Rilles (J. D. Burke, R. G. Brereton and P. M. Muller), 1234
  - Gaseous Species in Equilibrium with the Apollo 11 Holocrystalline Rocks during their Crystallization (T. R. Wellman), 716
  - Impact Craters and the Relative Ages of Earth and — (C. S. Beals), 368
  - Lunar Continental Migration and Maria Spreading (J. W. Elder), 842
  - Lunar Rilles and a Possible Terrestrial Analogue (G. H. J. McCall), 714
  - Thermal History of the — (J. J. Gilvarry), 623
  - Transient Lunar Phenomena and Electrostatic Glow Discharges (A. A. Mills), 929
- Moon: Conference, Houston, *news*, 321; Dust Stolen, *miscellaneous intelligence*, 894; French Lasers on, the —, *news*, 117; Geology, *news*, 127; Landing. Post Office Part, *miscellaneous intelligence*, 120; Mascons Plate-like Structure, *news*, 412; Origins, Houston Conference, *news* 221; Rocks Distribution, *news*, 782; Samples Report, *news*, 1194; Studies, Hazardous, *miscellaneous intelligence*, 8
- Moon Samples. Carbon Compounds in Apollo 11 Lunar Samples (Bartholomew Nagy, Ward M. Scott, Vincent Modzeleski, Lois Anne Nagy, Charles M. Drew, William S. McEwan, Joseph E. Thomas, Paul B. Hamilton and Harold C. Urey), 1028
- Moonrise. Sunrise and — at Stonehenge (Jack H. Robinson), 1236
- Morphine. Possible Mechanism of Action of — on Brain (E. Eidelberg and .A S. Schwartz), 1152
- Morphogenesis. — Substratum for Differentiation of Cartilage in Tissue Culture (Marshall R. Urist and Hiroshi Nogami), 1051
- Mortar Wreck, *news*, 404
- Mortars. Radiocarbon Dating of — (M. S. Baxter and A. Walton), 937
- Mössbauer Effect in a Smectic Liquid Crystal, *news*, 1001
- Mössbauer Study. — to find the Origin of Weak Ferromagnetism in Cassiterite (Subir K. Banerjee, C. E. Johnson and Miroslav Krs), 173
- Motile Cell. Eustigmatophyceae — a New Algal Class with Unique Organization of the — (David J. Hibberd and Gordon F. Leedale), 759
- Motivation. Brain Mechanisms and — (Keith Oatley), 797
- Motor Mechanisms. Two Innate Releasing Mechanisms subserving the Same Motor Pattern System (E. Curio, R. Blaich and N. Rieder), 750
- Motor Task. Movement Control in a Repetitive — (W. D. A. Beggs and C. I. Howarth), 752
- Mount Wilson Observatory Rechristening, *news*, 403
- Movement. Gliding in a Blue-Green Alga: a Possible Mechanism (Lawrence N. Halfen and Richard W. Castenholz), 1163
- MRC Cancer Research Coordination, *news*, 116
- Multi-author Papers in *Physics Letters*, *miscellaneous intelligence*, 784
- Murine Leukaemia Virus. Association of — from a Mouse Lymphoma (2731/L) with Reovirus Type 3 Infection (Jay A. Levy and Robert J. Huebner), 949
- Murine Lymphoma, Antigenic Expression of a — during Growth *in vitro* (Matko Cikeso), 645
- **Murine Sarcoma Viruses.** Transformation and Virus Growth by — in Human Cells (Stuart A. Aaronson and George J. Todaro), 450
  - Transformation of Hamster Cell Lines *in vitro* by a Hamster Sarcoma Virus (Jan Zavada and Ian Macpherson), 24
- **Muscle.** Effects on Smooth — Preparations of Unidentified Vasoactive Peptides from Intestine and Lung (Priscilla J. Piper, S. I. Said and J. R. Vane), 1144
  - Hydrolysis of Phospholipid Esters localized in Lysosomes and Related Structures of Rat — Cells in Culture (Grace Norris and Minocher Reporter), 1245
  - Myosin Filaments in Vertebrate Smooth — (J. Lowy, F. R. Poulsen and P. J. Vibert), 1053
  - Phosphorylase Activity in Acutely Exercised — (V. R. Edgerton, Donald Simpson, B. James Barnard and J. B. Peter), 866
- Muscular Disorders Sympsoium, *news*, 316
- Muscular Dystrophy Research, *news*, 316
- Museum. American — of Natural History at 100 (James A. Oliver), 1199
- **Mutagen.** Abnormal Zygote Development in Mice after Paternal Exposure to a Chemical — (Samuel S. Epstein, Sewa R. Joshi, Elsie Arnold, Elaine C. Page and Yvonne Bishop), 1260
  - Mutagenic Action of Aflatoxin B_1 on Transforming DNA and Inhibition of DNA Template Activity *in vitro* (Veronica M. Maher and William C. Summers), 68
- Mutagenesis. Complete — in a Bacterial Population induced by Thymine Starvation on Solid Media (S. Bresler, M. Mosevitsky and L. Vyacheslavov), 764
- Mutants. Courtship Behaviour of some *Drosophila melanogaster* — (S. Crossley and E. Zuill), 1064
- **Mutation.** Derivation of Partial Sequence of Human β-Chain Messenger RNA from — Data (Karl W. Lanks), 1244
  - Restoration of In-phase Translation by an Unlinked Suppressor of a Frameshift — in *Salmonella typhimurium* (Joseph Yourna and Shigegi Tanemura), 422
- *Mycoplasma laidlawii*. Isolation of a Virus infecting a Strain of — (R. N. Gourley), 1165
- G-Myeloma Proteins. Simple Method of Subtyping Human — based on Sensitivity to Pepsin Digestion (M. W. Turner, H. H. Bennich and J. B. Natvig), 853
- Myosin. — Filaments in Vertebrate Smooth Muscle (J. Lowy, F. R. Poulsen and P. J. Vibert), 1053
- Myxovirus. Self-annealing of Subgroup 2 — RNAs (William Robinson), 944

N

- NADP Isocitrate Dehydrogenase. Genetics of — in *Paramecium aurelia* (A. Tait), 181
- **Nannoplankton.** Late Maastrichtian — Provinces (T. Worsley and E. Martini), 1242
  - Standard Neogene Calcareous — Zonation (E. Martini and T. Worsley), 289
- Naphthalene. Oriented Grain Growth of Chlorinated — induced by an Electric Field (Keiji Miwa and Takeo Fukutomi), 271
- NAS Seismology Committee, *news*, 1098
- NASA: Budget, *news*, 305, 777; Budget Cuts, *news*, 685; Budget Process, *news*, 1097; Finance, *news*, 573; Finance, *news*, 582; Mars Experiments, *news*, 124; Projects, *news*, 994; Space Plan, *news*, 995; Space Programme, Next Decade, *news*, 1085; Space Reserch Plans, *news*, 790
- National Hospital for Nervous Diseases symposium on Muscular Diseases, *news*, 316
- National Institutes of Health, Budget, *news*, 996
- National Instrument Laboratories Inc., Publicity Drive for, *miscellaneous intelligence*, 120
- National Oceanic and Atmospheric Agency, *news*, 897
- National Science Board Finance, *news*, 789
- National Science Council of Ireland, *news*, 114
- National Science Foundation: Budget, *news*, 574, 777; Finance, *news*, 582; Funds, *news*, 785; Grant to Desert Research, *news*, 583
- Natta Medal, *news*, 213
- Natural History Museum. American Museum of Natural History at 100 (James A. Oliver). 1199
- Natural History Museum Open Day, *news*, 892
- Natural Selection. — and the Concept of a Protein Space (John Maynard Smith), 563
- Naval Research, *news*, 895
- Navy Deep Sea Diving Experiments, *news*, 1091
- Nebulae. Level Populations of Excited States of Hydrogen in Gaseous — (M. Brocklehurst), 618
- Neem Tree Planting to Improve soil, *news*, 131
- *Neobursaridium gigas*, *news*, 13
- Neogene. Standard — Calcareous Nannoplankton Zonation (E. Martini and T. Worsley), 289
- Neonates. Levels of Lysozyme in Human Foetuses and — (A. A. Glynn, W. Martin and M. Adinolfi), 77
- NERC: Grant for Cambridge Botany School, *news*, 680; Grants, National Distribution, *news*, 220; Goldfinger No Longer (R. S. Glover), *correspondence*, 570; support for University Research (R. I. H. Beverton), *correspondence*, 481
- Nerve Fibres. Neurotropic Control of Protein Synthesis in the Regenerating Limb of the Newt, *Triturus* (Paul Lebowitz and Marcus Singer), 824
- Nerve Function. Demonstration of Sodium and Potassium Conductance Changes during a Nerve Action Potential (E. Rojas, F. Bezanill and R. E. Taylor), 747
- Nerve Pulses Frequency Unit, Pitt (Alexandru T. Balaban), 1270
- Nervous Activity. — and RNA Metabolism in the Visual Cortex of Rat Brain (A. J. Dewar and H. W. Reading), 869
- Neuromuscular Junction. Effects of Black Widow Spider Venom on the Frog — (Herbert E. Longenecker, jun., William P. Hurlbut, Alexander Mauro and Allen W. Clarke), 701
- **Neurones.** Direct Postsynaptic Responses to Stimulation of Serotonin-containing — (G. A. Cottrell), 1060
  - Histochemical Differentiation of Motor — and Interneurones in the Anterior Horn of the Cat Spinal Cord (J. Fernandez-Campa and W. King Engel), 748
  - Re-evaluation of the Synaptic Activation of an Electrogenic Sodium Pump (JacSue Kehoe and P. Ascher), 820
  - Subcellular Localization of Serotonin in an Identified Serotonin-containing — (G. A. Cottrell and N. N. Osborne), 470
- Neurone Population Analysis. — of Somatosensory Thalamus in the Cat (Fredric A. Harris), 559
- Neurosecretion. —-like Material in the Hindbrain of Ageing Sheep and Sheep affected with Natural Scrapie (A. Bignami, Elizabeth Beck and H. B. Parry), 194
- Neutron Stars, *news*, 14
- **Neutron Stars.** Long Period Oscillations in Rotating — (Malvin Ruderman), 619
  - New Source of Intense Magnetic Fields in — (V. Canuto, H. Y. Chiu, C. Chiuderi and H. J. Lee), 47
  - Way for — to Pulse, *news*, 585

Neutron Starquakes. Pulsar Wobble and — (M. Ruderman), 836
Neutron Therapy for Cancer, *news*, 1182
Nevada Nuclear Tests and Earthquakes, *news*, 1098
Newcastle Disease Virus and Cell Fusion, *news*, 588
Newcastle University Department of Industrial Health, *news*, 406
Newmarket, Rabies at, *news*, 890
NHS Wigs, *news*, 217
NIH Budget, *news*, 996
Nitrite. Non-biological Reduction of — in Soil (L. A. Bulla, jun., C. M. Gilmour and W. B. Bollen), 664
Nitrogen. ν Indi a Weak Velocity Halo Star with Excess Deficiency of — (D. L. Harmer and B. E. J. Pagel), 349
  Interaction of Atomic Hydrogen and — with Graphite Surfaces (Bruce McCarroll and D. W. McKee), 722
Nitrogenase. Soluble — from Vegetative Cells of the Blue-Green Alga *Anabaena cylindrica* (R. V. Smith and M. C. W. Evans), 1253
Nitrosamines. Gas Chromatographic Determination of N-Alkyl — (J. K. Foreman, J. F. Palframan and E. A. Walker), 554
  — as Environmental Carcinogens (William Lijinsky and Samuel S. Epstein), 21
NOAA, *news*, 897
Noradrenaline. Changes in Enzymes involved in the Biosynthesis and Metabolism of — and Adrenaline after Psychosocial Stimulation (Julius Axelrod, Robert A. Mueller, James P. Henry and Patricia M. Stephens,) 1059
North America Continental Drift, *news*, 314
Norway Geology, *news*, 313
NSF Social Venture, *news*, 124
Nuclear Deterrent, Strategic, *parliamentary news*, 991
Nuclear Explosion. Detection and Location of an Atmospheric — by Microbarograph Arrays (T. G. Varghese and Vijai Kumar), 258
Nuclear Explosives in Engineering Conference, *news*, 218
Nuclear Fallout Deaths, *news*, 397, 779
Nuclear Fuel Business, *news*, 1089
Nuclear Information System, *news*, 791
Nuclear Magnetic Resonance Crystal Studies, *news*, 1000
Nuclear Power: Industry, *news*, 892; Reactor Development Speed, *news*, 1906; Report, Europe 1969, *news*, 1092
Nuclear Powered Ships, *parliamentary news*, 1184
Nuclear Research and AEC, *news*, 684
Nuclear Submarine Refitting, *news*, 306
Nuclear Tests and Earthquakes, *news*, 1099
Nuclei. Primordial Synthesis of Superheavy — in the Galaxy (B. Kichowics), 440
Nucleic Acid Hybridization, *news*, 414
Nucleogenesis. Evidence for the Existence of Heavy Nuclei Prior to the s-Process (H. D. Zeh), 361
Nucleoside Conformation. Changes in — (H. R. Wilson), 545
Nucleoside Stereochemistry, *news*, 1190
Nucleoside 5′-Triphosphatases. Four Base-specific — in the Subviral Core of Reovirus (A. M. Kapuler, N. Mendelsohn, H. Klett and G. Acs), 1209
Nucleotide Sequence. — from the Polypeptide Chain Termination Region of the Coat Protein Cistron in Bacteriophage R17 RNA (J. L. Nichols), 147
  Present within the 16S Ribosomal RNA of *Escherichia coli* (P. Fellner, C. Ehresmann and J. P. Ebel), 26
Nucleotide Sequence, R17 RNA, *news*, 125
Nucleus. Chromosome and Nuclear Location of Mouse Satellite DNA in Individual Cells (K. W. Jones), 912
NUT Proposals on Teacher Training, *news*, 399

O

OAO 2, *news*, 413
Obituaries
  Baldwin, Professor, E. H. F., 569
  Born, Professor Max, 669
  Carter, Dr. G. S., 1268
  Cosslett, Dr. Anna, 772
  Galloway, Dr. I. A., 978
  Greville, Dr. G. D., 977
  Hopwood, Dr. A. Tindell, 1172
  Hymen, Dr. Libbie Henrietta, 393
  Russel, Earl, 1080
Occupational Health, Pneumoconiosis, *parliamentary news*, 991
Ocean Undercurrent, *news*, 118
Oceans and Earth Conference, *news*, 591
Oceanography. Deep Sedimentary Basins proved in the Shetland-Hebridean Continental Shelf and Margin (M. H. P. Bott and A. B. Watts), 265
  Emperor Fracture Zone: a Newly Discovered Feature in the Central North Pacific (Barrett H. Erickson, Frederic P. Naugler and William H. Lucas), 53
Octopods, Marine, *news*, 1191
OECD Computer Industry Survey, *news*, 576
Oestrogen. Role of — in Spontaneous Ovulation demonstrated by Use of an Antagonist of Oestrogen, ICI 46, 474 (Anant P. Labhsetwar), 80
Oestrogen Antagonist. Role of Oestrogen in Spontaneous Ovulation demonstrated by Use of an Antagonist of Oestrogen, ICI 46, 474 (Anant P. Labhsetwar), 80
Oestrogen Receptors. Binding Activity of — destroyed by Iodination (G. A. Puca and F. Bresciani), 1251
Office of Health Economics Predictions, *miscellaneous intelligence*, 405
Office of Naval Research, *news*, 895
OGO-4. Harmonic Ion Cyclotron Resonances observed by — Satellite (H. Kikuchi), 257
Oil: Drillings Cautions, *news*, 683; in Liberia, *news*, 781; Pollution in Gulf of Mexico, *news*, 1096; Spill, Santa Barbara, *news*, 683; Well Regulations *news*, 1096
Oldest Men, *miscellaneous intelligence*, 120
Omega Project, *news*, 579
Oncogeny and Ontogeny, *news*, 588
Ontogeny and Oncogeny, *news*, 588
Opsin. Linkage of Retinal to — and Absence of Phospholipids in Purified Frog Visual Pigment$_{500}$ (Michael O. Hall and A. D. E. Bacharach), 637
Oral Contraceptives, *parliamentary news*, 405
Oral Intake. Cyclical "On Demand"—by Adults (Ian Oswald, Judith Merrington and Heather Lewis), 959
Orbiting Astronomical Observatory, *news*, 413
Ordered Systems. Magnetic Relaxation in — (J. R. Hansen and K. D. Lawson), 542
Ordovician Fauna. Faunal Provinces in the Ordovician of North Atlantic Areas (M. Lindström), 1158
Oriented Grain Growth. — of Chlorinated Naphthalene induced by an Electric Field (Keiji Miwa and Takeo Fukutomi), 271
Orienting. Possible Relationships between — and Diving Reflexes (David A. Goodman and Norman M. Weinberger), 1513
Ornitrol, *miscellaneous intelligence*, 894
Orogeny. Plate Tectonics, — and Continental Growth (J. F. Dewey and Brenda Horsfield), 521
Oscillating Chemical Reactions. Concentration Wave Propagation in Two-dimensional Liquid-phase Self-oscillating System (A. N. Zaikin and A. M. Zhabotinsky), 535
Osmoregulation. — by the Killifish, *Fundulus heteroclitus* in Fresh Water at Temperatures near Freezing (Bruce L. Umminger), 294
Overwacht Series. Pyrolysis of Early Pre-Cambrian — Organic Matter ($> 3 \times 10^9$ yr old) (Ward M. Scott, Vincent E. Modzeleski and Bartholomew Nagy), 1129
Ovulation. Role of Oestrogen in Spontaneous — demonstrated by Use of an Antagonist of Oestrogen ICI 46, 474 (Anant P. Labhsetwar), 80
Oxidation. Catalysis — of SO_2 on Carbon Films by Electrical Resistance Measurements (I. S. McLintock and J. C. Orr), 629
Oxidation State and Magnetic Polarization of Rocks, *news*, 1100
Oxygen. Assignment of Absolute Configuration from Anomalous Dispersion by — Atoms (S. Neidle and D. Rogers), 376
  Catalytic Inhibition by Adsorbed — (R. P. H. Gasser and P. R. Vaight), 933
  Molecular — as an Electron Trap in the Thermoluminescence of Saturated Hydrocarbons (I. Boustead), 846
Oxygen Sensors, *news*, 1104
Oxytocin. Gonadotrophin. Inhibition by the Synergistic Action of Vasopressin and — (L. J. Hipkin), 740
Ozone. Chlorotic Dwarf of Eastern White Pine caused by an — and Sulphur Dioxide Reaction (L. S. Dochinger, F. W. Bender, F. L. Fox and W. W. Heck), 476

P

Packing. Osculatory — of Finite Areas with Circles (H. H. Kausch-Blecken, von Schmeling and N. W. Tschoegl), 1119
Palaeoanthropology or Protein sequences for solving the problem of Human Evolution, *miscellaneous intelligence*, 8
Palaeomagnetism. — of some Middle Jurassic Lavas from South-east Argentina (D. A. Valencio and J. F. Vilas), 262
Palaeomagnetism, *news*, 1003
Palaeontological Data (I. G. Speden), *correspondence*, 302
Palaeontology. Vertebrate Fauna from the Lower Trias of Australia (Alan Bartholomai and Anne Howie), 1063
Palermo Cats and Rats, *miscellaneous intelligence*, 784
Palomar Observatory Rechristening, *news*, 403
Pandemic. Virus of the 1918 Influenza — (C. H. Stuart-Harris), 850
Papova-like Virus. Interaction between — and Paramyxovirus in Human Brain Cells: a Hypothesis (Hilary Koprowski, Giuseppe Barbanti-Brodano and Michael Katz), 1045
Paramyxovirus. Interaction between Papova-like Virus and — in Human Brain Cells: a Hypothesis (Hilary Koprowski, Giuseppe Barbanti-Brodano and Michael Katz), 1045
Parasites. Development of (Schizogony) *Eimeria tenella* in the Liver of Chickens treated with Corticosteroid (P. L. Long), 290
Parasitism. Hemiparasitic Flowering Plants: Phenotypic Canalization by Hosts (P. R. Atsatt), 1161
Parapsychology, *news*, 313
Parathyroid Hormone. — Secretion *in vitro*: Regulation by Calcium and Magnesium Ions (Louis M. Sherwood, Ingeborg Herrman and C. Andrew Bassett), 1056
Parkinson's Disease, *news*, 490
Parkinsonism. Aberrant mRNA in —: Support of Hypothesis from Studies with Chloramphenicol (C. N. Stefanis and M. Issidorides), 962
Parliament in Britain
  Aircraft Smoke Pollution, 680
  BAC 3-11, 894
  Beagle Aircraft Company, 784
  Britain's Space Programme, 1093
  Carbon Fibres and Lasers, 581
  CEGB Generating Capacity, 495
  Computers, 581
  Concorde, 784
  L-Dopa, 690
  Education Expenditure, 4-5
  Enzyme Detergents, 405
  Experiments on Animals, 495
  Fluoridation, 680, 991
  Gas Centrifuge Agreement, 991
  Gas Centrifuge Plant, 784
  Government R and D Savings, 405
  Government Research Establishments, 894
  Herbicide 2,4,5-T, 1093
  Ice Pollution, 581
  Kidney Machines, 1184
  Lung Cancer, 680
  Nuclear Powered Ships, 1184
  Oral Contraceptives, 405
  Pneumoconiosis, 991
  Polytechnics, 991
  Radiological Protection, 680
  Science Research Council, 1184
  Scowen Committee, 680
  Sea Bird Deaths, 784
  Strategic Nuclear Deterrent, 991
  Student Costs, 581
  Teacher Training, 784
  Teacher Training Grants, 495
  University Files, 1093
  University Places, 581
  Uranium Supplies, 1184
  VTOL Aircraft, 894
Passenger Train. British Rail Advanced — (S. Jones), 417
Peak District Traffic, *news*, 577
Pegasus Engine Award, *news*, 119
Penicillium stoloniferus. Three Types of Virus Particle in — (K. W. Buck and Gillian F. Kempson-Jones), 945
Pepper, Mr Curtis Bill, *miscellaneous intelligence*, 581
Pepsin. Simple Method of Subtyping Human G-Myeloma based on Sensitivity to — Digestion (M. W. Turner, H. H. Bennich and J. B. Natvig), 853
Peptide. Potent Peripheral and Splanchnic Vasodilator — from Normal Gut (Sami I. Said and Viktor Mutt), 863
Peptidyl Sites. Position of the Initiator and — in the *E. coli* Ribosome (Ernst Kuechler and Alexander Rich), 920
Peptidyl Transferase. Transesterification by — (E. Scolnick, G. Milman, M. Rosman and T. Caskey), 152
Perception. Acoustic Basis for Individual Recognition by Voice in the Gannet (Sheila J. White, R. E. C. White and W. H. Thorpe), 1156
  Inability to Perceive the Direction of Rotation Movement of Line Segments (Bela Julesz and Rosanne I. Hesse), 243
  The Detailed Texture of Eidetic Images (C. F. Stromeyer II and J. Psotka), 346
Perfumery, Soviet Union, *news*, 890
Peristalsis. Nature of Peristaltic Block produced by Magnesium (D. B. Beleslin), 383
Perpetual Secretary, *miscellaneous intelligence*, 1184
Personality Changes and Cannabis, *miscellaneous intelligence*, 991
Pesticides and Impotence, *news*, 796
Petrol Pollution of Air, *news*, 990, 1189
Petroleum. Transformations of Subsurface — (George Rudakov), 370
Phagocytic Cells. Induction of Immunity and Tolerance *in vitro* in the Absence of — (Erwin Diener, Ken Shortman and Pamela Russell), 731
Phase Behaviour. Liquid Phase Inversion (Benjamin C.-Y. Lu, Paul Yu and David P. L. Poon), 1128
Phase Separation. Effect of Electric Field on — of Glass (R. C. de Vekey and A. J. Majumdar), 172
Phase Transitions. Do — Exist? (B. U. Felderhof), 17
Phenotypes. Hemiparasitic Flowering Plants: Phenotypic Canalization by Hosts (P. R. Atsatt), 1161
Phenylthiocarbamate. Similar Polymorphism of Taste Sensitivity to — in Mice and Men (T. W. Klein and J. C. DeFries), 555
Pheromone. Alarm — and Avoidance Conditioning in Goldfish, *Carassius auratus* (G. McA. Kimbrell, Mark R. Weinrott, Edward K. Morris, jun., Jennifer Scheid and Deborah Sangster), 754
  Bark Beetle — (D. L. Wood and R. M. Silverstein), 557

  Primate Sex — of Vaginal Origin (Richard P. Michael and E. B. Keverne), 84
  Pheromones synthesized for Boll Weevil Control, *news*, 1100
Phosphate. Effect of — and Non-phosphate Buffers on Thermolability of Unstable Haemoglobins (Lawrence J. Schneiderman, Irene G. Junga and Dorothea F. Fawley), 1041
Phosphates. Possible Basis for the Cariostatic Effect of Inorganic — (K. M. Pruitt, A. D. Jamieson and R. C. Caldwell), 1249
Phospholipids. ATPase Activity of the Sodium Pump Needs Phosphatidylserine (K. P. Wheeler and R. Whittam), 449
  Linkage of Retinal and Opsin and Absence of — in Purified Frog Visual Pigment$_{500}$ (Michael O. Hall and A. D. E. Bacharach), 637
  Nuclear Relaxation in — and Biological Membranes (Stanley Kaufman, Joseph M. Steim and Julian H. Gibbs), 743
Phospholipid Bilayer Membranes. Effect of X-rays on the Electrical Conductance of —: a Retraction (A. M. Sutton and D. Rosen), 274
  Spin-Label Study of the Iodine-Lecithin Interaction (Gordon L. Jendrasiak and Robert Hayes), 278
Phospholipid Esters. Hydrolysis of — localized in Lysosomes and Related Structures of Rat Muscle Cells in Culture (Grace Norris and Minocher Reporter), 1246
Phosphorylase. — Activity on Acutely Exercised Muscle (V. R. Edgerton, Donald Simpson, R. James Barnard and J. B. Peter), 866
Photographic Memory, *news*, 227
Photons, *news*, 128
Photoreceptor. Rhodopsin Content of Frog — Outer Segments (D. Bownds and A. C. Gaide-Huguenin), 870
Photosphere. Abundance of Iron in the Solar — (John E. Ross), 610
Photosynthesis. Spatial Organization and Interactions of the Two Photosystems in — (W. P. Williams), 1214
Photosystems. Spatial Organization and Interactions of the Two — in Photosynthesis (W. P. Williams, 1214
Phyparus sculpturatus, *news*, 131
Physicists Employment, *news*, 897
Physics Exhibition, Computers, *news*, 891
Physics Institute Conference on Diffusion, *news*, 900
Physics Letters Multi-author Papers, *miscellaneous intelligence*, 784
Physics Productivity, *news*, 218
Physics Teaching in Universities, *news*, 988
Phytohaemagglutinin. Origin of Small Lymphocytes following Blastogenesis induced by Short-term — Stimulation (Peter R. Polgar and Sidney Kibrick), 857
Phytokinin. —like Activity of 8-Quinolinol Sulphate (S. E. Chue), 101
Phytophthora infestans. Vegetative Hybridity in — (Jean F. Malcolmson), 971
Pia Mater. Post-natal Growth of the Arterial Net in the Human Cerebral — (Deowattee Harnarine-Singh and John Baskerville Hyde), 86
Piezoelectricity. Piezoelectric Resonances in Amino-Acids (D. Vasilescu, R. Cornillon and G. Mallet), 635
Pigeon Contraceptive, *miscellaneous intelligence*, 894
Pigments. Plant — identified in the Common Blue Butterfly (John Feltwell and L. R. G. Valadon), 969
Pill: and Venereal Disease, *miscellaneous intelligence*, 784; Low Oestrogen, Speed of appearance on Market, *news*, 4
Pine. Chlorotic Dwarf of Eastern White — caused by an Ozone and Sulphur Dioxide Reaction (L. S. Dochinger, F. W. Bender, F. L. Fox and W. W. Heck), 476
Pitts. Proposed New Unit of Frequency, *correspondence*: (Lawrence Stark), 394; (Alexandru I. Balaban), 1270
Pituitary Growth Hormone. — Essential for Regulation of Serum Cholesterol (Meyer Friedman, Sanford O. Byers and Stephen R. Elek), 464
Placenta. Intrauterine Isoimmunization caused by Maternal IgA crossing the — (G. N. Vyas, A. S. Levin and H. H. Fudenberg), 275
Placental Lactogen, *news*, 591
Placental Porosity. Lack of Porosity of the Mouse Placenta to Maternal Cells (Mary J. Seller), 1254
Placentation. Histocompatibility and Human — (Markku Seppälä and Matti Tolonen), 950
Plagionite Group. Crystal Chemistry of the — (Seung-Am Cho and B. J. Wuensch), 444
Plan Calcul, Finance, *news*, 778
Planets. Chemical Homogeneity of Groups of Terrestrial — (J. C. Jamieson and J. V. Smith), 354
  Equatorial Jets in Planetary Atmosphere (R. Hide), 254
  Groups of Terrestrial — —a Reply (W. H. McCrea), 355
Planets: Grand Tour Planned, *news*, 996; Research, *news*, 995
Planetary Fission. — Events; Dynamical Constraints (J. M. Bailey), 48; *reply* by W. H. McCrea, 49
Planetary Life. Carbon Monoxide as a Basis for Primitive Life on Other Planets (Richard Wolfgang), 876
Planetary Nebulae Electron Densities, *news*, 1099
Planetary Systems. Dynamic Relaxation of — and Bode's Law (J. G. Hills), 840
Plants. Hemiparasitic Flowering —: Phenotypic Canalization by Hosts (P. R. Atsatt), 1161
  Root Exudation of Herbicides by Woody —: Allelopathic Implications (C. P. P. Reid and W. Hurtt), 291
Plant: Breeding Restrictions, *news*, 779; Cells, New, *news*, 692
Plant Growth Retardants. Differential Inhibition of Rat Liver Cholesterol Biosynthesis by — (L. G. Paleg), 1252
Plant Hormones. Brassins — a New Family of — from Rape Pollen (J. W. Mitchell, N. Mandeva, J. F. Worley, J. R. Plimmer and M. V. Smith), 1065
Plant Hormone. Ethylene, a — from Fluorescent Lighting (R. B. H. Wills and B. D. Patterson), 199
Plant Pigments. — identified in the Common Blue Butterfly (John Feltwell and L. R. G. Valadon), 969
Plant Protoplasts. Fusion of Isolated — (J. B. Power, S. E. Cummins and E. C. Cocking), 1016
Plasma. Two New — Instabilities in the Solar Wind (Joseph V. Hollweg and H. J. Völk), 441
Plasmapause. Longitudinal Structure in the —: VLF Goniometer Observations of Knee-whistlers (K. Bullough and J. L. Sagredo), 1038
Plasmodium berghei. Transmission of — (NK 65 Strain) by *Anopheles annulipes* Walker (Elizabeth C. Kalucy and B. McMillan), 97
Plate Tectonics. — Orogeny and Continental Growth (J. F. Dewey and Brenda Horsfield), 521
Pleistocene. — South-east Asiatic Pygmy Stegodonts (D. A. Hooijer), 474
Plio-Pleistocene Deposits. Early Elephantidae of Africa and a Tentative Correlation of African — (Vincent J. Maglio), 328
Plowshare Project Finance, *news*, 992
Pneumoconiosis, *parliamentary news*, 991
Poiseuille Flow. Migration of a Naturally Buoyant Particle in —: a Possible Explanation (Keishi Gotoh), 848
Polar Research, Soviet, *news*, 118
Polarization. Origin of the Optical — in the Nucleus of NGC 1068 (K. Nandy and R. D. Wolstencroft), 621
Political and Economic Planning Report on Third Airport Site, *news*, 1180
Pollen. Brassins — a New Family of Plant Hormone from Rape — (J. W. Mitchell, N. Mandeva, J. F. Worley, J. R. Plimmer and M. V. Smith), 1065
Pollen Grain Wall. Localization of Antigens associated with the — by Immunofluorescence (R. B. Knox, J. Heslop-Harrison and C. Reed), 1066
Pollution. Ecology of Fouling Organisms in a Polluted Area (Lydia Ignatiades and Th. Becacos-Kontos), 293
  Lead Accumulation in Roadside Soil and Grass (Tsaihwa J. Chow), 295
  Traffic — and Mental Efficiency (J. Lewis, A. D. Baddely, K. G. Bonham and D. Lovett), 95
Pollution: Aircraft Smoke, *parliamentary news*, 680; by Petrol Lead, *news*, 1181 ; Commission, *miscellaneous Intelligence*, 312; Control, *news*, 681, 885, 888; Crusade, *news*, 681; Environment, *Cleaning Our Environment. The Chemical Basis for Action* (American Chemical Society), *news*, 122; Ice, *parliamentary news*, 581; of Water Supplies, *news*, 1180; Prophets, *news* 397; River, *news*, 577; Teach-in at Edinburgh, *news*, 1004
Poly-I:C and Virus Infection, *news*, 1103
Polyelectrolytes. Physical Entrapment of — within Microporous Solids: the "Jack-in-the-Box" Effect (G. G. Allan, K. Akagane, A. N. Neogi, W. M. Reif and T. Mattila), 175
Polymers. Hückel-level Calculations on the Band Structure of Poly-AT (R. L. Flurry, jun., and Donna Breen), 729
  Pulsatile Flow Instrument for Viscoelasticity in Dilute — Solutions (G. E. Gadd), 724
  Radiation Graffing of Polysulphides to Wool (C. Samways and G. W. Hastings), 634
Polymer Solutions. Karman Vortices in the Flow of Drag-reducing — (V. N. Kalashnikov and A. M. Kudin), 445
Polymerases, Function, *news*, 586
Polynucleotides. Catalysis of — in Ionic Reactions (Norio Ise), 66
Polyoma Virus. Control of the Reversion of Properties in Transformed Cells (Zelig Rabinowitz and Leo Sachs), 136
Polyoma Virus, *news*, 794
Polypeptide Chains. Orientation of Polypeptide Side Chains in Solution (E. M. Bradbury, B. G. Carpenter, C. Crane-Robinson and H. Goldman), 65
Polypropylene. New Flame Retardant Finish for — Textiles (A. Adler and W. Brenner), 60
Polysaccharide. Antitumour — derived Chemically from Natural Glucan (Pachyman) (Goro Chihara, Junji Hamuro, Yukido Maeda, Yoshiko Arai and Fumiko Fukuoka), 943
  Effects of Cell Wall — on the Mating Ability of *Salmonella typhimurium* (Tsutomu Watanabe, Toshihoko Atai and Takanori Hattori), 70
Polysaccharide Vaccine, *news*, 1193
Polytechnics: Building, *parliamentary news*, 991; Directors Committee, *news*, 8; Policy, *news*, 573
Polytetrafluoroethylene. Production of Oxygen Atoms in Quartz and — Discharge Tubes (A. M. Mearns and A. J. Morris), 59
Polyvinyl Chloride Symposium, *miscellaneous intelligence*, 120
Pontostratiotes glaber, *news*, 224
Population. Annual Production and Respiration in Animal — (S. McNeill and J. H. Lawton), 472
  — Growth and the Multi-type Galton-Watson Process (E. Seneta), 766
  Problems of Expanding — (D. Wolfers), 593
Population: and Conservation (Eliot Slater), *correspondence*, 773; and Select Committee, *news*, 437; Predictions for GB, *news*, 410; versus Agricultural Developments, 1
Post Office: and Baird's Invention, *miscellaneous intelligence*, 435; Part in Moon Landing, *miscellaneous intelligence*, 120
Postgraduate Course in Environmental Conservation, *news*, 580
Potassium. Demonstration of Sodium and — Conductance Changes during a Nerve Action Potential (E. Rojas, F. Bezanilla and R. E. Taylor), 747
  Self-induced Transparency and Dispersion in — Vapour (D. J. Bradley, G. M. Gale and P. D. Smith), 719
Potassium Ion. Allosteric Interactions in ($Na^+ + K^+$) ATPase (T. Tobin, S. P. Banerjee and A. K. Sen), 745
  Relation of Intracellular Ca^{2+} to Retention of — by Liver Slices (G. D. V. van Rossum), 638
Potassium Ion Pump, *news*, 500
Prague Symposium Problems, *miscellaneous intelligence*, 120
Pratt and Stevenson, *news*, 313
Pregnancy. Effect of — Sera on Isolated Lysosomes (Karl H. Hempel, Linda A. Fernandez and Robert H. Persellin), 955
  Production of Duodenal Ulcers and Hypertrophic Pyloric Stenosis by Administration of Pentagastrin to Pregnant and Newborn Dogs (J. A. Dodge), 284
Pressure Chamber Experiments, *news*, 1091
Primate Diseases, *news*, 319
Primates. — Sex Pheromones of Vaginal Origin (Richard P. Michael and E. B. Keverne), 84
Probability. —, Information and Entropy, (J. S. Rowlinson), 1196
Probenecid. 5-Hydroxyindoleacetic Acid Levels in the Cerebrospinal Fluid of Depressive Patients treated with — (H. M. van Praag, J. Korf and J. Puite), 1259
s-Process. Evidence for the Existence of Heavy Nuclei prior to the — (H. D. Zeh), 361
Procreation and Coronary Infarction. Miscellaneous Intelligence (A. E. M. McLean and E. K. McLean), *correspondence*, 206
Progesterone. Synthesis of — by the Mammary Gland of the Goat (Cecilia A. Slotin, R. B. Heap, J. M. Christiansen and J. L. Linzell), 385
Proline. — in the Vesicles and Sporelings of *Valonia ventricosa* and the Concept of Cell Wall Protein (F. C. Steward, R. L. Mott, H. W. Israel and P. M. Ludford), 760
Proline Hydroxylase and Liver, *news*, 500
Prostaglandins. Inactivation of — by the Lungs (Priscilla J. Piper , J. R. Vane and J. H. Wyllie), 600
Prostaglandin Injection for Abortion, *news*, 415
Protein Kinase. Stimulation by Cyclic AMP of Intrinsic — Activity in Ox Brain Membrane Preparations (M. Weller and R. Rodnight), 187
Protein Space. Natural Selection and the Concept of a — (John Maynard Smith), 563
Protein Synthesis. Effects of Actinomycin D and Cycloheximide on RNA and — in Cleavage Stage Mouse Embryos (Richard J. Tasca and Nina Hillman), 1022
  Neurotropic Control of — in the Regenerating Limb of the Newt, *Triturus* (Paul Lebowitz and Marcus Singer), 824
Protein Synthesis: Controlling Translation, *news* 317; Synthesis, Initiation, *news*, 317; Synthesis, Initiation Factors, *news*, 903
Proteolytic Enzymes. Histochemistry of some Lysosomal — of Human Rheumatoid Synovia (J. Chayen, Lucille Bitensky and L. W. Poulter), 1050
Protoplasts. Fusion of Isolated Plant — (J. B. Power S. E. Cummins and E. C. Cocking), 1016
Pseudosymmetry. Five-fold — (B. G. Bagley), 1040
Psychosis. Investigation of *p*-Methoxyamphetamine Excretion in Amphetamine Induced — (B. M. Angrist, J. W. Schweitzer, A. J. Friedhoff and S. Gershon), 651
Public Health Information Conference, *news*, 676
Publicity Drive for National Instrument Laboratories Incorporated, *miscellaneous intelligence*, 120
Pulsars. Bouncing-core Theory of — (E. R. Harrison), 44
  Bremsstrahlung Radiation in an Intense Magnetic Field and Emission from — (Hong-Yee Chiu and Vittorio Canuto), 1230
  Can Pulsar Masses be Determined? (Peter Sutherland, Gordon Baym, Christopher Pethic and David Pines), 353
  Five New — (A. E. Vaughan and M. I. Large), 167

  Improved Parameters for Seven — (R. N. Manchester), 1124
  Long Term Variations of — Intensities (T. W. Cole, H. K. Hesse and C. G. Page), 712
  Low Mode Coherent Synchrotron Radiation and — Phenomena (Bernard J. Eastlund), 430
  On the Connexion between — and Supernovae (A. J. R. Prentice), 437
  Possible Mechanism for — Radio Emission (M. M. Komesarov), 612
  — and the Universal X-ray Background (M. V. Krishna Apparao), 836
  — Periods and Rapid Changes in the Terrestrial Rotation Rate: a Reply (Jörg Pfleiderer), 437
  — Wobble and Neutron Starquakes (M. Ruderman), 836
  Search for High Energy Gamma Rays from Four — (B. K. Chatterjee, G. T. Murthy, P. V. Ramana Murthy, B. V. Sreekantan and S. C. Tonwar), 839
  Secondary Periodicity in — (K. H. Hesse), 837
Pulsars: *news*, 222; Crab Nebula, *news*, 14; Crab, and its Planet, *news*, 590
Pulsar CP 0329. Long Baseline Interferometer Measurements of — (J. A. Galt, N. W. Broten, T. H. Legg, J. L. Locke and J. L. Yen), 530
Pulsar CP 0808. Short Duration Pulsations of — and Main Features of its Radio Emission in the Metre Band (V. V. Vitkevich and Yu. P. Shitov), 248
Pulsar CP 1133. Size and Motion of the Interstellar Scintillation Pattern from Observations of — (Kenneth R. Lang and B. J. Rickett), 528
Pulsar, Crab. Polarization of the Radio Pulses from the — (D. A. Graham, A. G. Lyne and F. G. Smith), 526
  X-ray Scattering by Grains in the Direction of the — and Sco XR-1 (C. S. Bowyer, J. Mack and M. Lampton), 1125
Pulsar, *NP* **0532.** Nature of Emission from — (I. S. Shklovsky), 251
  —: Average Polarization and Daily Variability at 430 MHz (D. B. Campbell, C. Heiles and J. M. Rankin), 527
  Scattering of X-rays from — by Interstellar Grains (S. Naranan and G. A. Shah), 834
Pulsars *PSR* 0833–45. Age of — (I. S. Shkovsky), 252
Pulsar Sco X-1. Short-term Periodic Variations following an Optical Flare of — (J. R. Gribbin, P. A. Feldman and S. H. Plagemann), 1123
Pulsar. Sco XR-1. X-ray Scattering by Grains in the Direction of the Crab Pulsar and — (C. S. Bowyer, J. Mack and M. Lampton), 1125
Pump. Mechanical — with a Suction Lift of 17 Metres (A. T. J. Hayward), 376
Pyloric Stenosis. Production of Duodenal Ulcers and Hypertrophic — by Administration of Pentagastrin to Pregnant and Newborn Dogs (J. A. Dodge), 284
Pyridoxine. Positive α_1-Foetoprotein Tests in — deprived Baboons: Relevance to Liver Carcinoma in Africans (Henry Foy, Athena Kondi, C. A. Linsell, Alison M. Parker and P. Sizaret), 952
Pyrolysis. — of Early Pre-Cambrian Onverwacht Organic Matter ($>3\times10^9$ yr old), (Ward M. Scott, Vincent E. Modzeleski and Bartholomew Nagy), 1129

Q

Quarks. Difficulties with Fusion Catalysis by — (E. E. Saltpeter), 165
Quartz. Ionic Diffusion in — (S. White), 375
  Production of Oxygen Atoms in — and Polytetrafluoroethylene Discharge Tubes (A. M. Mearns and A. J. Morris), 59
Quasars. Apparent Periodicities in the Redshifts of — (T. J. Deeming), 620
  Evidence of Radio Luminosity Evolution of Quasi-stellar Radio Sources, (M. A. Arakelian), 458
  How Significant are the Sharp Peaks in the Frequency Distribution of QSO Redshifts? (A. J. Wesselink), 927
Quasar 3C 345: Reply to Kinman concerning — (James H. Hunter, jun., and Philip K. Lü), 366
8-Quininolinol Sulphate. Phytokinin-like Activity of — (S. E. Chue), 101

R

Rabies: at Newmarket, *news*, 890; Regulations, *news*, 987
Radar: Probing of the Atmosphere, *news*, 7; Scanning of the Sun, *news*, 401
Radiation. — Catastrophe (E. R. Harrison), 245
Radiation Effects. Bonding of Small Molecules to DNA following Ionizing Radiation (John E. Byfield, Young C. Lee and Leslie R. Bennett), 859
  Effect of Serum from Irradiated Rats on Gastrointestinal Function (Charles K. Levy, Frank R. Irvin and John Garcia), 463
Radio Emission. Possible Mechanism of Pulsar — (M. M. Komesarov), 612
Radio Luminosity Evolution. Evidence of — of Quasi-stellar Radio Sources (M. A. Arakelian), 358
Radio Pulses. Frequency Spectrum of Air Shower — (H. R. Allan, R. W. Clay and J. K. Jones), 253
Radio Sources. Evidence of Radio Luminosity Evolution of Quasi-Stellar — (M. A. Arakelian), 358
  Excess Surface Density of Extragalactic — at 8 GHz (G. W. Brandie), 352
  Low-frequency Spectrum in the Crab Nebula (A. H. Bridle), 1035
  Percentage Number of Nonthermal Sources in the Northern and Southern Halves of the Galaxy (W. J. Altenhoff and T. L. Wilson), 245
  Variations of Linear Polarization in Extragalactic — (Hugh D. Aller), 440
Radio Sources. 3C 84. Decametric Variability of Cas A and — (A. H. Bridle and J. L. Caswell), 356
Radio Sources. Cas A. Decametric Variability of — and 3C 84 (A. H. Bridle and J. L. Caswell), 356
Cassiopeia A. Radio Source — Absorption Spectrum and the Interarm Medium (G. De Jaeger), 622
Radio Telescope. Observation of Atmospheric Turbulence with a — at 5GHz (R. A. Hinder), 614
Radiocarbon Dating. — of Mortars (M. S. Baxter and A. Walton), 937
Radio-interferometer, French, *news*, 117
Radiological Protection. *parliamentary news*, 680
Radiolysis. Effects due to Non-equilibrium *pH* Conditions in the — of Aqueous Systems (E. M. Fielden, G. C. Stevens, J. M. Phillips, G. Scholes and R. L. Willson), 632
Ramapithecus. 'Newly' Recognized Mandible of — (L. S. B. Leakey), 199
Rats and Cats, Palermo, *miscellaneous intelligence*, 784
Reactor Development Speed, *news*, 1096
Reading and Kew, Adacemic Cooperation, *news*, 780
Redshifts. Apparent Periodicities in the — of Quasi-stellar Objects (T. J. Deeming), 620
  How Significant are the Sharp Peaks in the Frequency Distribution of QSO —? (A. J. Wesselink), 927
  — of Companion Galaxies (Halton Arp), 1033
"Redsnow" Algae, *news*, 587
Reflexes. Possible Relationships between Orienting and Diving — (David A. Goodman and Norman M. Weinberger), 1153
Regeneration. Neurotropic Control of Protein Synthesis in the Regenerating Limb of the Newt, *Triturus* (Paul Lebowitz and Marcus Singer), 824
Renal Dialysis Units Shortage, *news*, 1183
Renin. Active Water insoluble Derivatives of — (T. Seki, T. A. Jenssen, Y. Lvein and E. G. Erdös), 864
Reovirus. Association of Murine Leukaemia Virus from a Mouse Lymphoma (2731/L) with — Type 3 Infection (Jay A. Levy and Robert J. Huebner) 949
  Four Base-specific Nucleoside 5′-Triphosphatases in the Subviral Core of — (A. M. Kapuler, N. Mendelsohn, H. Klett and G. Acs), 1209
Replication, Chromosome, *news*, 499
Report Fee, *miscellaneous intelligence*, 1184
Reprints. "Evolution"—"Development"—Anatomical and Cerebral Features and the Pathological Consequences (D. Davies, D. P. McKenzie and J. S. Turner), 636
  Prostaglandins—an Experiment (V. R. Pickles), 636
Reproduction. Effects of Cell Wall Polysaccharide on the Mating Ability of *Salmonella typhimurium* (Tsutomu Watanabe, Toshihiko Arai and Takanori Hattori), 70
  Reproductive Cycle of a Neotropical Insectivorous Bat, *Myotis nigricans* (Don E. Wilson and James S. Findley), 1155
Reptiles Evolution, *news*, 1101
Research. Value of Curiosity-oriented — (M. Gibbons, J. R. Greer, F. R. Jevons, J. Langrish and D. S. Watkins), 1005
Research: and Development Corporation, *news*, 209; Association Future, *news*, 673; Associations Collaboration, *news*, 399; Budget, *news*, 1177; Budget and Mansfield, *news*, 1185; Budget, French, *news*, 778; Expenditure, NSF, *news*, 785; for Technology, *news*, 404; Government Help for Industrially Sponsored projects, *news*, 783; Management Advisory Panel, *news*, 1186; Relevancy and Finance, *news*, 777
Reservoirs, Geological effects, *news*, 7
Resonance Lines. The Shape and Shift of the — of Ca^+ produced by Electron Collisions (K. S. Barnes and G. Peach), 374
Respiration. Annual Production and — in Animal Populations (S. McNeill and J. H. Lawton), 472
Retina. Action spectrum of the Human Early Receptor Potential (Ronald E. Carr and Irwin M. Siegel), 89
  Elevation of Vision Threshold by Displacement of Retinal Image (D. M. McKay), 90
  Interocular Transfer of Suppressive Effects of — Image Displacement (D. M. MacKay), 872
Retinal. Linkage of — to Opsin and Absence of Phospholipids in Purified Frog Visual Pigment$_{500}$ (Michael O. Hall and A. D. E. Bacharach), 637
Retine in Cell Division Inhibition, *news*, 413
Retro-reflexion (M. Minnaert), 718
Reykjanes Ridge. Heat Flow Measurements on the — (Ki-Iti Horai, Mary Chessman and Gene Simmons), 264
Rhenium. Anomalous — Isotopic Ratio in the Solar Wind: Detection at the Nano gram Level (J. W. Morgan), 1037
Rhesus Factor. Anti-Rh Activity of Human γG4 Proteins (Marion Frame, P. L. Mollison and William D. Terry), 641
Rheumatoid Arthritis. Histochemistry of some "Proteolytic" Enzymes of Human Rheumatoid Synovia (J. Chayen, Lucille Bitensky and L. W. Poulter), 1050
Rheumatoid Factors. Rheumatoid Anti Gm Factors with Specificity for the pFc′ Subfragment of Human Immunoglobulin G (J. B. Natvig and M. W. Turner), 855
Rhinoceroses for Whipsnade, *news*, 1181
Rhinotracheatic Virus. Probable Occurrence of Infectious Bovine. — in Tanzania in Wildlife and Cattle (M. M. Rweyemamu), 738
Rhodopsin. — Content of Frog Photoreceptor Outer Segments (D. Bownds and A. C. Gaide-Huguenin), 870
Ribosomal RNA. Observations on the Primary Structure of the 23S Ribosomal RNA from *E. coli* (Ward M. Scott, Vincent E. Modzeleski and Bartholomew Nagy), 1129
  Structural Comparison of 26S and 17S — of Yeast (R. C. van den Bos and R. J. Planta), 183
Ribosomes. Position of the Initiator and Peptidyl Sites in the E. coli — (Ernst Kuechler and Alexander Rich), 920
  Temperature Sensitive Alterations of 30S Subunits demonstrated by *in vitro* Reassociation of Functional — (Soo-Sang Kang), 1132
Ribosome Ambiguity, *correspondence* (Bernard D. David), 206; (F. Varricchio), 206
Rifampicin. Anti-trachoma Activity of — and Rifamycin SV Derivatives (Yechiel Becker Yael Asher Nehama Himmel and Zichriya Zakay-Rones), 454
Rifamycin. Anti-trachoma Activity of Rifampicin and — SV Derivatives (Yechiel Becker, Yael Asher, Nehama Himmel and Zichriya Zakay-Rones), 454
Rift Valley, East Africa, *news*, 314
Rijswijk, Laboratory Primate Disease Conference, *news*, 319
Rilles. Desert Stream Channels resembling Lunar — (J. D. Burke, R. G. Brereton and P. M. Muller), 1234
  Lunar — and a Possible Terrestrial Analogue (G. H. J. McCall), 714
Ring's Problem, *news*, 492
River Pollution, *news*, 577
RNA. Effect of an Imprinting Procedure on Regional Incorporation of Tritiated Uracil into Chick Brain — S. P. R. Rose, P. P. G. Bateson, Ann, L. D. Horn and G. Horn), 650
  Self-annealing of Subgroup 2 Myxovirus — (William S. Robinson), 944
RNA Chain Synthesis. Reinitiation of — *in vitro* (John P. Richardson), 1109
RNA Metabolism. Nervous Activity and — in the Visual Cortex of Rat Brain (A. J. Dewar and H. W. Reading), 869
RNA Polymerase. — and the Shut-off Host RNA and Protein Synthesis in T4 Phage Infection (Shirley Su, Fanyela Weinberg, Antero G. So and Earl W. Davie), 62
  — σ-Factor and the Selection of Initiation Site (M. Sugiura, T. Okamoto and M. Takanami), 598
  Specific Action of α-Amanitin on Mammalian — Protein (S. T. Jacob, E. M. Sajdel and H. N. Munro), 60
RNA Structure. Structural Comparison of 25S and 17S Ribosomal RNA of Yeast (R. C. van den Bos and R. J. Planta), 183
RNA Structure, *news*, 497
RNA Synthesis. Dosage Compensation and Effect for — in Chromosome Puffs of *Drosophila melanogaster* (K. Gorge), 386
  Effects of Actinomycin D and Cycloheximide on RNA and Protein Synthesis in Cleavage Stage Mouse Embryos (Richard J. Tasca and Nina Hillman), 1022
RNA Tumour Viruses, *news*, 690
RNA Translation. Translation of RNA from Encephalomyocarditis Virus in a Mammalian Cell-free System (A. E. Smith, K. A. Marcker and M. B. Mathews), 184
fMet-tRNA. Complementary Oligonucleotide Binding to the Anticodon Loop of — (Olke C. Uhlenbeck, Julie Baller and Paul Doty), 508
mRNA. Aberrant — in Parkinsonism: Support for Hypothesis from Studies with Chloramphenicol (C. N. Stefanis and M. Issidorides), 962
  Derivation of Partial Sequence of Human β-Chain — from Mutation Data (Karl W. Lanks), 1244
  Formation of the Initiation Complex using Muscle — (Stuart M. Heywood), 694
  Model for Quantitative and Qualitative Control of — Translation in Eukaryotes (Maurice Sussman), 1245
rRNA. Autoradiographic Detection of Molecular Hybrids between — and DNA in Tissue Sections (M. Buongiorno-Nardelli and F. Amaldi), 946
  Nucleotide Sequences Present within the 16S — of *Escherichia coli* (P. Fellner, C. Ehresmann and J. P. Ebel), 26
R17 RNA Nucleotide Sequence, *news*, 125

tRNA. High-resolution X-ray Diffraction by Single Crystals of Mixtures of — (Richard D. Blake, Jacques R. Fresco and Robert Langridge), 32
tRNA: crystals, *news*, 11; Structure, *news*, 414
Roadside. Lead Accumulation in — Soil and Grass (Tsaihwa J. Chow), 295
Rockall Plateau. New Sedimentary Basin on — (D. G. Roberts, D. G. Bishop, A. S. Laughton, A. M. Ziolkowski, R. A. Scrutton and D. H. Matthews), 172
Rockets, Japanese, *news*, 678
Rodents. Variations in the DNA from Two — Families (Cricetidae and Muridae), (W. Hennig and P. M. B. Walker), 915
Rolling Circle Model of Bacteriophage Chromosome Replication, *news*, 499
Root Exudation. — of Herbicides by Woody Plants: Allelopathic Implications (C. P. P. Reid and W. Hurtt), 291
Root Nodules Haem Synthesis, *news*, 415
Rotation. Inability to Perceive the Direction of — Movement of Line Segments (Bela Jules and Rosanne I. Hesse), 243
Rothschild's Sperms. *miscellaneous intelligence*, 1184
Royal Astronomical Society: *news*, 498; Anniversary Commemorative Stamp, *news*, 686; Meeting, *news*, 222; Meeting on Earth's Core-mantle Interface, *news*, 793; Presidential Address, *news*, 686
Royal Commission on Environment, *news*, 780
Royal Society: Discussions on Strong Fibrous Solids, *news*, 592; Education Committee, *news*, 579; Meeting on Rift, *news*, 314; New Members, *news*, 1182
Russian Spacemen, Deaths, *news*, 216

S

Saccharin, Cyclamates and Tumours, *miscellaneous intelligence*, 1184
Saharan Glaciation, *news*, 792
Salk Institute, Human Affairs Biology Council, *news*, 788
Salmon. Serum Lipids and the Death of Spawning Pacific — (Stuart Patton, G. F. Crozier and A. A. Benson), 754
Salmon Atlantic Fishing Battle, *news*, 887
SALT: *news*, 211; Prospects, *news*, 1178
Santa Barbara Oil Spill, *news*, 683
Sarcoma. Demonstration in — Patients of Anti-tumour Antibodies which Fix only Human Complement (F. R. Eilber and D. L. Morton), 1137
Satellite DNA. Chromosomal and Nuclear Location of Mouse — in Individual Cells (K. W. Jones), 912
Satellite DNA, *news*, 414, 899
Satellite Engineer Advertisement, for SRC, *news*, 1089
Satellites: Interkosmos, *news*, 117; Japanese, *news*, 678
Saturn's Rings Ammonia Ice, *news*, 687
Scanning Electron Microscope. Electron Channelling Patterns from the Small (10 μm) Selected Areas in the — (C. G. van Essen, E. M. Schulson and R. H. Donaghy), 847
Schizophrenia. — as a Genetic Morphism (William H. James), 1153
Scholarships for Young US Scientists, *news*, 998
Science: and Irish Economic Development Report, *news*, 114; Graduates Employment, *news*, 488; Libel Action, *news*, 1079; *correspondence* (John R. Lewis, John S. Gray, Leland W. Pollock and P. Geoffrey Moore), 1081; Policy and Dainton, *news*, 679; Policy Research Unit, *news*, 487
Science Students. Career Choices by — (D. O. Edge), 506
Scientific Inference. Statistical Method in — (Allan Birnbaum), 1033
Scientific Information Explosion: *correspondence* (F. Bruce Dandford), 979; (A. Johnson), 1173
Scientists: Appointment in College Administration, *news*, 998; Appointment Service Closure, *news* 213; Free Travel (O. Wichterle), *correspondence*, 773; Intelligence Quotient, *miscellaneous intelligence*, 784; Returning to Britain, *news*, 213; Scholarships, US, *news*, 998
Scottish Brain Drain, *news*, 116
Scowen Committee, *parliamentary news*, 680
Scrapie. Neurosecretion-like Material in the Hindbrain of Ageing Sheep and Sheep Affected with Natural — (A. Bignami, Elizabeth Beck and H. B. Barry), 194
Scrapie Agent. Evidence for the Multiplication of — in Cell Cutlture (M. C. Clarke and D. A. Haig), 100
Scurvy. Formation of Epithelial Basement Membrane is restricted by — *in vitro* and is stimulated by Vitamin C (Robert E. Priest), 744
Sea Bed Monopolies, *news*, 411
Sea Bird Deaths, *parliamentary news*, 784
Seafloor Spreading, *news*, 11, 223
Seamounts. Emperor Fracture Zone: a Newly Discovered Feature in the Central North Pacific (Barrett H. Erickson, Frederic P. Naugler and William H. Lucas), 53
Seat Belts and Safety, *news*, 228
Seawater. Occurrence of Indium in — and some Marine Sediments (A. D. Matthews and J. P. Riley), 1242
  Tin in Organisms and Water in the Gulf of Naples (J. David Smith), 103
Sediments. Occurrence of Indium in Seawater and Some Marine — (A. D. Matthews and J. P. Riley), 1242
Sedimentary Basins. Deep — proved in the Shetland-Hebridean Continental Shelf and Margin (M. H. P. Bott and A. B. Watts), 265
  New — on Rockall Plateau (D. G. Roberts, D. G. Bishop, A. S. Laughton, A. M. Ziolkowski, R. A. Scrutton and D. H. Matthews), 172
Sedimentology, Saharan, *news*, 792
Seed. Alkaloids of an *Ipomoea* — known as *Kaladana* in Pakistan (Charles I. Abou-Chaar), 663
Seismology. Seismicity of the Earth (C. F. Richter), 170
  Some Aspects of the Structure of Southern India based on Recent Bhadrachalam Earthquakes (S. K. Arora, T. G. Varghese and C. A. Krishnan), 261
Seismology Committee, NAS, *news*, 1098
Select Committee: Computer Policy, *news*, 497, 575, 1080; Computer Industry, *news*, 679; sees British Computer Society, *news*, 1088; sees Honeywell, *news*, 986; sees IBM, *news*, 889
Semenov Medal, *news*, 213
Seminiferous Tubules. Localization of Irradiation Damage in Rat — (Elizabeth R. Hall and E. W. Hupp), 85
'Sepharose'. Fractionation of Antibodies against Staphylococcal Nuclease on — Immuno-adsorbents (Gilbert C. Omenn, David A. Ontjes and Christian B. Anfisen), 189
Serine Proteases. Amino-acid Sequence of Porcine Pancreatic Elastase and its Homologies with other — (D. M. Shotton and B. S. Hartley), 802
Serine Proteases, *news*, 791
Serotonin. Direct Postsynaptic Responses to Stimulation of — containing Neurones (G. A. Cottrell), 1060
  Subcellular Localization of — in an Identified Serotonin-containing Neurone (G. A. Cottrell and N. N. Osborne), 470
Serpukhov, Antihelium at, *news*, 678
Serum Cholesterol. Pituitary Growth Hormone Essential for Regulation of — (Meyer Friedman, Sanford O. Byers and Stephen R. Elek), 464
SESER. Source of Electrons in a Selected Energy Range — (J. F. Andrews and R. H. Varey), 270
Severn Bridge Superstructure Award, *news*, 119
Sewage Disposal, *news*, 577
Sex. Chromosome Loss compared with Chromosome Size, Age and — of Subjects (P. Neurath, K. DeRemer, B. Bell, L. Jarvick and T. Kato), 281
Sex Ratio Inheritance. — in the Butterfly *Acraea encedon* (D. P. Owen), 662
Seyfert Galaxy. Yearly Variations of 3C 120 (P. D. Usher, B. S. P. Shen, and F. W. Wright), 365
Seyfert Galaxy, *news*, 410
Sigma Factor. Involvement of a Phage T4 σ Factor and Anti-terminator Protein in the Transcription of Early T4 Genes *in vivo* (D. A. Schmidt, A. J. Mazaitis, T. Kasai and E. K. F. Bautz), 1012
  Positive Control of Transcription by a Bacteriophage — (Andrew A. Travers), 1009
  RNA Polymerase — and the Selection of Initiation Site (M. Sugiura, T. Okamoto and M. Takanami), 598
Sigma Factor: *news*, 999; Function, *news*, 585
Silicon Carbide. Field Emission from — (Francis S. Baker), 539
Silicon Sheet. Silicon Tetrahedral Sheet in Stilpnomelane (R. A. Eggleton), 625
Simian Virus 40, *news*, 794
Simian Virus Reference Centre, *news*, 1103
Ship Excavated, *news*, 1090
Shock Deformation. Influence of — on the Transverse Magnetoresistivity of Polycrystalline Iron (A. Cristou), 372
SI Units (David Davies), *correspondence*, 980
Sickle Cells. Surface Ultramicroscopy of — (P. N. Farnsworth, M. R. Nadel and B. J. Stoll), 190
Silicate Cement. Formation of Dental — (A. D. Wilson, B. E. Kent, R. J. Mesley, R. P. Miller, D. Clinton and K. E. Fletcher), 272
Skin Reactive Factor. Release of — from Guinea-pig Lymphocytes by Mitogens (E. Pick, J. Krejci and J. L. Turk), 236
Skynet Satellite, *news*, 309
Sleep. Effect of Presenting Novel Verbal Material during Slow-wave — (D. J. Bruce, C. R. Evans, P. B. C. Fenwick and Valerie Spencer), 873
Smoke and London Taxis, *miscellaneous intelligence*, 217
Social Anthropology (John Buettner-Janusch), *correspondence*, 775
Social Sciences (John Buettner-Janusch), *correspondence*, 775
Society and Technology, Harvard Program, *news*, 219
Society of General Microbiology Symposium on Virology, *news*, 318
Sociological Association, Sociology of Science Study Group Conference, *news*, 212
Sociology of Science Study Group Conference, *news*, 212
Sodium. Demonstration of — and Potassium Conductance Changes during a Nerve Action Potential (E. Rojas, F. Bezanilla and R. E. Taylor), 747
Sodium Ion. Allosteric Interactions in $(Na^+ + K^+)$-ATPase (T. Tobin, S. P. Banerjee and A. K. Sen), 745
  Effects of General Anaesthetics on Na^+ Transport in Human Red Cells (M. J. Halsey, E. B. Smith and T. E. Wood), 1151
Sodium Pump. ATPase Activity of the — needs Phosphatidylserine (K. P. Wheeler and R. Whittam), 449
  Net Synthesis of ATP by Reversal of the — (V. L. Lew, I. M. Glynn and J. C. Ellory), 865
  Re-evaluation of the Synaptic Activation of an Electrogenic — (JacSue Kehoe and P. Ascher), 820
Soft Particle Fluxes. Satellite Observations of — in the Auroral Zone (W. J. Heikkila), 369
Soil. Lead Accumulation in Roadside — and Grass (Tsaihwa J. Chow), 295
  Meteoritic Spherules in the — surrounding Terrestrial Impact Craters (Paul W. Hodge and Frances W. Wright), 717
  Non-biological Reduction of Nitrate in — (L. A. Bulla, jun., C. M. Gilmour and W. B. Bollen), 664
Soil Bacteria. Bacterial Mechanism of Gley Formation in Artificially Submerged Soil (J. C. G. Ottow), 103
Soil Fungistasis. Volatile Factor in — (T. S. Hora and Ralph Baker), 1071
Soil Improvement by Neem Tree Planting, *news*, 131
Solar Flare. Penetration of Solar Particles to Ionospheric Heights at Low Lattitudes (S. Ganguly and Manoranjan Rao), 169
Solar Particles. Penetration of — to Ionospheric Heights at Low Latitudes (S. Ganguly and Manoranjan Rao), 169
Solar Physics, *news*, 401
Solar Wind. Anomalous Rhenium Isotopic Ratio in the —: Detection at the Nanogram Level (J. V. Morgan), 1037
  Topside Ionosphere of Venus and its Interaction with the — (S. J. Bauer, R. E. Hartle and J. R. Herman), 533
  Two New Plasma Instabilities in the — (Joseph V. Hollweg and H. J. Völk), 441
Solar Wind: *news*, 15; Instabilities, *news*, 1003
Solid State, Electroluminescent Devices, *news*, 1004
Solid State Chemistry. — and Colloid Chemistry (E. P. Honig), 537
Solid State Physics: Conference, *news*, 315; Light Carrying Electrons (Helmut Schwarz), *correspondence*, 1173
Somatic Cell Fusion, *news*, 588
Soviet: Academic Village, *news*, 782; and US Space Collaboration, *news*, 1094; Anti-Helium Nuclei, *news*, 678; Azerbaijan, Oldest Men, *miscellaneous intelligence*, 120; Chemistry Awards, *news*, 213; Communication Satellite, *news*, 1092; Oceanography 'Discovery', *news*, 118; Perfumery, *news*, 890; Polar Research, *news*, 119
Soviet-French Space Collaboration, *news*, 117
Space: Collaboration Chronology, US and Soviet Union, *news*, 1095; Plan, NASA, *news*, 995; Programme, Next Decade, *news*, 1085; Programme, UK, *parliamentary news*, 1093; Research, International Collaboration, *news*, 1094; Research, Planets, *news*, 996; Research Plans, NASA, *news*, 790; Research, UK, *news*, 987; Shuttles, *news*, 685; Strategy, US, *news*, 995
Spacecraft, Economics, *news*, 685
Spaghetti Consumption and Exercise, *miscellaneous intelligence*, 581
Spectral Lines Broadening, *news*, 590
Spectroscopy: *news*, 590; Symposium, *news*, 1102
Sperm. — Transfer by Spermatophores in *Glossinia austeni* Newstead (J. N. Pollock), 1063
Spermatophores. Sperm Transfer by — in *Glossinia austeni* Newstead (J. N. Pollock), 1063
Spermatozoa. Abnormal Development in Mice after Paternal Exposure to a Chemical Mutagen (Samuel S. Epstein, Sewa R. Joshi, Elsie Arnold, Elaine C. Page and Yvonne Bishop), 1260
Spermatozoon. Probable Haploid Expression of *HL-A* Antigens on Human — (M. Fellous and J. Dausset), 191
Spherical Harmonic Analysis, *news*, 501
Spinal Cord. Effects of Histamine on Toad — (A. K. Tebcis), 196
  Histochemical Differentiation of Motor Neurones and Interneurones in the Anterior Horn of the Cat — (J. Fernancez-Campa and W. King Engel), 748
Spleen. Growth of Haemopoietic — Colonies after Grafting of Human Bone Marrow in Mice (A. C. Louwagie and R. L. Verwilghen), 383
SRC: *parliamentary news*, 1184; and Engineering, *news*, 577; Budget, *news*, 218; Fellowships Altered, *news*, 781; Ion Implantation Grant, *news*, 889; Mathematics Committee Report, *news*, 989; Satellite Engineer Advertisement, *news*, 1089
Stamp Commemorating Royal Astronomical Society's 150 Anniversary, *news*, 686
Standards Bureau: Error, *news*, 684; Growth, Annual Report, *news*, 1187
Standing Royal Commission on Environmental Pollution, *miscellaneous intelligence*, 312

Staphylococcal Nuclease. Fractionation of Antibodies against — on 'Sepharose' Immunoadsorbents (Gilbert C. Omenn, David A. Ontjes and Christian B. Anfisen), 189
Staphylococcus aureus. Altered Cell Walls of — resistant to Methicillin (L. D. Sabath, C. D. Leaf, D. A. Gerstein and M. Finland), 1074
Stars, 73 Draconis. Heavy Elements in — (Mercedes Jaschek and Stella Malaroda), 245
Statistics. Statistical Method in Scientific Inference (Allan Birnbaum), 1033
Stegodonts. Pleistocene South-east Asiatic Pygmy — (D. A. Hoijer), 474
Sternglass; *news*, 397; and Thames TV (Ian Martin), *correspondence*, 882; (Philip L. Taylor), *correspondence*, 881; Assumptions (Patricia J. Lindop and J. Rotblat), *correspondence*, 979; Predictions, *news*, 779
Sterol. — Metabolism as a Basis for a Mutualistic Symbiosis (Loke Tuck Kok, Dale M. Norris and Hsien Ming Chu), 661
Stilpnomelane. Silicon Tetrahedral Sheet in — (R. A. Eggleton), 625
Stomata Ion Pump, *news*, 500
Stomias lampropeltis, *news*, 589
Stone Artefacts. — from Swartkrans (M. D. Leakey), 1222
Stonehenge. Sunrise and Moonrise at — (Jack H. Robinson), 1236
Stoneley Waves. — in Anisotropic Media (T. C. Lim and M. J. P. Musgrave), 372
Strategic Arms Limitation Talks, *news*, 211
Strawberries. Achene Spacing of — as an Aid to Calculating Potential Yield (A. J. Abbott and R. W. Webb), 663
Streptomycin Action Site, *news*, 902
Stress. Measurements of Electrical Potential for Detecting Crack Extension in COD Tests (J. Davies, D. F. Cannon and R. J. Allen), 1243
Strong Fibrous Solids, Discussion, *news*, 592
Strychnine. — Antagonism and Glycine: a Reply (S. Roper and J. Diamond), 1259
  —, Glycine and Vertebrate Postsynaptic Inhibition (D. R. Curtis and G. A. R. Johnston), 1258
Student Costs, *parliamentary news*, 581
Subacute Sclerosing Panencephalitis. Adaptation to Hamsters of Neurotropic Measles Virus from — (D. P. Byington, A. E. Castro and T. Burnstein), 554
Sublittoral Reef Phenomena. — of Aldabra (J. Barnes, E. Drew and J. Lythgoe), 268
Sucrose. Dietary — and Platelet Behaviour (Stephen Szanto and John Yudkin), 467
"Sulphation Factor". Production of — by the Perfused Liver (Paddy McConaghey and Clement B. Sledge), 1249
Sulphur Dioxide. Catalysis Oxidation of — on Carbon Films by Electrcial Resistance Measurements (I. S. McLintock and J. C. Orr), 629
  Chlorotic Dwarf of Eastern White Pine caused by an Ozone and — Reaction (L. S. Dochinger, F. W. Bender, F. L. Fox and W. W. Heck), 476
Sun. Abundance of Iron in the Solar Photosphere (John E. Ross), 610
  Anomalous Rhenium Isotopic Ratio in the Solar Wind: Detection at the Nanogra Level (J. V. Morgan), 1037
  High Resolution Observations of Solar Microwave Bursts (Haruo Tanaka and Shinzo Énomé), 435
  Identification of the ν_3 NO_2 Band in the Solar Spectrum observed from a Balloon Borne Spectrometer (A. Goldman, D. G. Murcray, F. H. Murcray, W. J. Williams and F. S. Bonomo), 443
  Two New Plasma Instabilities in the Solar Wind (Joseph V. Hollweg and H. J. Völk), 441
Sun: Ground-based Observation, *news*, 431; Solar Wind Instabilities, *news*, 1003
Sun Eclipse: Observations, *news*, 997; Path, *news*, 584
Sunrise. — and Moonrise at Stonehenge (Jack H. Robinson), 1236
Superconductivity in Thin Films, *news*, 792
Supernova Bands. An Explanation for the Broad — (Donald R. Huffman), 833
Supernovae. Ejection of Companion Objects by — (Stirling A. Colgate), 247
  On the Connexion between Pulsars and — (A. J. R. Prentice), 437
Supersonic Aircraft not to view Eclipse, *news*, 886
Suppressor. Restoration of In-phase Translation by an Unlinked — of a Frameshift Mutation in *Salmonella typhimurium* (Joseph Yourno and Shigegi Tanemura), 422
Surveyor Examined, *news*, 782
Sussex Science Policy Research Unit, *news*, 487
Swann Report Criticized, *news*, 311
Swanscombe Village Early Man, *news*, 589
Swartkrans. More Evidence of an Advanced Hominid at — (R.J. Clarke, F. Clark Howell and C. K. Brain), 1219
  New Finds at the — Australopithecine Site (C. K. Brain), 1112
  Stone Artefacts from — (M. D. Leakey), 1222
  Two New Hominid Vertebrae from — (J. T. Robinson), 1217
Swartkrans Site: Importance, *news*, 1188; New Finds, *news*, 1217
Swiss Research Institute for Avalanches, *news*, 678
Symbiosis. Sterol Metabolism as a Basis for a Mutualistic — (Loke Tuck Kok, Dale M. Norris and Hsien Ming Chu), 661
Synapse. Strychnine, Glycine and Vertebrate Postsynaptic Inhibition (D. R. Curtis and G. A. R. Johnston), 1258
Synapse Function. Chemospecific Repression as a Possible Memory Store (R. F. Mark), 178
Synchrotron Emission. Coherent — in the Crab Nebula (Lawrence J. Caroff and Jeffrey D. Scargle), 169
Synchrotron Radiation. Low Mode Coherent — and Pulsar Phenomena (Bernard J. Eastlund), 430
Synovia. Histochemistry of some "Proteolytic" Enzymes of Human Rheumatoid — (J. Chayen, Lucille Bitensky and L. W. Poulter), 1050
Synovial Fluid. Features of the — Film in Human Joint Lubrication (P. S. Walker, J. Sikorski, D. Dowson, M. D. Longfield and V. Wright), 956
Systemic Lupus Erythematosus. Lymphocyte Cytotoxic Antibodies in — (Kamal L. Mittal, Roger D. Rosen, John T. Sharp, Martin D. Lidsky and William T. Butler), 1255

T

Tago-Sato-Kosaka, *news*, 413
Taste. Similar Polymorphism of — Sensitivity to PTC in Mice and Men (T. W. Klein and J. C. DeFries), 555
Taxis and Smoke, *miscellaneous intelligence*, 217
Teacher's Pay, *news*, 113
Teacher Training: *news*, 399; *parliamentary news*, 784; Grants, *parliamentary news*, 495
Teaching Profession Structure, *news*, 113
Technological Innovation, Case Histories of Innovations (R. W. Cahn), 693
Technology and Society, Harvard Program on, *news*, 210
Technology Decision Making, *news*, 890
Tectonics of the Indian Ocean, *news*, 11
Teeth. Formation of Dental Silicate Cement (A. D. Wilson, B. E. Kent, R. J. Mesley, R. Miller D. Clinton and K. E. Fletcher), 272
  New Approach to Zahnreihen (J. W. Osborn), 343
  Rates of Evolutionary Change in the Hominid Canine — (W. G. Kinzey), 296
Teichoic Acids. — and Membrane Function in Bacteria (S. Hepinstall, A. R. Archibald and J. Baddiley), 519
Tektites, *news*, 17
Telecommunications Satellites Organization Conference, *news*, 1187
Telephone Story, *miscellaneous intelligence*, 894
Television Broadcasting, *news*, 489
Temperature. Characterization of the Germination Response to — of Species and Ecotypes (P. A. Thompson), 827
Temperature Effects. Exchange of bound Tritium between DNA and Water at Different Temperatures (Robert E. Bird, Karl G. Lark, Basil Curnutte and John E. Maxfield), 1043
  Osmoregulation by the Killifrish, *Fundulus heteroclitus*, in Fresh Water at Temperatures near Freezing (Bruce L. Umminger), 294
  Temperature Sensitive Alteration of 30S Subunits demonstrated by *in vitro* Reassociation of Functional Ribosomes (Soo-Sang Kang), 1132
Teratogens. Teratogenic Effect of Lithium Carbonate in the Foetal Mouse (K. T. Szabo), 73
Test Tube Baby, *news*, 886
$\Delta^{1(6)}$-Tetrahydrocannabinol. Metabolism of —, an Active Marihuana Constituent (Sumner H. Burstein, Franco Menezes, Ethel Williamson and Raphael Mechoulam), 88
Textiles. New Flame Retardant Finish for Polypropylene — (A. Alder and W. Brenner), 60
Textiles' Merger, *news*, 114
Thalamus. Population Analysis of Somatosensory — in the Cat (Fredric A. Harris), 559
Thames Barrier, *news*, 403
Thames TV and Sternglass; *news*, 779; (Ian Martin), *correspondence*, 882
Theories. Generation of Hypothesis and — (Bernard Meltzer), 972
Thermal Protons. Origin and Dynamical Behaviour of — in the Venusian Ionosphere (P. M. Banks and W. I. Axford), 924
Thermal Shock and Fracture Mechanics, *news*, 688
Thermodynamics. Entropy of Microbial Growth (W. W. Forest), 1165
  — makes Progress (P. T. Landsberg), 1204
Thermodynamics: of Enzyme Reactions, *news*, 690; Misunderstanding (J. W. Warren), *correspondence*, 1082
Thermoelasticity. — of Elastin (D. Volpin and A. Cifferi), 382
Thermogravimetry. Derivation of Rate Equations used in — (J. R. MacCallum and J. Tanner), 1127
Thermolability. Effect of Phosphate and Non-phosphate Buffers on — of Unstable Haemoglobins (Lawrence J. Schneiderman, Irene G. Junga and Dorothea F. Fawley), 1041
Thermoluminescence. Molecular Oxygen as an Electron Trap in the — of Saturated Hydrocarbons (I. Boustead), 846
Thermonuclear Research, AEC, *news*, 684
Thermoplastic Elastomers. Macro-lattice from Segregated Amorphous Phases of a Three Block Copolymer (A. Keller, E. Pedemonte and F. N. Willmouth), 538
Thin Films Superconductivity, *news*, 792
^{14}C-Thiosemicarbazide. Distribution of — in the Rat Brain: an Attempt to localize Sites of γ-Aminobutyric Acid Production (Bertalan Csillik and Elizabeth Knyihar), 562
Thiostrepton. The Structure of — (Bryan Anderson Dorothy Crowfoot Hodgkin and M. A. Viswamitra), 233
Third London Airport, *news*, 436; 1180; (W. R. P. Bourne), *correspondence*, 773
Thymectomy. Cellular Deficit in Thymectomized Mice (D. E. Mosier, F. W. Fitch, D. A. Rowley and A. J. S. Davies), 276
  In vitro Reconstitution of the Immune Response to Thymus-deprived Mice to Sheep Red Blood Cells (Alan Munro and Philippa Hunter), 277
Thymidine. Electron Reactions in γ-Irradiated — and Cystamine (A. D. Lenherr and M. G. Ormerod), 545
^{3}H-Thymidine. Inhibition of — Incorporation into Rat Liver Nuclei by *E. coli* L-Asparaginase (Siegfried Seeber and Ulrich Weser), 652
Thymine. Complete Mutagenesis in a Bacteria Population induced by — Starvation on Solid Media (S. Bresler, M. Mosevitsky and L. Vyacheslavov), 764
Tin. — in Organisms and Water in the Gulf of Naples (J. David Smith), 103
Tissue Culture. Morphogenetic Substratum for Differentiation of Cartilage in Tissue Culture. (Marshall R. Urist and Hiroshi Nogani), 1051
Toad Species, *news*, 794
Tobacco Wilt Disease. Host Response of Tobacco to *Verticillium dahliae* (tob.) Kleb.—a Cytological Method of assaying Resistance (H. K. Mahanty), 755
Tobacco Mosaic Virus. Antigenic Properties of a Homogeneous — Hapten Conjugate (L. I. Slobin), 698
Tobacco Mosaic Virus Subunits Explored, *news*, 130
Tokyo Dogs, *miscellaneous intelligence*, 784
Top Bloody Secret (Stanley Hyland), *miscellaneous intelligence*, 217
Toxicity. Acute — of Aflatoxin B_1 in Wethers (Bernard H. Armbrecht, William T. Shalkop, Larry D. Rollins, Albert E. Pohland and Leonard Stoloff), 1062
Toxins. Extract of *Ipomoea pes-caprae* (Convolvulaceae) antagonistic to Histamine and Jelly-fish Poison (S. Wasuwat), 758
Trachoma. Anti- — Activity of Rifampicin and Rifamycin SV Derivatives (Yechiel Becker, Yael Asher, Nehama Hummel and Zichriya Zakay-Rones), 454
Tracked Hovercraft Limited, *news*, 214
Traffic in Peak District, *news*, 578
Train. British Rail Advanced Passenger —. (S. Jones), 417
Training. Actinomycin-*D*: Effects on Memory at Different Tines after — (Larry R. Squires and Samuel H. Barondes), 649
Transcription. Involvement of a Phage T4 σ Factor and Anti-terminator Protein in the — of Early T4 Genes *in vivo* (D. A. Schmidt, A. J. Maziatis, T. Kasai and E. K. F. Bautz), 1012
  Positive Control of — by a Bacteriophage Sigma Factor (Andrew A. Travers), 1009
Transesterification. — by Peptidyl Transferase (E. Scolnick, G. Milman, M. Rosman and T. Caskey), 152
Transfer Cells, *news*, 692
Transformation. — and Virus Growth by Murine Sarcoma Viruses in Human Cells (Stuart A. Aaronson and George J. Todaro), 458
  — of Hamster Cell Lines *in vitro* by a Hamster Sarcoma Virus (Jan Zavada and Ian Macpherson), 24
  — of Hamster Macrophages into Giant Cells with Antimacrophage Serum (W. Ptak, Z. Porwit-Bòbr and Z. Chlap), 655
Transformation and Transcription, *news*, 794
Transformed Cells. Control of the Reversion of the Properties in — (Zelig Rabinowitz and Leo Sachs), 136
Transient Lunar Phenomena. — and Electrostatic Glow Discharges (A. A. Mills), 929
Transition. — Probabilities for a Forest (Paul E. Waggoner and George R. Stephens), 1160
Transition Metal Band Structure, *news*, 900
Translation. Cover-to-cover — (Ole V. Groos), *correspondence*, 482
Transparency. Self-induced — and Dispersion Delays in Potassium Vapour (D. J. Bradley, G. M. Gale and P. D. Smith), 719
Transplantation. Acute Childhood Leukaemia in Relation to the HL-A Human — Genes (Roy L. Walford, Simon Finkelstein, Robert Neerhout, Pat Konrad and Edward Shanbrom), 461
  Adding a Ventricle Instead of Transplanting a Heart (Kenneth Bloor), 1142
  Effect of Route of Administration of Antilymphocytic Globulin on Skin Allograft Survival in Rats (R. G. Dalton, J. L. Touraine, N. F. Anderson and M. F. A. Woodruff), 1140
  Production of Chick Chimaeras (G. Marzullo), 72
Transplantation Antigens. — in the Central Nervous System (A. G. Gervais), 647
Transplantation Immunity. Comparison of the Fates of Ag-B Locus Compatible Homografts of Skin and Hearts in Inbred Rats (Clyde F. Barker and R. E. Billingham), 851

INDEX

Trees to Improve Soil, *news*, 131
Trias. Vertebrate Fauna from the Lower — of Australia (Alan Bartholomai and Anne Howie), 1063
Tribology Profits Symposium, *news*, 1194
Tritium. Exchange of Bound — between DNA and Water at Different Temperatures (Robert E. Bird, Karl G. Lark, Basil Curnutte and John E. Maxfield), 1043
Tropopause. Vertical Distribution of CO near the — (Eigil Hesstvedt), 50
Trypanosoma cruzi. Delayed Hypersensitivity in — Infection (Stanley Seah), 1256
Tumours. Cytotoxic Efficiency and Effect on Tumour Growth of Heterospecific Antilymphocytic and Antitumour Sera (Michael Woodruff and Lindsay Hamilton Smith), 377
  Effect of Antilymphocytic Serum on the Appearance of Reticular Neoplasms in SJL/L Mice (N. A. Burstein and A. C. Allison), 1139
  Evidence against the Presence of H2 Histocompatibility Antigens in Ehrlich Ascites — Cells (L. Chen and J. F. Watkins), 734
Tumours, Cyclamates and Saccharin, *miscellaneous intelligence*, 1184
Tumour Immunology. Cytotoxic Efficiency and Effect on Tumour Growth of Heterospecific Antilymphocytic and Antitumour Sera (Michael Woodruff and Lindsay Hamilton Smith), 377
Tumour for Virology, *news*, 794
Tumour Virus. Effect of a DNA — on Antibody Forming Cells in Neonatally Infected Hamsters (Herman Friedman and Herman Goldner), 455
Tumour Viruses, *news*, 690
Tungsten-181. — in Fallout in Finland (L. Blomqvist and M. Asikainen), 58
Tungsten Lamps, Luminous Gas Flux Error, *news*, 684
Tyrosine Aminotransferase. Induction of Hepatic — mediated by a Cholinergic Agent (Ira B. Black), 648

U

UGC: Annual Report, *news*, 409; Annual Survey, *news*, 398
UK Drugs Legislation, *news*, 485
UKAEA, Evidence as Computer user, *news*, 679
Ultramicroanalysis. Absolute, Unambiguous — of Metabolites present in Complex Biological Extracts (J. R. Majer and Alan A. Boulton), 658
Ultramicroscopy. Surface — of Sickle Cells (P. N. Farnsworth, M. P. Nadel and B. J. Stoll), 190
Ultraviolet. Dye Lasers in the — (James A. Myer Irving Itzkan and E. Lierstead), 544
Umbellulidae. — Distribution extended in the Atlantic (W. Jahn), 1068
Unidentified Airborne Organisms, *news*, 126
United States Weather Bureau Centenary, *news*, 683
Universal Deceleration Parameter, *news*, 13
Universe Theories. Radiation Catastrophe (E. R. Harrison), 245
University: Expansion, *news*, 407; Files, *parliamentary news*, 1093; Finance, *news*, 398; Independent, Costs, *news*, 988; Lists Comparison, *miscellaneous intelligence*, 312; of London Enquiry, *news*, 491; Physics Teaching, *news*, 988; Places, *parliamentary news*, 581; Reform (R. S. Smith), *correspondence*, 206; Research Grants, *news*, 492; Research, NERC support for (R. J. H. Beverton), *correspondence*, 481; University Veterinary Science Conference, *news*, 988
University Performance. A-Levels and — (G. D. Bagg), 1105
Uracil. Effect of an Imprinting Procedure on Regional Incorporation of Tritiated — into Chick Brain RNA (S. P. R. Rose, P. P. G. Bateson, Ann L. D. Horn and G. Horn), 650
Uranium: Enriched, *news*, 490; Enrichment Agreement, *parliamentary news*, 991; Supplies, *parliamentary news*, 1184
US: and Soviet Space Collaboration, *news*, 1094; Antarctic Research, *news*, 121; Budget, *news*, 582; Budget Forecast, *news*, 7; Environmental Pollution, *news*, 122; Physicists Productivity, *news*, 218; Research Budget and Mansfield, *news*, 1185; Research Finance, *news*, 1177
Uterus. Cyclic 3′,5′-AMP and Uterine Contractility (Lubos Triner, N. I. A. Overweg and Gabriel G. Nahas), 282

V

Vaccines, Polysaccharide, *news*, 1193
Vagina. Primate Sex Pheromones of Vaginal Origin (Richard P. Michael and E. B. Keverne), 84
Valonia ventricosa. Proline in the Vesicles and Sporelings of — and the Concept of Cell Wall Protein (F. C. Steward, R. L. Mott, H. W. Israel and P. M. Ludford), 760
Vapour Pressure. — of $H_2^{18}O$ Ice (I) (-17° C to 0° C) and $H_2^{18}O$ Water (0° C to 16° C), (Gabor Jancso, Jovan Pupezin and W. Alexander Van Hook), 723
Vascular Permeability. Correlation of Bradykinin Structure with Ability to increase — (G. J. H. Melrose, H. K. Muller and W. J. Vagg), 547
Vasoactive Peptides. Effects on Smooth Muscle Preparations of Unidentified — from Intestine and Lung (Priscilla J. Piper, S. I. Said and J. R. Vane), 1144
Vasodilator Peptide. Potent Peripheral and Splanchnic — from Normal Gut (Sami I. Said and Viktor Mutt), 863
Vasopressin. Gonadotrophin Inhibition by the Synergistic Action of — and Oxytocin (L. J. Hipkin), 740
Vector Dominance Interaction Model, *news*, 128
Venereal Disease and the Pill, *miscellaneous intelligence*, 784
Venom. Effects of Black Widow Spider — on the Frog Neuromuscular Junction (Herbert E. Longenecker, jun., William P. Hurlbut, Alexander Mauro and Allen W. Clarke), 701
Ventricle. Adding a — Instead of Transplanting a Heart (Kenneth Bloor), 1142
Venus. Origin and Dynamical Behaviour of Thermal Protons in the — Ionosphere (P. M. Banks and W. I. Axford), 924
  Topside Ionosphere of — and its Interaction with the Solar Wind (S. J. Bauer, R. E. Hartle and J. R. Herman), 533
Venus' Atmosphere, *news*, 689
Vertebrate. Flying Speed of the Largest Ariel — (Cherrie D. Bramwell and G. R. Whitfield), 660
Vertebrate Fauna. — from the Lower Trias of Australia (Alan Bartholomai and Anne Howie), 1063
Veterinary Science Conference, *news*, 988
Vertical Take-off and Landing Aircraft, *news*, 577
Vibrational Energy. Observations of Vibration—Vibration Energy Pumping between Diatomic Molecules (J. D. Teare, R. L. Taylor and C. W. von Rosenberg, jun.), 241
Viking Project: *news*, 995; Experiments, *news*, 124
Viral Subunits Expolired, *news*, 130
Virology: Symposium, *news*, 1103; Tumour, *news*, 690
Virus. Introduction of a New Virus Disease of the Coconut Rhinocerus Beetle in Western Samoa (K. J. Marschall), 288
  Three Types of — Particle in *Penicillium stoloniferum* (K. W. Buck and Gillian F. Kempson-Jones), 945
  Transformation and — Growth by Murine Sarcoma in Human Cells (Stuart A. Aaronson and George J. Todaro), 450
Virus: Group Symposium of the Society of General Microbiology, *news*, 318; Infection and Poly-I: C. *news*, 1103; Reference Centre, *news*, 1103
Virus Component. Is there a — in the Genetic Background? (Hans Grüneberg), 39
Virus Isolation. Isolation of a Virus infecting a Strain of *Mycoplasma laidlawii* (R. N. Gourlay), 1163
Viruses and Cancer, *news*, 226
Viruses and Cell Fusion, *news*, 588
Viscoelasticity. Pulsatile Flow Instrument for detecting — in Dilute Polymer Solutions (G. E. Gadd), 724
Vision. Cells sensitive to Binocular Depth in Area 18 of the Macaque Monkey Cortex (D. H. Hubel and T. N. Wiesel), 41
  Electrophysiological Correlate of Binocular Depth Perception in Man (D. Regan and H. Spekreijse) 92
  Elevation of — Threshold by Displacement of Retinal Image (D. M. McKay), 90
  Eye Dominance in the Visual Cortex (Colin Blakemore and John D. Pettigrew), 426
  Inability to perceive the Direction of Rotation Movement of Line Segments (Bela Julesz and Rosanne I. Hesse), 243
  Interocular Transfer of Suppressive Effects of Retinal Image Displacement (D. M. McKay), 872
  Lack of Eye to Eye Transfer of an Early Response Modification in Birds (H. Zeier), 708
  Nervous Activity and RNA Metabolism in the Visual Cortex of Rat Brain (A. J. Dewar and H. W. Reading), 869
  Spread of Responses in the Cerebral Cortex to Meaningful Stimuli (B. Delisle Burns and A. C. Webb), 469
  Stereoscopic — in the Macaque Monkey: a Behavioural Demonstration (Edward W. Bough), 42
Vision. Stereoscopic, in Macaque Monkey, *news*, 41
Visual Cortex. Eye Dominance in the — (Colin Blakemore and John D. Pettigrew), 426
Visual Feedback Control. Movement Control in Repetitive Motor Task (W. D. A. Beggs and C. I. Howarth), 742
Visual Pigment$_{500}$. Absorptivity and Quantum Yield of Bleaching in Bovine — (Joram Heller), 636
  Linkage of Retinal and Opsin and Absence of Phospholipids in Purified Frog — (Michael O. Hall and A. D. E. Bacharach), 637
Vitamin B_{12}. Biological Properties of Model Molecules for — (N. Satgni, B. De Bernard, G. Costa and G. Mestroni), 942
Vitamin C. Formation of Epithelial Basement Membrane is restricted by Scurvy *in vitro* and is Stimulated by — (Robert E. Priest), 744
VLF. — Wave Generation by the Čerenkov Process in the Inner Magnetosphere (R. P. Singh and R. N. Singh), 49
Voice Recognition. Acoustic Basis for Individual Recognition by Voice in the Gannet (Sheila J. White, R. C. E. White and W. H. Thorpe), 1156
VTOL Aircraft: *news*, 577; *parliamentary news*, 894

W

Washington Communications Satellites Conference, *news*, 789
Water. Exchange of Bound Tritium between DNA and — at Different Temperatures (Robert E. Bird, Karl G. Lark, Basil Curnutte and John E. Maxfield), 1043
  Vapour Pressure of $H_2^{18}O$ Ice (I) (—17° C to 0° C) and $H_2^{18}O$ — (0° C to 16° C) (Gabor Jancso, Jovan Pupezin and W. Alexander Van Hook), 723
Water Supplies Pollution, *news*, 1180
Water Uptake. — from Subsaturated Atmosphere: its Site in Insects (J. Noble-Nesbitt), 754
Watson at Cold Spring Harbour, *news*, 896
Watson-Crick Theory Criticism. The Chemical Basis of Inheritance (P. Fleischman), 30
Wattle Research, *miscellaneous intelligence*, 581
Wave Propagation. Concentration — in Two-dimensional Liquid Phase Self-oscillating System (A. N. Zaikin and A. M. Zhabotinsky), 535
Weather Bureau: *news*, 897; Centenary, *news*, 683
Wheeler-Feynman Electrodynamics. Quantization of — (F. Hoyle and J. V. Narliker), 1233
Whipsnade Rhinoceroses, *news*, 1181
WHO: CBW Report, *news*, 400; Heart Research, *news*, 217
Why Britain Needs a Population Policy. The Conservation Society, *news*, 213
Wigs from Hong Kong, *miscellaneous intelligence*, 217
Wildlife. Probable Occurrence of Infectious Bovine Rhinotracheitis Virus in Tanzania in — and Cattle (M. M. Rweyemamu), 738
Wilt Disease. Host Response of Tobacco to *Verticillium dahliae* (tob.) Kleb.—a Cytological Method of assaying Resistance (H. K. Mahanty), 755
Wind Dispersal. Windbourne Dispersal of Foot and Mouth Virus (K. P. Norris and G. J. Harper), 68
Wolfson Foundation Grants, *news*, 492
Wood Decay. Kinetics of Growth of Filamentous Fungi with Particular Reference to — (T. Gillespie and M. A. Hulme), 102
Wool. Radiation Grafting of Polysulphides to — (C. Samways and G. W. Hastings), 634
World List of Universities, *miscellaneous intelligence*, 312
World of Learning, *miscellaneous intelligence*, 312
World Wildlife Fund. Anonymous Donation, *news*, 783; Endowment (Peter Scott), *correspondence*, 1174; Protest, *news*, 215
Worms, Ice, *news*, 587

X

Xenon Hexafluoride, *news*, 316
X-rays. Effect of — on the Electrical Conductance of Phospholipid Bilayer Membranes: a Retraction (A. M. Sutton and D. Rosen), 274
  Scattering of — from Pulsar NP 0532 by Interstellar Grains (S. Naranan and G. A. Shah), 834
X-ray Astronomy. Intensity of the Soft X-ray Background Flux: Reply to Bowyer and Field (R. C. Henry, G. Fritz, J. F. Meekins, H. Friedman and E. T. Byram), *reply* by C. Stuart Bowyer and George B. Field, 362
X-ray Astronomy, *news*, 14, 223, 1002
X-ray Background. Pulsars and the Universal — (M. V. Krishna Apparao), 836
X-ray Background Flux. Intensity of the Soft —: Reply to Bowyer and Field with comment by the same (R. C. Henry, G. Fritz, J. F. Meekins, H. Friedman and E. T. Byram), 362
X-ray Crystallography. High Resolution X-ray Diffraction by Single Crystals of Mixtures of tRNAs (Richard D. Blake, Jacques R. Fresco and Robert Langridge), 32
X-ray Scattering. — by Grains in the Direction of the Crab Pulsar and Sco XR-1 (C. S. Bowyer, J. Mack and M. Lampton), 1125
X-ray Stars. — and Infrared Excess (Robert F. O'Connell and Satya Dev Verma), *correspondence*, 671
X-ray Stars, Scorpius X-1, *news*, 223

Y

Yaks or Naks, *miscellaneous intelligence*, 991
Your Environment, *news*, 5

Z

Zahnreihen. New Approach to — (J. W. Osborn), 343
Zodiacal Light. Possibility of a Terrestrial Component in the Doppler Shifted — (V. Vanysek and M. Harwit), 1231
Zinc Oxide. Decomposition of Isopropyl Alcohol photosensitized by — (Heinrich Dieter Müller and Friedrich Steinbach), 728
Zygote. Abnormal — Development in Mice after Paternal Exposure to a Chemical Mutagen (Samuel S. Epstein, Sewa R. Joshi, Elsie Arnold, Elaine C. Page and Yvonne Bishop), 1260

ERRATA INDEX

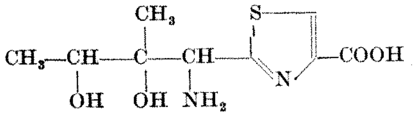
Erratum. In the article "Nucleotide Sequences Present within the 16S Ribosomal RNA of *Escherichia coli*" by P. Fellner, C. Ehresmann and J. P. Ebel (*Nature*, **225**, 26; 1970), the figure 69 on line 5, page 28, should read 59.

Erratum. In the paper "Genetics of NADP Isocitrate Dehydrogenase in *Paramecium aurelia*" (*Nature*, **225**, 181; 1970), line 16 on page 182 should read "heterozygote pattern (IDH_m 1–2) is consistent with".

Erratum. In the article "Stimulation by Cyclic AMP of Intrinsic Protein Kinase Activity in Ox Brain Membrane Preparations", by M. Weller and R. Rodnight (*Nature*, **225**, 187; 1970), the ordinate of Fig. 1 should read: nmoles [^{32}P]-phosphorylserine/mg protein.

Erratum. The author of the article "Effects of Histamine on the Toad Spinal Cord" (*Nature*, **225**, 196; 1970) is A. K. Tebēcis.

Erratum. The following corrections should be made to the article "The Structure of Thiostrepton" (*Nature*, **225**, 233; 1970): In Table 1, page 234, all minus (−) signs should be replaced by the letter L and plus (+) signs by the letter D; similar corrections should be made in Fig. 2, page 234, and on line 33 of page 235. In Table 1, the chemical formula of thiostreptine is



In ref. 7, the name of the first author is Cross, D. F. W.

Erratum. In the article "The Moon at Houston" (*Nature*, **225**, 321; 1970), the second sentence under the sub-heading Chemical Composition on page 326 should read: "Of the ninety-two elements in the periodic table, results are now available for seventy-nine".

Erratum. In the article "Activity of Various Fractions of Bradykinin Potentiating Factor against Angiotensin I Converting Enzyme" by S. H. Ferreira, L. J. Green, V. A. Alabaster, Y. S. Bakhle and J. R. Vane (*Nature*, **225**, 379; 1970), the abbreviation pGa on line 12 should read PCA (pyrrolidone carboxylic acid).

Erratum. In the article "Growing Pains in British Universities" (*Nature*, **225**, 407; 1970), the second and third sentences in the third paragraph should read: "Fig. 1 shows that between 1959 and 1965, expenditure increased at an average annual rate of about 18 per cent, while student numbers rose by about 7 per cent a year. But after 1965, when Colleges of Advanced Technology received university status, the increase in expenditure seems to have been reduced to about 9 per cent a year, while student numbers continued to rise at an average yearly rate of about 8 per cent".

Corrigendum. At a meeting of the Parkinson's Disease Society on February 11 the impression arose that patients involved in trials of L-dopa would have to stop taking the drug when trials are completed. (*Nature*, **225**, 675; 1970.) Professor J. N. Walton, chairman of the MRC working party on L-dopa, has pointed out that this is not correct, and that patients who benefit from L-dopa have been guaranteed a continuing supply after completion of trials.

Erratum. The title of the article by Jay A. Levy and Robert J. Huebner (*Nature*, **225**, 949; 1960) should read "Association of a Murine Leukaemia Virus from a Mouse Lymphoma (2731/L) associated with Reovirus Type 3 Infection".

Erratum. In the title of the article by J. Ken McDonald, Benjamin B. Zeitman and Stanley Ellis (*Nature*, **225**, 1048; 1970), the word "appropriate" should read "inappropriate".

Erratum. In the review of "A Short History of Botany in the United States" (*Nature*, **225**, 1078; 1970), the second and third sentences of the first paragraph should read: "The eleventh International Botanical Congress was held in Seattle in August 1968. This volume outlines the growth of knowledge . . ."

Erratum. In the article "New Finds at the Swartkrans Australopithecine Site" by C. K. Brain (*Nature*, **225**, 1112; 1970), a penultimate sentence should be added to the paragraph beginning "In 1958 . . . " on page 1113: "A concentration of coarse material is visible in the outer cave filling, against the N. wall, just east of the face worked by Broom and Robinson".

Erratum. In the article "The Man who Lived with Australopithecines" (*Nature*, **225**, 1188; 1970), it was implied that the stone industry recovered from the Swartkrans cave site was contemporaneous with the Oldowan culture from Bed I at Olduvai, thus making it about 1·75 million years old. The comparison should, however, have been with the much younger culture from Bed II at Olduvai.

Erratum. In the article "Bremsstrahlung Radiation in an Intense Magnetic Field and Emission from Pulsars" by Hong-Yee Chiu and Vittorio Canuto (*Nature*, **225**, 1230; 1970), equation 5 should read:

$$\alpha_\omega = \frac{8\pi^3 c^2}{n_r^2 \omega^2}\, 2m \int_0^\infty \left[f(p_z')\, \frac{\partial}{\partial p_z'} \left(\frac{Q_\omega(p_z')}{p_z' + p_z}\right) + f(-p_z')\, \frac{\partial}{\partial p_z'} \left(\frac{Q_\omega(p_z')}{p_z' - p_z}\right)\right] \mathrm{d}p_z' \qquad (5)$$

Erratum. The book *Matrix Algebra for Business and Economics* was incorrectly priced at 40*s* (*Nature*, **225**, xxxii; 1970). It costs 140*s*.

Printed in Great Britain by FISHER, KNIGHT & CO. LTD., St. Albans, and published by
MACMILLAN (JOURNALS), LTD. at 4 Little Essex Street, London, W.C.2.
Saturday, July 18, 1970.

NATURE

MACMILLAN (JOURNALS) LTD

No. 5231 SATURDAY JANUARY 31 1970 Vol. 225

CONTENTS

THE PROPHETS OF POLLUTION—ACADEMICS IN LIMBO—100 Years Ago—Research Associations—Teacher Training—Dragon—CB Weapons—Solar Physics—Geology—Linnean Society—Carnegie Observatories—London—Archaeology—Industrial Research—Parliament in Britain—Miscellaneous Intelligence . . 397

UNIVERSITY RUBS SHOULDERS WITH INDUSTRY 406

GROWING PAINS IN BRITISH UNIVERSITIES 407

SIGNS OF CHANGE AT UGC 409

News and Views—UP OR DOWN ?—Seyfert Galaxies—Structure—Magnetic Monopoles—Moon—Sea Floor Spreading—Comets—Cell Division—Nucleic Acids—Transfer RNA—Root Nodules—Gynaecology—Ichthyology 410

BRITISH RAIL ADVANCED PASSENGER TRAIN by S. Jones 417

DIFFUSION IN EMBRYOGENESIS by Francis Crick 420

RESTORATION OF IN-PHASE TRANSLATION BY AN UNLINKED SUPPRESSOR OF A FRAMESHIFT MUTATION IN *Salmonella typhimurium* by Joseph Yourno and Shigegi Tanemura 422

EYE DOMINANCE IN THE VISUAL CORTEX by Colin Blakemore and John D. Pettigrew 426

LOW MODE COHERENT SYNCHROTRON RADIATION AND PULSAR PHENOMENA by Bernard J. Eastlund 430

Letters to the Editor (see overleaf) 435

Book Reviews by Frank Greenaway, R. A. Crowson, A. D. Buckingham, C. Bodsworth, George Rowlands, Daphne F. Jackson, F. H. Ludlam, J. A. Joule, E. H. R. Ford, D. R. Newth, Daphne J. Osborne and J. D. Carthy 477

Correspondence—NERC Support for University Research by R. J. H. Beverton; Mild Thaw on Disarmament by Elizabeth Young; Cover-to-cover Translation by Ole V. Groos; Numerical Notation: Decimalization by F. W. Derwent . . 481

University News—Appointments—Announcements—Sabbatical Itinerants . . 483

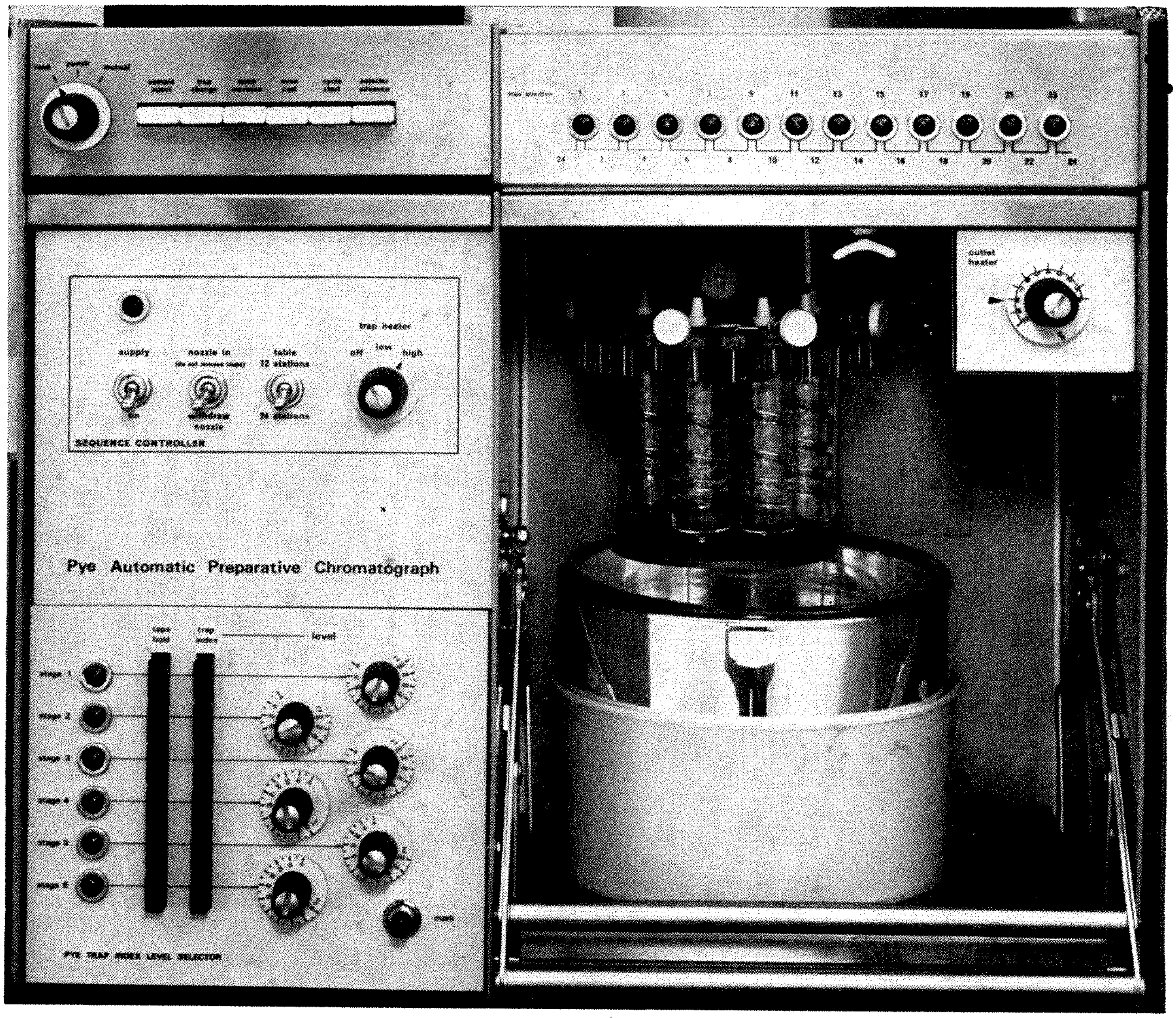
RECENT SCIENTIFIC AND TECHNICAL BOOKS xix

Nature in North America : From the beginning of next week, *Nature* will maintain an office in Washington DC. Contributors in the United States and Canada are invited to submit manuscripts for publication either to the address given below or to the present London office. The intention is that each office will be able to answer inquiries about all manuscripts and other matters, although a strictly symmetrical situation will not obtain until mid-February, from which point, for half a year, the editor of *Nature* will be based in Washington. This is one of several steps by which it is hoped to provide a better service for readers and contributors.

London Office
4 Little Essex Street,
London WC2.
01-826-6633

Washington Office
711 National Press Building,
Washington DC 20004

(Continued overleaf)



An Index of Quality

The Pye Trap Index Level Selector is a brilliant new addition to the accessories available with the Series 105 Automatic Preparative Chromatograph. In conjunction with the instrument's punch tape control system, this highly sophisticated control unit, with an electric comparator circuit, achieves an exceptional degree of accuracy in component and fraction collection – even at the end of a separation lasting several hours. Trap rotation and sample collection at any level on the chromatogram are initiated independently of the recorder by the amplifier's signal. And up to six different actuation stages are available. Programming is straightforward – you press the command buttons either to synchronise the tape with the chromatogram and index a trap, or simply to index a trap, then dial the amplifier output level at which the operation is to be carried out.

Write or 'phone for full details of the Pye Series 105 Automatic Preparative Chromatograph and its Index of Quality.

PYE UNICAM

1966 1969
THE QUEEN'S AWARD TO INDUSTRY

Precision Instruments

Pye Unicam Ltd
York Street Cambridge, CB1 2PX, England
Telephone (0223) 58866 Telex 81215

LETTERS TO THE EDITOR

Page

PHYSICAL SCIENCES

Solar bursts—A new phenomenon—TANAKA and ÉNOMÉ (Nagoya) . . . 435

Pulsars—Measurement of changes in length of day: a reply—PFLEIDERER (US National Radio Astronomy Observatory) . . . 437

Pulsars—More associations with supernovae suspected—PRENTICE (Oxford) . . . 438

Extragalactic radio sources — Interpretation of polarization variations—ALLER (Michigan) . . . 440

Superheavy nuclei—Primordial synthesis in the galaxy—KUCHOWICZ (Warsaw) . . . 440

Solar wind—Two new plasma instabilities—HOLLWEG and VÖLK (Max-Planck-Institut) . . . 441

Spectroscopy—Identification of the ν_3 NO_2 band in the solar spectrum—GOLDMAN, MURCRAY, MURCRAY, WILLIAMS and BONOMO (Denver) . . . 443

Plagionite—Crystal structure of the plagionite group—CHO and WUENSCH (MIT) . . . 444

Turbulence—Karman vortices in the flow of drag-reducing polymer solutions—KALASHNIKOV and KUDIN (USSR Academy of Sciences) . . . 445

Dry friction—Effect of rotational phase transformations—SIMKINS (US Army) . . . 446

Flow friction—Friction reduction characteristics of fibrous and colloidal substances—ARRANAGA (US Naval Undersea Research and Development Center) . . . 447

BIOLOGICAL SCIENCES

Sodium pump—ATPase specifically requires phosphatidylserine—WHEELER and WHITTAM (Leicester) . . . 449

Ion transport—Electrons and protons in active transport across biological membranes—MANDEL (Pennsylvania) . . . 450

Model membranes—Conductance changes in the presence of antibiotics—HLADKY and HAYDON (Cambridge) . . . 451

Fungal genetics—Blindness of *Phycomyces* cured genetically—VILLET (California Institute of Technology) . . . 453

Rifamycin—Anti-trachoma activity of derivatives related to side chain structure—BECKER, ASHER, HIMMEL and ZAKAY-RONES (Hebrew University–Hadassah Medical School) . . . 454

DNA tumour virus—Immune suppression not significant in SV40 oncogenesis—FRIEDMAN and GOLDNER (Albert Einstein Medical Center and Temple) . . . 455

Page

Adenoviruses—Effects of adenoviruses on human cells—McDOUGALL (MRC) . . . 456

Murine sarcoma viruses — Transformation and growth in human cells—AARONSON and TODARO (US National Cancer Institute) . . . 458

ALS—Effects on amino-acid uptake in lymphocytes contrast with those of PHA—BÉTUEL, RICHARD, COLOBERT and REVOL (Lyon) . . . 459

Transplantation genes—Relation of genotype to childhood leukaemia—WALFORD, FINKELSTEIN, NEERHOUT, KONRAD and SHANBROM (California and Hyland Laboratories) . . . 461

Polymorphism—Enzyme patterns in tetraparental mouse liver—WEGMANN (Harvard) . . . 462

Radiobiology—Aversion conditioning of rats caused by effect of histamine on gut—LEVY (Boston), ERVIN (Harvard) and GARCIA (State University of New York) . . . 463

Serum cholesterol—Pituitary growth hormone essential for regulation—FRIEDMAN, BYERS (Mount Zion Hospital, San Francisco) and ELEK (Southern California) . . . 464

Nutrition—Dietary sucrose causes changes in blood platelets typical of atherosclerosis—SZANTO and YUDKIN (London) . . . 467

Animal behaviour—Effects of malnutrition early in life—LEVITSKY and BARNES (Cornell) . . . 468

Cerebral cortex—Spread of responses to meaningful stimuli—DELISLE BURNS and WEBB (MRC) . . . 469

Neurones—Serotonin in a subcellular position—COTTRELL and OSBORNE (St Andrews) . . . 470

Ecology—Annual production and respiration in animal populations—McNEILL (London) and LAWTON (Oxford) . . . 472

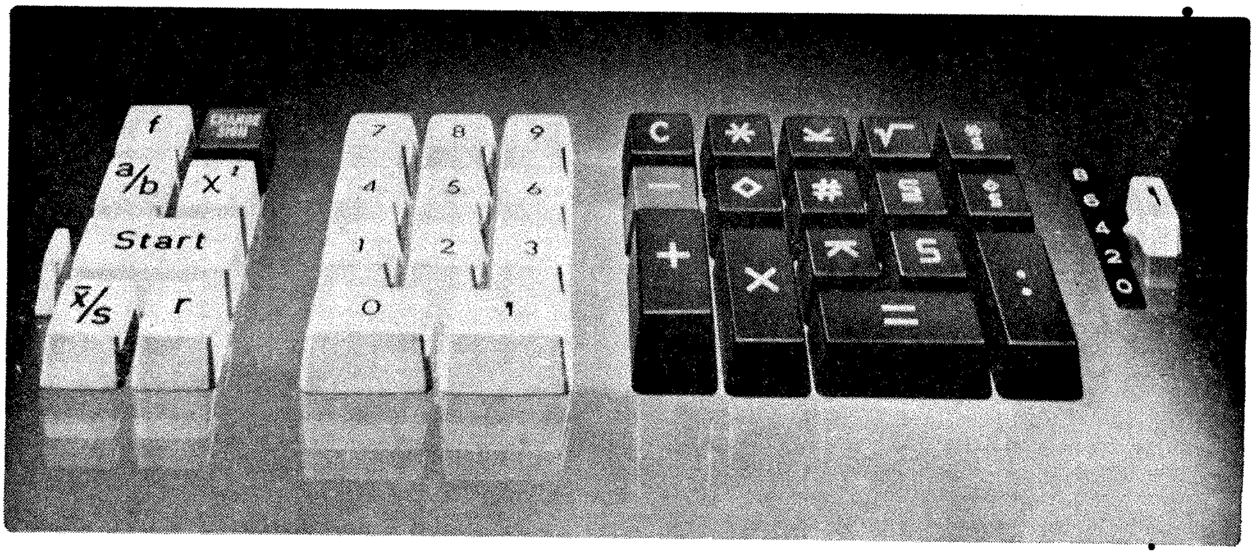
Palaeontology—Pygmy elephants in the Malay Archipelago—HOOIJER (Rijksmuseum van Natuurlijke Historie, Leiden) . . . 474

Air pollution—Ozone and sulphur dioxide together cause chlorotic dwarf disease of pines—DOCHINGER, BENDER (US Department of Agriculture), FOX and HECK (US Department of Health) . . . 476

The titles on this page are prepared by the staff of *Nature* on the basis of the titles submitted by authors which appear in full on the pages indicated

DIEHL sigmatron

Electronic Calculating System with Pre-programmed Statistical functions



DIEHL sigmatron is the first calculating system specifically designed for Statistical calculations. By pre-programmed functions, Statistical calculations are reduced to one key operations. For instance, to obtain the Arithmetic Mean and Standard Deviation enter data on the keyboard and touch the pre-programmed key. After entry of the data is completed you need only touch the same pre-programmed key to obtain the results immediately. Linear Regression, Chi Square, Coefficient of Correlation are all reduced to the simplicity of one key operation. DIEHL sigmatron also gives you a powerful and effective calculating performance with ten separate memories, automatic square root, automatic decimal selection up to 8 places and a complete check of each figure, each step and each result.
If you prefer to make your own programs, DIEHL combitron S has 10 Program/Memory registers providing a capacity of 120 steps with branching and looping.
For external input of data, DIEHL dilector – punched tape reader – is available.

Please cut out!

DIEHL Calculating Systems 8500 Nürnberg 2 W · Germany

Please send me details of DIEHL Calculating Systems

Name ______________________ Title ______________________

Address ____

City ______________________ Country ______________________

Diehl

NATURE Volume 225 JANUARY 31, 1970

The Prophets of Pollution

If anyone doubted that pollution has now usurped the place of original sin in the public consciousness, he has only to read the diatribes of one of the more colourful of contemporary prophets, Professor Ernest Sternglass. Like Moses calling down the death of the Egyptian firstborn, Professor Sternglass is convinced beyond all reasonable doubt that the fall-out from nuclear testing has caused the death of sometimes 400,000 and sometimes two million children in the United States. A prophet, however, is not honoured in his own country, and this week Professor Sternglass was in London to attend the screening of a film on pollution made by Thames television and entitled, with suitable Old Testament flavour, "And on the Eighth Day".

Professor Sternglass is not the run of the mill kind of prophet. He has been vice-president of research at the Westinghouse Research Laboratory for 15 years and is now professor of radiation physics at the University of Pittsburgh. He is a forceful speaker, both in public and private, especially when a gust of indignation lends a gleam to his eye that would outface Savonarola. That he is sincere in what he believes goes without saying, and if he deliberately courts publicity it is not for delight of being in the public eye but because he considers his views have not received a fair hearing.

The difficulty of getting a fair hearing seems to be an important plank of Professor Sternglass's platform. This week he was complaining that *Nature* had refused to publish both the original statement of his case and a reply to the critique by Professor J. Rotblat and Dr Patricia L. Lindop (*Nature*, **224**, 1257; 1969). He also affirmed that Professor Rotblat had neglected the courtesy of sending a copy of his critique to him before publication.

Among the documents on public display were xerox copies of a letter from Dr H. J. Dunstan, secretary of the forthcoming International Radiation Protection Association Congress, saying that "the pressure of papers submitted" has made it impossible to accept an abstract of one of Professor Sternglass's articles. Dr Dunstan adds his hope "that it will still be possible for you to attend the Congress and participate in the discussions". At the same time Professor Sternglass is eager to demonstrate the acceptance of his views by the scientific community, and he also produced a letter from Professor John T. Edsall of Harvard University saying that, if Professor Sternglass could take care of certain minor points in a manuscript, "I would be glad to send a letter to Philip Abelson [editor of *Science*] urging him to publish your article so that the evidence you have to present could be brought out into the open for all interested persons to debate".

Is there really a conspiracy against Professor Sternglass and all his works? The young film producers at Thames television, which sponsored Professor Sternglass's appearance, believe that there is and even dropped hints that, were it not for the refusal of *Nature* to publish Sternglass's articles, his visit to London might not have been necessary. It certainly seems that Professor Sternglass has decided to appeal to the public over the heads of his scientific colleagues in the belief, perhaps, that if you can't join them you can beat them. Professor Sternglass does not himself claim that there is a conspiracy against him, but in allowing others to so believe he is paying possibly too little heed to the ways and means of scientific communication.

The truth is that the truth is hard to find. It does not lie on plates or hang from trees. This is why over the years various conventions and by-laws have been formulated whereby to keep the congested channels of scientific communication reasonably free from spurious traffic. Once a false result gains a place in that sacred corpus known as "the literature", it can on occasions do untold harm and waste innumerable hours of valuable time. It is hardly surprising that editors of scientific journals try to exclude articles which fall in this category, particularly when they carry the implications of Professor Sternglass's hypothesis. That is a long way from conspiracy, and the fact that more than one editor has rejected Professor Sternglass's papers whether on these or other grounds could be a sign of the looseness of Professor Sternglass's arguments rather than of the tightness of the conspiracy. Professor Sternglass's original paper was rejected by *Nature* because of its inherent deficiencies. Far from not being shown to Professor Sternglass before publication, the critique by Lindop and Rotblat was held up

Nature in North America

From the beginning of next week, *Nature* will maintain an office in Washington DC. Contributors in the United States and Canada are invited to submit manuscripts for publication either to the address given below or to the present London office. The intention is that each office will be able to answer inquiries about all manuscripts and other matters, although a strictly symmetrical situation will not obtain until mid-February, from which point, for half a year, the editor of *Nature* will be based in Washington. This is one of several steps by which it is hoped to provide a better service for readers and contributors.

London Office
4 Little Essex Street,
London WC2.
01-826-6633

Washington Office
711 National Press Building,
Washington DC 20004.

for several weeks to allow him the chance of replying. The reply was rejected because it restated the original case instead of answering the criticisms. None of this implies that Professor Sternglass is wrong in what he has to say. He may indeed be right. But he has not yet argued his case in a scientifically acceptable style. In other words, there is a substantial possibility that he is entirely wrong.

To publish a hypothesis in this state of uncertainty is to risk the usual confusion created by a false result together with the dangers of crying wolf too often. In the present climate of concern about pollution it is all too easy to misinform the gullible and add fuel to the sense of hysteria in which, for example, the Minister of Agriculture recently banned cyclamates and Lady Summerskill called in the House of Lords for a complete withdrawal of all contraceptive pills. People such as Professor Sternglass should perhaps ask themselves exactly what cause they are serving when they simplify a complex issue and present it in emotive terms. To say, for example, as Professor Sternglass does in the Thames television programme on pollution, that "something like 100,000 children in Britain" have died because of nuclear testing is the statement of a propagandist, not of a scientist. Professor Sternglass doubtless knows when he is playing one part and when the other, but other people will inevitably be confused.

The upshot of this confusion is vividly illustrated in the television programme that brought Professor Sternglass to London. "Report on Pollution—And on the Eighth Day" is not so much a report as a wholly uncritical exposition of what various scientists interested in pollution problems have to say. The film, in consequence, is no better than its raw material, which means that when Dr Kenneth Mellanby is being interviewed it is excellent and when Professor Sternglass's views on infant mortality are followed by the triple cliché of a baby in a cot, a pregnant woman and falling rain, all to the sound of a song hinting at radioactive contamination, the result is a complete abnegation of the principles of balanced reporting.

Those who feel that a little overstatement here and there will do the cause of protecting the environment no harm should consider carefully some of the confusion that too much propaganda creates, both in the minds of television producers and in those of their audience. Like the primitive who blames the weapon instead of the murderer, the Thames television film seems to be arguing that the environment is polluted not because society has failed to control technology, but because technology exists. "However ingenious our technology, the natural laws will exact their price no matter how we try to postpone or circumvent them" is a statement marvellously redolent with ancient anti-Promethean undertones.

Must man really be forbidden to use fire? Is technology an affront to the natural order that will attract the retribution of gods? Quaint as this view may be, the harking after the ideals of the noble savage is all too easily turned into an attack on rationalism and the pursuit of scientific knowledge. Indeed, the Thames television film explicitly questions the scientific method: "The scientist, learned in one thing, may be ignorant of all else. . . . With the population exploding, knowledge scarce, the scientific method inappropriate, it is little wonder we are in this environmental mess." It is little wonder that if people of the undoubted ability and conviction of Professor Sternglass go around in girt-up loincloths prophesying fire and brimstone in advance of the basic facts being agreed upon, some of the bystanders will think men of science strange and dangerous.

Academics in Limbo

Where will it all end? As the months go by, the sense in the British universities that serious trouble can be only just around the corner begins to give way to the more awesome prospect that radical changes may take place without being recognized as such. The latest annual survey of the University Grants Committee, which is a useful document not merely for its liberality with factual information, is unfortunately unlikely to help keep people on their toes. Statistically (see page 409), there is every reason to believe that the financial squeeze of the past few years has begun to take effect. The staff–student ratio, for example, has begun to decline—not before time, perhaps. Qualitatively, the University Grants Committee has made a beginning, however slow, on the painful task of providing a better coordination for the activities of British universities, and in the process it is inevitable that some institutions will feel that their freedom is being restricted. The trouble, of course, is that there are plainly too many universities all trying to do the same kinds of things, so that the avoidance of duplication is a serious problem. But the most serious sign to show in the UGC's courtly document is the uncertainty which it discloses about the future scale of growth. Will the British Government remain committed, in the 1970s, to the Robbins doctrine that all suitably qualified people should be provided with a university place (which begs the question of what is meant by suitably qualified)? Or will there instead be a tendency to look for cheaper solutions? Much, no doubt, will depend on how the universities respond to the request made of the universities last September to consider new ways of organization. Collectively, however, the universities have less freedom of manoeuvre than they are inclined to think.

So might it not be simpler, as it would certainly be more courageous, to begin by tackling the more serious issues which overhang the British system of higher education? The lack of any mention of these important matters is the gravest defect of the UGC's new document. What, for example, is to be the relationship between the universities and the polytechnics on which, three years ago, the government falsely pinned a good deal of its hope? Will these institutions be encouraged (as they should be so long as they remain autonomous) to become so good that they resemble universities, will there instead be a foolish attempt to make them second-class institutions, or will the government (or its successor) confess the error of its ways and find some way of linking the two systems (with colleges of education included) into an integrated whole? Then what is to happen to the relationship between universities and the government? Will utter financial dependence continue to be the almost universal rule? The benefits of a broadening of the financial base consist not of mythical academic freedom but a built-in tendency towards the kind of academic diversity which is at present missing. Then there are questions of the financial relationship between universities and their students on the one hand and their teachers on the other. Is it right that British students should be financially dependent either on the local government or their parents? Is there not some halfway house in which the direct cost of university education would not be simply related to the size of its output? And is it not time that the present rigid scales of pay for academics were softened by more than the tiny percentage which has survived from the recommendation of the Prices and Incomes Board that four per cent of the salary bill should be spent on special awards to hard-working teachers? To say that the neglect of these matters for the proper coordination of postgraduate courses is to fiddle while Rome burns would be to exaggerate—but not by very much.

100 Years Ago

BOSTON

Society of Natural History, November 17.—The president in the chair. Prof. N. S. Shaler read a note on the occurrence of the remains of *Tarandus rangijer* Gray, at Big Bone Lick, in Kentucky. At a previous meeting was presented the evidence in support of the conclusion that one of the large mammals of North America, the buffalo, had recently changed its limits, and had only ranged in the Ohio valley within the past few centuries. The same locality supplies us with evidence that the caribou existed in abundance in this river basin at a time anterior to the coming of the buffalo, and probably not very long after the disappearance of the *Elephas primigenius*. Since the coming of civilised man into America, the caribou has been confined to a narrow area in the north-east part of the continent; it is questionable whether it has ever ranged during this time south of the southern limit of the State of Maine. The position in which these remains were found leaves the precise relationship in time of this species to the mammoths and mastodons a little questionable. There is, however, little doubt in my mind that, if not in existence during the later part of the time of these pachyderms, it came immediately after them. Its bones are always found below the line of the buffalo and the Virginia deer. The remains of this latter species are found only among the most recent deposits of the swamp. The disappearance from this region of this eminently boreal animal immediately after the passing away of the ancient elephants from the Mississippi valley, goes to confirm the conclusion that the climatic change which closed the period of the mammoths was from cold to warmth, and not an alteration of the opposite character.

From Nature, 1, 369, *February 3, 1870. Shaler was professor of palaeontology at Harvard.*

RESEARCH ASSOCIATIONS

Strength in Unison

THE industrial research associations, long since accustomed to life on the edges of public munificence, seem to have embarked on a strategy that will allow them at least to complain about their lot more effectively. Earlier this week, the associations collaborated to form what is known as the Conference of Industrial Research Associations, evidently intended to serve as a more effective voice than the Committee of Directors of Research Associations has been able to be—in some eyes, at least, directors are lowly fellows because they are formally the employees of the autonomous councils which are formally the fountain-heads of policy. The director of the new body is the Earl of Shannon, and it is intended that there shall be a deliberate attempt to provide a solid base from which the case for the research associations can be advanced.

There seems no doubt that the publication of the Ministry of Technology Green Paper on the reorganization of civilian research within the government has fired the zeal of the research associations, which are almost conspicuously left untouched by the proposals for reorganization. For the past few years, the central government's contribution to the finances of the research associations has been fixed at £4 million a year, which works out at about a quarter of the total budget, the remainder of which is supplied by industrial contributions. Although this kind of treatment is familiar enough among the pensioners of governments who have fallen out of favour, it seems to be felt among the research associations that the Ministry of Technology has an influence over policy which is incommensurately great compared with its financial contribution. It will be interesting to see whether the new arrangements will tempt the research associations to make a dash for freedom and do without a subvention. It may be easier to tell which way the wind is blowing on February 11, when the Conference of Industrial Research Associations plans to hold a public meeting at which Dr E. A. Davies, Joint Parliamentary Secretary at the Ministry of Technology, will be one of the speakers.

TEACHER TRAINING

Comprehensive Degrees?

by our Education Correspondent

TEACHERS have very little opportunity to help formulate policy on teacher training. This claim was made by representatives of the National Union of Teachers when they met the Select Committee on Education and Science on January 27. The NUT does, however, have strong views about the way that teacher training should be run, and submitted these to the Select Committee in a detailed document setting out a number of radical proposals. Like the National Union of Students and the Association of University Teachers, the NUT wants to see an end to the binary system. The proposals call for a major restructuring of the whole sphere of higher education, the final result being a "comprehensive university". Colleges of education should be grouped together into schools of education, attached to a university, and the NUT argues that all education students should take a four year course leading eventually to a degree.

The NUT suggests that responsibility for financing all spheres of higher education should be vested in one body, on the lines suggested by the Select Committee itself in its report on student relations. Such a system would remove the present colleges of education from the financial control of the local authorities. If they were grouped together into schools of education and brought under the control of individual universities, the NUT argues that this would "avoid wasteful duplication of courses", and would facilitate coordination of entrance requirements for higher education to "relieve the pressures which result in unnecessary specialization in secondary schools".

As far as course content is concerned, the NUT argues that there should be no dichotomy between academic and professional matters. What matters is the nature of the knowledge acquired and the understanding of how to communicate this knowledge to different age groups. Placing the schools of education in a comprehensive university would give potential teachers the opportunity to mix with students from other disciplines and this would help them to gain maximum benefit from their education. But the NUT suggests that the best way to provide links between the academic and professional aspects of training would be to appoint teacher–tutors. These would be teachers on the staff of schools where teaching practice is carried out, who would be responsible for a few students, and would work closely with college tutors.

The NUT believes that the present three year courses are too short and that they should be lengthened to four years. The NUT also suggests that the "general recognition of the need for a four year course would be a major step towards the union's ultimate goal of an all-graduate profession". The union also suggests that schools of education could provide one year courses of education for students who are unable or do not wish to take a degree course in education. Such courses are at present virtually a monopoly of the universities.

DRAGON

Now We are Ten

An opportune moment has been chosen for the publication of the tenth annual report of the Dragon high temperature reactor project, released last week, but the future probably bodes less well for the steam generating heavy water reactor which the UKAEA has on its books. Dragon is just across the way from the SGHWR at Winfrith, but the Dragon reactor is a joint effort of twelve European states and run by the European Nuclear Energy Agency. The point about Dragon is that the metal fuel cans have been done away with so that much higher temperatures can be allowed in the core—up to 1,500° C. One of the by-products of the project has been a boom in the technology of graphite which is used for the structure of the core and moderator. The high temperatures mean a high conversion efficiency in a compact reactor. Cooling is by helium gas, and one possibility which people have been talking about for this type of reactor is to use the helium to drive the turbines directly, thus cutting out the mandatory intermediate steam cycle with a further gain in efficiency.

Since the encouraging remarks about high temperature reactors made by Sir Stanley Brown at a conference in Zurich last year, the outlook has been bright for projects such as Dragon. Prototype reactors working on similar principles are in operation in Germany and the United States. Sir Stanley is chairman of the Central Electricity Generating Board, and he said that high temperature systems show an advantage of about £10 per kW over the advanced gas-cooled reactors (AGRs) such as are being built for the board at present. The point seems to have been taken by the participants in Dragon, who recently agreed to extend the project for a further three years to March 31, 1973, within a total budget for the period of £38 million.

The view of the CEGB is that an enquiry for a 1,300 MW HTR station with two reactors is likely to be issued toward the end of 1970, with the first reactor intended to come into service in 1976. But there is not going to be a rapid transfer to HTR systems and the board is probably going to continue with AGR stations to the end of the decade. Instead, the first HTR will be what is being called a "leader" station, and its performance will be watched carefully before the board goes further. The way the 20 MW Dragon project has gone seems to have persuaded the board that there is no need for a bigger prototype reactor before the leader station.

One of the charms of HTR systems as far as the CEGB is concerned seems to be that they are likely to be better than AGRs at coping with the large daily fluctuations in demand which are a feature of the British power scene. Meanwhile, with Winfrith's Dragon in a happy state, negotiations are still going on with Greece to barter an SGHWR for a consignment of tobacco.

CB WEAPONS

More Shocks from WHO

The latest contribution of the World Health Organization to the debate about chemical and biological weapons is the most alarming so far (*Health Aspects of Chemical and Biological Weapons*, WHO agencies, £1·20). Together with a catalogue of the properties of some hitherto obscure agents, the document contains a detailed calculation of the potential effects of chemical and biological agents directed at hypothetical targets—mostly densely populated cities. The document is a by-product of the work of the eighteen member advisory committee which drafted the WHO advice to the United States, later embodied in the UN report on the subject published last year.

One of the most vivid of the examples of the effects of chemical and biological weapons assumes that the agent would be the bacterium responsible for tularaemia. Although human beings do not often come into contact with the insect vectors of the disease, it is known that as few as twenty-five bacterial cells can cause infection. The report points out that even the susceptibility of the bacterium to antibiotics such as streptomycin need not prevent its use as an incapacitating agent, but that a death rate of about 25 per cent among infected people would otherwise be expected. The bacteria are easily grown, infection is easy by several routes, immunization in advance is unlikely to be effective and one consequence of the use of such an agent would probably be the establishment of pools of infection among wild mammals.

An attack with tularaemia bacteria on a city of five million people would, the report estimates, produce 250,000 infected cases and probably many more. Although most of those affected would be treated by antibiotics within 48 hours, something like 25,000 people would need to go to hospital and something like 2,500 people would die. The greatest casualties, however, would be among those escaping treatment early on, possibly by fleeing the city—the committee estimates that between 7 and 10 per cent, or possibly 2,000 people, would die, making a total death roll of 4,500 spread over 10 days. Given that a modern city with a population of 5 million is likely to include 10,000 doctors and 60,000 nurses, the report concludes that it would probably be possible for existing medical staffs to handle the consequences of the attack, at least so long as panic and severe disorganization could be avoided. This case study shows how much more resistant to an attack would be a well provided city than a city of the same size in a developing country—there, the calculation goes, 30,000 deaths within ten days would be expected.

The use of plague as an infecting agent could be expected to be more damaging. It is estimated that in a city of five million people, there would probably be 150,000 cases infected with pneumonic plague and up to 100,000 hospital cases of whom 36,000 would die. One of the most serious causes of death would be secondary infection, responsible for, say, 100,000 deaths. The report says that "burial facilities would be taxed and some difficulties could be expected". In cities of the same size without medical services for providing those initially infected with antibiotics and without hospitals on a modern scale, it is estimated that 250,000 people would die in the first and succeeding waves of infection.

Nerve gases could be as damaging as pneumonic plague, but faster. The WHO report estimates that if 150,000 people were exposed to a lethal concentration of the persistent nerve gas VX, 80,000 of them would die before help arrived and that a further 40,000 would die before help could be effective. One of the particular hazards of VX is that its persistence would play havoc with the helpers. The fact that most of the deaths would be concentrated in the first two days would only aggravate the strain on the hospitals and medical facilities.

The panel responsible for the WHO report has also calculated the likely consequences of attempts to infect public drinking water systems with various organisms. One kilogram of freeze-dried typhoid, containing more than 10^{14} organisms, is assumed to lose all but five per cent of its infectiveness on being introduced into the public water system of a city. In a modern city, something like 35,000 people would receive a sufficiently high dose to be assured of developing typhoid, and 200 or so of these would die. In less advanced cities, the same amount of freeze-dried bacillus could cause 130,000 cases and 4,500 deaths. The use of type A botulin toxin is likely to yield less certain consequences—because the first effects are likely to appear within 6 to 8 hours of ingestion, much will depend on how quickly public authorities recognize what has happened. It would be responsible, however, to conclude that 0·24 kg of toxin could cause 30,000 deaths, allowing a six-fold safety factor to account for unequal distribution.

SOLAR PHYSICS

Plans for More Facilities

A SENSIBLE plan for the development of ground-based observations of solar–terrestrial phenomena has now been published by a panel of the National Research Council in Washington (*Physics of the Earth in Space—The Role of Ground-Based Research*, available from the National Academy of Sciences). The Space Sciences Board of the NRC published in 1968 an analysis of the ways in which rockets and satellites could advance the cause of solar–terrestrial physics, but the new report, produced under the guidance of Dr Herbert Friedman, is almost lyrical in its description of what remains to be done by deploying more effective equipment on the ground.

Most of the recommendations in the new report are concerned with conventional equipment. The committee is, however, strong in its advocacy of direct observation of the Sun at radar wavelengths, and asks for the development of a scanning radiometer operating in the mid-centimetre range which would be able to resolve to within a minute of arc or better and which would produce what would be television pictures (at radio wavelengths) at the rate of one a minute. The object of this instrument would be to make possible direct observations of those regions in the chromosphere of the Sun which lie at the base of solar flares or which surround them. The panel says that the most direct attack on the problem of solar flares could be mounted by means of simultaneous X-ray mapping of the Sun from satellites and ground-based observations at radio wavelengths of features no more than a second of arc across, but the committee asks at this stage only for the study of the radio-interferometers which could help in the design of such an expensive piece of equipment. In the same way, the panel would like to see an improvement of facilities for the optical observation of the Sun, especially at places where continuous observation is possible, and it is even more enthusiastic about the need for a new instrument for the spectroscopic mapping of the magnetic field on the surface of the Sun. Less hopefully, the panel asks for money for the exchange of information from solar observations and for the exchange of people, graduate students as well as independent investigators.

The panel is downcast at the way in which financial support for cosmic ray research is declining, and asks that this tendency should be reversed, with the Environmental Science Services Administration paying hard cash for some of the uses which it makes of cosmic ray data. The National Center for Atmospheric Research has advocated work on the development of cosmic ray balloons of great endurance capable of flying at more than 120,000 feet—there is apparently great promise in a scheme for launching balloons which use liquid hydrogen as ballast—controlled evaporation could turn ballast into lift. Flights lasting several days over the polar caps (and especially in the Antarctic, where national boundaries create no problem) come high on the list, as does the need for developing instruments for balloon flights which can yield information about the momentum, charge and directional distribution of cosmic ray particles which is comparable with that obtained from ground-based equipment. For the rest, where cosmic ray studies are concerned, the panel would like to see an all-round development of present work.

Throughout the panel's report, there is a constant repetition of the need for mobile facilities for the measurement of cosmic ray neutron flux, the measurement of geomagnetic disturbances and the recording of some kinds of solar phenomena. It seems to be acknowledged that the handling of geomagnetic data is a special problem, and the panel suggests that a world-wide communication link based on a COMSAT circuit would be sensible (but there is no estimate of cost). There is, the report says, a great need for the development of programmes which will continuously monitor whistler signals received at very low frequency.

The panel has several expensive proposals for the better study of the ionosphere, the chief of which is the building in the northern United States (at the edge of the magnetic shell $L=4$) of a transmitter–receiver capable of exploiting incoherent scatter techniques in the study of the motion of plasma in the ionosphere. The cost of this proposal is said to be potentially comparable with that of the Arecibo station in Puerto Rico. The panel also asks that there should be a smaller station in the auroral zone, and suggests that this might be established by moving the incoherent scatter equipment now at Palo Alto to College, Alaska. The panel is clearly anxious that the stations at Jicamarca (Peru), Millstone and Arecibo will be kept on tight budgets, especially now that the last of the three is due to be moved from the Advanced Research Projects Agency to the National Science Foundation. The financial position of these three stations seems also to concern those who would make simultaneous optical and radio measurements of the atmosphere from stations on the ground, but the panel also urges that there should be more detailed laboratory studies of important processes in aeronomy.

To the extent that the panel's report will help agencies such as the National Science Foundation to work out a strategy for solar–terrestrial physics, the report will be valuable. Inevitably, however, it is bound to seem like wishful thinking or even special pleading so long as it lacks a detailed costing. This deficiency is no doubt one of the inevitable features of reports prepared in the splendid isolation of Aspen, Colorado.

GEOLOGY

Applied Mapping

WITH an eye on the practical applications of its services, the Institute of Geological Sciences has been putting its surveying and mapping work to good use despite difficulties in recruiting staff and in re-siting numerous offices. One economically important project mentioned in the institute's recently published annual report on 1968 (HMSO, £1·2; 1969) is a comprehensive survey of the Humberside area with a view to the optimum development of its industries, services and amenities. The field unit is revising the six-inch mapping of the area, a new marine geophysics unit has made its first ship-borne survey in the estuary and the hydrogeological department is studying the underground resources of the entire Humber drainage basin.

The engineering geology unit has played its part with a geotechnical study of the area of the new town of Milton Keynes, and maps and tables showing the variation of physical properties over the area will be ready within the next few months. This seems to have been helpful in planning the drainage for the town although too late to influence any of the major planning decisions. The institute is, however, involved right from the earliest stages of planning with all road projects costing more than a million pounds, by agreement with the Ministry of Transport.

In its programme for shallow geological mapping of the sea bed of the continental shelf, the institute hopes to encourage commercial firms to carry out more detailed deep surveys in areas which seem likely to have mineral deposits. The institute publishes these maps in a new IGS report series, and it has so far surveyed the North Irish Sea, the sea of the Hebrides and Minch, the Clyde and the Firth of Forth. One deep reflexion seismic survey was commissioned in Cardigan Bay, a deep sedimentary basin of potential interest to oil companies.

The radiogeological department, under a £250,000 contract from the United Kingdom Atomic Energy Authority, is examining two areas in North Scotland and the Midlands for uranium deposits. The results have not yet been fully analysed, but at least four areas have been identified where the radioactivity can be attributed to uranium and one where there is thorium. Hydrogeochemical reconnaissance seems to be an effective prospecting technique in this environment. A new twelve-inch mass spectrometer in the isotope geology unit is being used mainly for geochronological work using K-Ar, Rb-Sr and U-Pb dating techniques. An interesting development has been work on stable isotope ratios. The institute hopes, for instance, to set up a new unit to study the relationship between O^{16}/O^{18} ratios and the temperatures of formation of rocks and mineral deposits and is also interested in examining sulphur and hydrogen isotopes which may give information about the fluid inclusions in rocks and minerals.

LINNEAN SOCIETY

Successful Appeal

by our Botany Correspondent

FIFTEEN months after it was launched, the appeal for development of the rooms of the Linnean Society in London has drawn gifts and promises of more, totalling £69,000. So successful has it been that the first phase of alterations at Burlington House is already under way. The new strongroom is complete and only has to dry out before the collections of plants, shells, insects and fishes, made by Linnaeus in the eighteenth century, can be moved in from the wooden cabinets they have occupied since the society moved into its rooms in 1857.

The latest piece of good news for the organizers of the appeal came just before Christmas when they heard that the Swedish Government had approved a grant of 100,000 Swedish crowns (about £8,000) towards the provision of suitable premises for the collections. Linnaeus was of course a Swede, and the impetus for the gift seems to have come from the Royal Swedish Academy of Sciences at the instigation of Professor S. O. Horstadius, a foreign member of the Linnean Society. Other gifts from Sweden have come from HM the King, the University of Uppsala and the Swedish Match Co., as well as the Academy of Sciences.

The most generous single British donations have come from the Royal Society (£4,970) and from the Drapers Company (£5,000) towards the cost of a new council room as a memorial to the late Sir Frederick Stern, former master of the company and treasurer of the society. In its report on the appeal the society notes that the response of industry and commerce has been reasonable in view of the present financial climate and the rather specialized nature of the appeal. BP and ICI were the most generous donors, giving £1,000 and £500 respectively. Many scientific societies have also contributed, and the Linnean Society hopes that some of them will be able to make use of the modernized rooms for their meetings.

The work already completed or under way in the basement, which as well as the strongroom includes the provision of new cloakrooms and heating and ventilation systems, will cost about £42,000. Further plans have been drawn up for improvements to the library and lecture room and the provision of a council room and offices. This work, due to begin soon, will cost more than £30,000, and so the appeal is by no means closed. If sufficient funds are available there are a few more improvements, particularly to the library, which the society would like to see effected.

The library of the Linnean Society has considerable historical interest, because it is founded on the libraries of Linnaeus himself and J. E. Smith, the wealthy friend of Sir Joseph Banks, who bought the collections and books for £1,088 5*s* from Linnaeus's family in 1783. The Linnean Society was formed five years later, meeting at the Marlborough Coffee House in London, with Smith as one of the seven founder members. After Smith's death in 1828 the society bought the collections and books from his executors, and has had them ever since.

CARNEGIE OBSERVATORIES

Rechristening Mount Wilson

THE Mount Wilson and Palomar Observatories in California are in future to be known jointly as the Hale Observatories. This was announced recently by the Carnegie Institution of Washington and the California Institute of Technology. The immediate benefit of the change will be that innocents will not in future fall into the trap of calling Palomar Mountain Mount Palomar, but it is also intended that there should be a new method of planning work at the observatories, which will separately be known by their old and separate names. The director of the observatories will remain Dr Horace W. Babcock, but the new arrangements specify that there should always be a director from one of the observatories and an associate director from the other. In fact, Dr J. Beverly Oke has been appointed associate director of the Hale Observatories. The allotment of observing time will be entrusted to an observatory committee of eight people.

The permanent staff of the observatories is now more than 120 strong and includes nineteen astronomers, many of whom are also members of the faculty at the California Institute of Technology. The naming of the observatories after George Ellery Hale is of course appropriate—Hale founded not merely the Mount Wilson and Palomar Observatories but also those at Kenwood and Yerkes. Although experienced people say that seeing conditions at Mount Wilson have never been as good as in the years during the war when Los Angeles was blacked out, the two observatories remain remarkably usable. The 200-inch Hale telescope on Palomar Mountain, for example, had 238 complete nights of seeing in 1968 and 62 nights when it could be used for part of the time.

LONDON

Preventing a Flood

by our Planning Correspondent

WHILE the British Government has not yet firmly committed itself to the form or the location of a barrage or barrier across the River Thames as a defence against a tidal flood, it has agreed that London and Lower Thames-side must be protected in some form, although it does not regard the likelihood of a serious flood with quite the same alarm as does the Greater London Council. Nevertheless, the Minister of Housing and Local Government, Mr Greenwood, did say in a written parliamentary answer on January 20 that no time should be lost in completing the further studies called for in a report on various defence systems which the GLC has just submitted to the minister, and that the government and the responsible authorities "will then decide as a matter of urgency on the nature and siting of the defences required".

The GLC report to which the minister referred contains the council's preliminary conclusions from a detailed study into what it believes to be the growing threat of a flood caused by a combination of circumstances—the sinking of the south-east at the rate of about a foot a century; the increasing height of the tides in the Thames (about 3 feet a century at London Bridge) compared with the general sea level; the reclamation of low lying land alongside the river over the centuries which has stopped the build-up of silt; and the channelling of the river through the building of flood walls which has raised the general level of water. A high spring tide, a "surge" of moving water from the North Atlantic, an adverse wind, heavy rainfall and drainage off the land could, in the council's opinion, cause a disastrous flood.

The council has worked on the basis of providing protection against a high water level six feet above the levels reached by the Thames in the floods of 1953, taking into account the sinking of the land, the increase in tidal range and so on. This new high water level, the council says, would put the risk of flooding at about 1 in 10,000 in 1980, about 1 in 6,000 in 2010 and about 1 in 4,000 in 2030. At present, 55 square miles of London, with a population of 1,200,000 people, are below this new level. Walls an extra six feet high along the river banks would, however, be unacceptable from the amenity point of view in most parts of London. The council has therefore looked into other ways of dealing with the problem and, since 1968, has spent £250,000 (half of which is being paid for by the government) on investigations which have included the commissioning of a 400-foot concrete model of the Thames at the Hydraulics Research Station (see *Nature*, **221**, 507; 1969), computer predictions of surge tides which might be expected in the future, measurements of silting and water movement in the river, and other field studies. Of all the various types and locations of a fixed barrage or a movable barrier which could be used in conjunction with a raising of the riparian walls in places, the council favours a tide-control barrier in the Woolwich/Limehouse area just upstream of the Royal Docks; or failing this, a similar barrier in the Crayfordness area farther downstream. The barrier in either site would operate by closing at times of great risk between September and March at a point during the ebb tide, reopening on the following flood tide unless there was a warning of a surge, when it would remain shut. It would be provided with a lock so that medium-sized ships could pass through. It is estimated that the Woolwich barrier would take about 7 years to build at a cost of about £50 million; at Crayfordness, the barrier would take about 11 years to build and would cost about £60 million.

While the GLC's suggestions for flood protection have been generally welcomed, there are fears about the extra cost to shipping if the barriers are built—reckoned to be £250,000 a year in the case of a Woolwich barrier, and £1 million through shipping delays if the Crayfordness barrier is built—and that the barrier if built at Woolwich would be an eyesore. Presumably, these economic and environmental matters will be sorted out in the next stage of the GLC's investigations.

ARCHAEOLOGY

Cargo of Mortars

It is original of the Gollcher Foundation administered by the National Museum of Malta to choose as the subject of the first publication in its archaeological monograph series an underwater wreck site. Captain Olof Gollcher, a Swedish nobleman and Knight of St John who died in 1962, left his home in Malta and its contents as a national collection and established a fund in support of archaeological studies. He was an enthusiast of underwater archaeology, so it is appropriate in several ways that the first volume of the Gollcher series deals with a wreck off Malta studied by Miss Honor Frost, who has won her international reputation in this exacting science through work in the Mediterranean. *The Mortar Wreck in Mellieha Bay*, just out from the Appleton Press (EC1) (38 pp., 25*s*), deals with a second century Roman wreck. The ship was apparently bound from southern Italy to North Africa when it was blown by a *gregale* (north-easterly wind) on to "rocks awash", as they are currently labelled on the marine chart, in the middle of the bay.

The prime cargo consisted, rather improbably, of mortars of various sizes but distinctive manufacture. Into the paste was mixed, before firing, sizable grits to give "bite" in the pounding up of pulses and grains. The grit material has been identified as *andularia*, a type of feldspar of very limited distribution in Europe. Piedmont appears the most likely source in this case. That Roman merchants had an export trade of mortaria to the colonies is surprising. But they have been found on land in places as far apart as Syria, Jerusalem, Strasbourg and even Newcastle. Some of the Mellieha wreck mortars are 1·3 metres across. The cargo also included glass, the dating and analysis of which have proved a valuable contribution to the history of glass technology.

Miss Frost observes that the scholarly value of wreck sites is still scarcely appreciated, largely because of slow and inadequate publication. Wrecks, being "closed groups", are important both for dating and for deducing the provenance of antiquities. When their controlled excavation becomes the rule, they should be such a reliable source of information that archaeologists debating attributions may one day ask: "Is such and such a view confirmed by findings from *wreck* excavation?" At least Miss Frost cannot be faulted on publication.

INDUSTRIAL RESEARCH

Keep Technology Pure

The translation of ideas from pure research into technology must occur in a research environment, according to Dr A. P. Speiser, the director of research of Brown, Boveri and Company, speaking at the Institution of Electrical Engineers in London recently. He explained how Brown, Boveri, which is an international concern, has been applying this concept in the design of its new research centre in Switzerland, and why it is vital to get the right balance and coupling between the pure and applied aspects of a company's research effort.

Dr Speiser was quite critical of some of the methods used to select research projects in industry. A common pitfall, he said, was to justify the lack of scientific content in a piece of research by its possible practical benefit, and to justify the lack of the latter by possible scientific benefit. He thought that some work on controlled nuclear fusion fell into this category. An important criterion for evaluating the worth of a project should be the standing of the group involved, he said, and it is also a good test if the research workers are well respected in university circles.

A sound test of the value of a technological project is whether it fits in somehow with the overall strategy of a company, Dr Speiser said. Close personal contacts between research and production departments are vital for this, but he was still convinced that the majority of technologists in a firm should work within the research laboratories.

Brown, Boveri has an annual turnover of about £400 million, most of which comes from Switzerland, West Germany and France. Dr Speiser pointed out that it had been the aim of his company to concentrate its whole research effort into the new research centre, but that in practice this policy is a hindrance in attracting government grants and in dealing with national orders. But a major proportion of the £2-4 million being spent on research by Brown, Boveri would still be channelled into the new centre, he said, leaving perhaps 30 per cent of the total research budget to be divided between smaller research departments in France and West Germany.

Parliament in Britain

Enzyme Detergents

THE Home Office is conducting enquiries into possible harmful effects of enzyme washing powders, and it will decide on the basis of these enquiries whether any action is necessary. This was announced by the Joint Under Secretary of State, Mr Merlyn Rees, who also said that a few members of the public have complained that they have been adversely affected by these powders. (Written answer, January 19.)

Education Expenditure

PUBLIC expenditure on education, in real terms, rose on average by about 6 per cent a year between 1958–59 and 1968–69. Mr Edward Short, Secretary of State for Education and Science, gave this estimate in reply to a question from Mr David Lane, but he said that technical problems prevent a comparative estimate of educational expenditure being made. He therefore could not give an estimate for the increase in public expenditure on primary education, secondary education, further education or university education during the past decade. Mr Short also said that although raising the school leaving age to sixteen would not require more teachers it would cost about £13·5 million a year. (Written answer, January 23.)

Oral Contraceptives

THE Committee on the Safety of Drugs is reviewing the arrangements for conveying its views about adverse reactions to drugs to the professions concerned. This assurance was given by Mr Richard Crossman, Secretary of State for Social Services, after Mr Laurence Pavitt had expressed concern that major decisions of the committee, such as the advice on oral contraceptives, reach pharmacists via the national newspapers. Baroness Summerskill had previously provided a good example of the extreme reactions that have followed from the scare over harmful effects of oral contraceptives. She asked the government to issue instructions that all contraceptive pills should be withdrawn from the market because the United States has withdrawn the oestrogen-free contraceptive Normenal. Baroness Llewelyn-Davies of Hastoe said, however, that the government sees no justification for withdrawing all oral contraceptives from the market, and Baroness Gaitskill suggested that Baroness Summerskill lacked a sense of proportion in asking for this extreme measure. Baroness Birk also pointed out that if the government were panicked into withdrawing all contraceptive pills, the increase in deaths arising from pregnancy or abortions would be greater than the number of deaths from side effects of the pill, and she suggested that the anwer is to put more money into research to find a safer pill. (Written answers, January 23, and oral answers, Lords, January 22.)

Government R and D Savings

DR ERNEST DAVIES, Joint Parliamentary Secretary to the Ministry of Technology, said that reductions will be made in expenditure on civil nuclear and aerospace research, and development in AEA and Mintech establishments. Existing projects are not expected to be cut back, however. Government research and development is estimated to cost £66·8 million in 1971–72, compared with £71 million in 1969–70. (Written answers, January 19.)

Miscellaneous Intelligence

MR ROBERT GRAVES, the poet, has a bone to pick with science. "My main objection to science," he explains in the current issue of *Impact of Science on Society*, "is that it has spoilt the relation between the sexes. . . . Science and technology have interfered with natural sexual expression by making men and women no longer sure of themselves as men and women. . . . (They) have diminished the romantic relationship . . . and all but destroyed the pleasure and joy of sex, which is where everything good in life starts." Scientists who are mortified by this plangent indictment may take heart at the equally unrestrained pronouncement delivered in the same journal by Miró: "Scientists reveal nature's secrets as we artists do, but from another angle. Those who reflect and explore belong to a superior race. They are the princes . . . the real princes of today". To practise science, it seems, is to be roundly misunderstood on all sides, but better perhaps to be thought a Mrs Grundy or one of nature's aristocrats than to be ignored altogether.

IF any of the social sciences is a science, social anthropology is, but its rigour seems to depend on the long period of introspection that intervenes between studying the tribe and writing up the results. Articles in the current issue of *Man*, for example, are based on fieldwork carried out among the Limba in 1963–64, in the Coimbatore District of Madras State in 1965–66, and in Arnhem Land in 1965.

THE brave new image of the British Post Office has received so severe a tarnishing that it seems scarcely fair to drag even an ancient skeleton from its cupboard. But an article in the July 3, 1926, issue of *Nature* is too topical to wait its turn in *100 Years Ago*. In that week, *Nature*'s intrepid reporter visited the rooms of a Mr J. L. Baird in Upper St Martin's Lane, London WC, and was agreeably surprised to see the progress he had made in the problem of "seeing by telegraph" or television. "It is natural that Mr Baird and his partner, Captain Hutchinson, should contemplate a great future for television. Every possessor of a 'televisor' will be in a position to see on his screen the performers in operas and plays as well as hearing them. The new discovery will in no way interfere with the ordinary British broadcasting. The Post Office officials, seeing the probable advent of a new British industry, regard the scheme with *benevolent neutrality*." Let the faces of all servants of the NRDC blush pillar box red at thought of it.

FUTUROLOGY, it seems, is just wishful thinking after all. Almost the only surprising prediction of *Medicine in the 1990's*, a 'Delphi' method forecast produced by the Office of Health Economics, is that "the large international drug companies will develop greater political awareness, so that they may be able, for example, to exert considerable influence in the framing of national regulations for the control of medicines. . . . The dangers of state participation as a stifling influence on competition and innovation are all recognized in the advanced countries of the Western World". Nobody would guess it, but the Office of Health Economics was founded and is supported by the Association of British Pharmaceutical Industry.

University Rubs Shoulders with Industry

from our Social Medicine Correspondent

Newcastle upon Tyne, January

TWENTY-FIVE years ago the Department of Industrial Health was set up at the University of Newcastle upon Tyne (then King's College, University of Durham) with the aid of a £40,000 grant from the Nuffield Foundation. Housed, to begin with, in the medical school and staffed by a skeleton team of twelve, the department has since grown under the guidance of Professor R. C. Browne into a large and active assembly of semi-autonomous sections, inconveniently divided between the medical school and an elegant row of buildings less than a quarter of a mile away. In spite of the increasing teaching load, applied research continues to receive heavy emphasis and it is refreshing to see the way in which medical and non-medical staff work harmoniously together.

In addition to university financing, now approaching £50,000 a year, the department has also received generous support from the Science and Medical Research Councils, the National Coal Board, the Central Electricity Generating Board and the Nuffield Provincial Hospitals Trust among others. The largest grant so far—£200,000—has come from the Department of Health and Social Security. Made over to the department in late 1969, the grant will make possible the setting up of a new Medical Care Research Unit, which is to be jointly directed by Professor D. J. Newell, currently head of the department's medical statistics section, and Dr John H. Walton. The general interests of the unit will be directed towards problems inherent in the unification of health services: attempts will be made to establish norms in the provision of medical care and the response to its provision; to determine the pattern and efficiency of transfer between different sources of medical care; and to investigate the relationship of preventive, medical and social care, particularly in childhood. Work currently in progress in the medical statistics section includes a statistical analysis of the causes and methods of treatment of low-back pain—part of a multi-centre trial coordinated by Professor Newell. As well as carrying out research, the section's staff provides a consulting service for the design and analysis of clinical trials and medical surveys in the medical school and teaching hospitals.

Professor R. C. Browne, an expert on coal miners' pneumoconiosis, is head of the Department of Industrial Health.

Dr J. Steel heads the active and dual functioning occupational hygiene section. On the one hand, research is carried out into the various problems linked up with health and hygiene in industry. For example, much effort is concentrated on predicting the health risk to welders and burners who are exposed to fumes containing oxides of the toxic elements copper, zinc, nickel and cobalt which result from the decomposition of surface coatings during application of intense heat. These elements can cause, among other things, metal fume fever and metal poisoning. As a result of this work, the section has acquired a national role for the pre-testing of paints and electrodes before manufacturers put the products on the market. Another current line of research is the development of a sensitive method for detecting lead poisoning among workers using small samples of blood. Current methods require 5–10 ml. of blood. By looking at the way in which lead is in part organically bound to blood and urine, Dr Steel hopes to be able to predict susceptibility to lead poisoning in certain persons.

The section also tackled particular problems for local industries on a fee-for-service basis, as part of the North of England Industrial Health Service. This service, which is based in the Department of Industrial Health, has its own management committee consisting of representatives of the university, management and the Trades Union Congress and is virtually self-supporting. Although most of the work is done on a contract or consultative basis, some firms have joined as members, paying so much a head. This entitles them to advice on any matter relating to health at work and they also receive a regular visit from one of the service's medical officers. A new feature of the service is the launching this month, in Washington New Town, an industrial estate, of a group occupational health service under which an appointed nurse will make daily visits to member firms on the estate to administer first aid and to help in social and welfare matters. She will be backed up by a medical officer and may in future be joined by staff offering physiotherapy, chiropody and ophthalmic services.

Dr R. I. McCallum is currently perfecting a technique for measuring directly the amount of antimony trioxide in the lungs of process workers using an iodine radioactive isotope and a scintillation counter. Antimony pneumoconiosis, which is more of an inconvenience than a serious clinical problem, affects workers in one factory in the north-east, but the detection technique can be applied equally well to measuring tin in the lungs—a more common hazard. Dr McCallum is attempting to induce antimony pneumoconiosis in cats so that the amount of the element measured in living lungs can be compared with the amount present at autopsy as measured by, for example, atomic absorption spectroscopy. This will indicate the accuracy of the X-ray method.

Two other aspects of the department's varied research programme include an investigation into the effects on the lungs of breathing in radioactive xenon—headed by Dr H. L. Leathart—and a continued ergonomic study of industrial component inspection using a conveyor belt. This study is being conducted by Mrs Judy Lion.

Also housed in the department is the Decompression Sickness Registry, directed by Dr P. D. Griffiths. Although the Medical Research Council grant supporting the registry is made to Professor D. N. Walder, in the Department of Surgery, who is concerned with the significance of gas micronuclei in the aetiology of decompression sickness (see *Nature*, **222**, 251; 1969), the records and radiographs of thousands of men who have worked in compressed air are kept in the department. So are the new decompression tables giving the most suitable length of time for decompression, which are being widely used in compressed air work on an experimental basis. Under these tables, a man working at 44–46 pounds per square inch—almost the limit for civil engineering work—is required to decompress for 270 minutes compared with the 127 minutes required under government tables. Working in close association with other members of the MRC Decompression Sickness Panel, Dr Griffiths and Dr McCallum periodically arrange for X-rays to be taken of the limbs of men in compressed air work in the hope of detecting, at an early stage, aseptic necrosis of the bones.

Growing Pains in British Universities

by our Education Correspondent

THE next few years could see major changes in the British system of higher education. The number of student places must double in the next ten years: that now seems to be recognized both by the government and the universities, and it is unlikely that the system will come through such expansion unchanged. But the changes will inevitably be accompanied by their fair share of arguments. Preliminary skirmishes have already been made—the Department of Education and Science, in October and December last year, discussed, with the Committee of Vice-Chancellors and with the Association of University Teachers, various ways to achieve the expansion as cheaply as possible. It seems likely, however, that both bodies will reject most of the proposals put forward, and Mr Gerald Fowler, Minister of State with responsibility for higher education, has already complained that academics are being hypersensitive to alleged threats to academic freedom.

The controversy has been brought to a head by the latest predictions of the demand for higher education in the 1970s. Using the Robbins basis of relating entry to higher education with numbers of school leavers, the ministry predicts that 437,000 students will be engaged in higher education in 1971–72, 557,000 in 1976–77 and 727,000 in 1981–82. It suggests, however, that colleges of education will only increase their intake by about 20,000 in the next ten years, and the universities and further education establishments will therefore bear the brunt of the expansion. Such an expansion will require about a 7 per cent annual increase in student numbers, and it is unlikely that expenditure on higher education will be allowed to grow at the same rate. Faced with the demand for places outstripping the available resources, Mr Fowler has been casting around for ways to reduce the costs per student, and it is this which has stirred up the rumblings in the universities.

Analysis of the Department of Education and Science's figures for 1959–60 to 1967–68 suggests, however, that the government may already be reducing the costs per student. Fig. 1 shows that expenditure on the universities rose at about the same rate as student numbers between 1959 and 1964. But after 1965, when the Colleges of Advanced Technology were given university status, costs rose less sharply than student numbers. Even if these trends were continued, it is doubtful whether it would effect the saving which Mr Fowler wants, and moreover, expenditure on new buildings will inevitably tend to push costs up. For one thing, expansion of this order would require that several new universities must be established. It is reasonable to expect that existing institutions can do no more than treble in the next decade (only Reading and Strathclyde have done this in the past ten years), and most universities believe that about 12,000 is the maximum size that they could achieve without becoming completely impersonal establishments. Even if existing universities expand as quickly and as far as possible, the expansion would therefore fall short of the target for 1980.

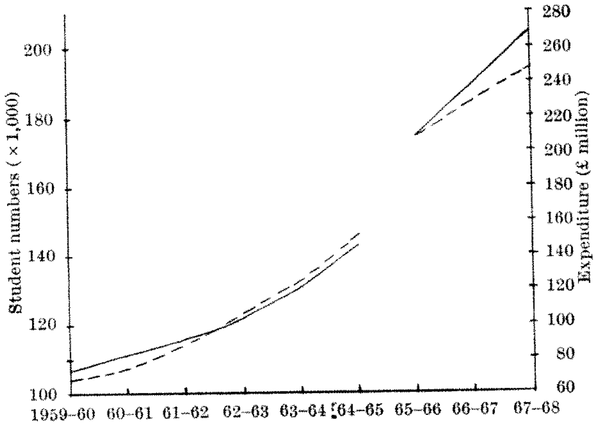


Fig. 1. Student numbers and university expenditure, 1959-68. ———, Student numbers; — — — —, university expenditure.

Some change in the system therefore seems inevitable, and the Department of Education and Science has suggested thirteen proposals to the vice-chancellors and the AUT for their consideration. Individual vice-chancellors will submit their own views to the Vice-Chancellors Committee probably early in February, and the AUT has set up a working party to formulate a policy for the universities in the 1970s. This policy will be presented to an extraordinary council meeting probably in March. At least twelve of the thirteen points are likely to be rejected by both bodies.

The proposals which seem to have caused the most concern are those which seek to alter the length of degree courses and the number of terms in a year. Among the points put to the vice-chancellors were proposals for replacing the present three-year courses with two-year courses of four terms a year, and for introducing a "box and cox" arrangement, in which the university timetable would be arranged in such a way that two sets of students could be taught in the same year. A similar arrangement is at present being operated by some colleges, one set of students being taught when the other is on vacation or industrial training. The vice-chancellors maintain, however, that alteration of the university year would inevitably reduce the time available for research and would place an intolerable burden on teaching staff. Sir Derman Christopherson, chairman of the Committee of Vice-Chancellors, speaking at the North of England Education Conference on January 9, also maintained that the three-year degree course and the present low drop-out rate would become more important in the future.

The Department of Education and Science also canvassed the possibility of student loans and of increasing the percentage of students living at home as measures likely to effect some reduction of cost, but both these suggestions will be bound to antagonize the National Union of Students, and it seems that the Association of University Teachers and the Vice-Chancellors would also oppose them. Sir Derman Christopherson, for example, said that students and their parents are already finding about £20 million a year towards making up their grants, and that simple commercial loans would tend to restrict higher education to those who could afford to pay for it. Professor Michael Swann, vice-chancellor of Edinburgh University, commenting on this, said that loans would not save very much money, but he suggests that neither students nor staff are as violently opposed to their introduction as is usually claimed.

Unless the number of staff increases at the same rate as the number of students in higher education, the staff/student ratio will inevitably suffer, and this is an aspect of the proposed expansion which seems to alarm many academics. The ratio in British universities, about 1 : 8 or 9, is, however, much lower than in most other countries, and Sir Derman hinted at the Northern Education Conference that he is prepared to see it raised slightly. He suggested, for example, that some departments could accept more students without parallel staff increases and without loss of quality. Professor Swann also suggests that if research facilities were expanded at the same rate as student intake, then some deterioration in research quality may be unavoidable. He therefore sees the higher education system developing more along the lines of the American system, with postgraduate schools becoming further removed from undergraduate teaching, but he indicated that he thought this to be a step in the wrong direction.

None of the proposals put to the vice-chancellors suggests that the government has altered its thinking on the binary system in higher education. One proposal did suggest, however, that there should be more sharing of resources between the universities and the polytechnics, and this is unlikely to be opposed by the vice-chancellors or by the AUT. There are indications, however, that the universities may be prepared to see the binary system eroded away. Sir Derman told the Northern Education Conference that "the separation of the education and training of most teachers from that of other students is an unsatisfactory arrangement which ought to be brought to an end as soon as possible", and he suggested that colleges of education should open their doors to students taking general degree courses, courses in social services and possibly science courses as well.

The AUT is likely to oppose the binary system outright. Like the National Union of Students, it sees the system as wasteful of resources, and it is bound to perpetuate the elitism of the universities. The AUT working party on policy for the universities in the 1970s suggested to the AUT annual council in December that an expanded University Grants Committee should distribute capital and recurrent finance to universities, polytechnics and in due course to colleges of education. A very similar system was proposed by the Select Committee on Education and Science last year, when it suggested that a Higher Education Commission should finance all sectors of higher education. But the government has given no indication that it intends to act on the Select Committee report, and indeed, maintaining the binary system seems to be central to its thinking on higher education expansion.

In a speech to the NUS Universities Conference on January 6, Mr Jack Straw, NUS president, argued that expansion is absolutely vital, and that the NUS regards the figures for student numbers in 1980 as a low basic minimum. The NUS also believes that the binary system should go, and Mr Straw argued for comprehensive institutions of higher education to be established. These institutions, called "polyversities", should provide a variety of courses, as wide as the universities, the polytechnics and the colleges of education combined, and they should accommodate both full and part-time students.

Both the NUS and the AUT are, however, less explicit about where the necessary finance should be found. Indeed, Mr Straw throws that problem back at the government by suggesting that it is not the duty of a pressure group like the NUS to do the work of the Treasury. He maintained at the Northern Universities Conference, however, that Britain lags behind most other advanced countries in an international league table of education expenditure. Using 1962–63 figures, Mr Straw said that the USSR spends about 8 per cent of its GNP on education, Japan, Holland and Canada each spend about 7 per cent and the United States spends about 6·3 per cent. Britain, on the other hand, devotes only about 5·8 per cent of its GNP to education. Mr Straw therefore suggests that the government is throwing a "smokescreen of panic" over the whole issue, and that the proposed expansion would do no more than bring Britain into line with other countries.

Although it seems that there is to be considerable controversy about the means of achieving the proposed expansion in higher education, both the government and the universities accept that it is necessary, and that doubling the numbers of students will not necessarily cause a deterioration of standards. Sir Derman Christopherson maintains that standards have been rising during the 1960s, although student numbers increased at an unprecedented rate, and Mr Fowler said that the doctrine that "more means worse" is patently untrue if one looks at the experience of other countries or of Britain during the past decade. If the projected figures for student intake in the 1970s are not met, competition for places in 1980 would be even stiffer than it is now, and the proportion of students undergoing some form of post-school education would be well below that of most other advanced countries. Moreover, even if provision in higher education were made for everybody who has minimum entry requirements now, Britain still would not have a mass higher education system equivalent to that in the United States.

Signs of Change at UGC

THE shortage of funds of which British academics have been complaining in the past year seems now to be real enough to have caused an actual decline in the cost of university education. This is apparent from the latest Annual Survey (for 1968–69) of the University Grants Committee (HMSO, 4*s*), which shows that in terms of 1967–68 prices, the cost of educating a full-time student fell from its peak of £900 a year in 1966–67 to £877 a year in 1968–69. The cost of paying academics has decreased steadily for the past four years, and now amounts to £357 per student per year, chiefly because of the slow increase of the ratio of students to staff, which is now greater than at any time since 1962 even if the British ratio remains, as the UGC reminds its pensioners.

The UGC is now half-way through the most turbulent quinquennium for a long time. The new report is the first to be presented by the new chairman, Mr Kenneth Berrill, who succeeded Sir John Wolfenden in January 1969. The quinquennium has already seen the controversy about the report of the Prices and Incomes Board on academic pay (December 1968), a strike by university technicians, a running battle (or skirmish) between the UGC and the universities over attempts to assess the productivity of academic people, the decision that the accounts of universities should be open to inspection by the government's inspector, the Comptroller General, and the perpetual quarrel about money. The new survey looks all these issues in the face but seems to dodge altogether more important issues of public policy—the relationship between the universities and the polytechnics. Nobody will take offence.

The intervention of the Prices and Incomes Board has clearly sharpened the aim of the Association of University Teachers to participate in formal machinery for the negotiation of salaries, although it seems clear that the question has not yet been settled of whom the AUT should negotiate with. The UGC is the obvious adversary, but everybody knows that it does not have the power to summon up the money needed to settle pay increases which may be forced on it, yet negotiation directly with the Treasury, which really holds the purse-strings, would undermine the doctrine of the autonomy of the university system. In default of a solution, the Prices and Incomes Board has been retained as referee, and a pay settlement based on data soon to be available will be back-dated to the beginning of the current academic year.

The UGC continues to provide funds for schemes which foster collaboration between universities and industry—many of the 38 grants now current (which will cost £190,000 in the current year) are for training courses in subjects such as cryogenics and food engineering, but three universities (Bath, Heriot-Watt and Dundee) have been blessed with industrial liaison officers while others have acquired lecturers in subjects such as management. These grants are only available for periods of three years, and it is laid down that the Science Research Council will often be involved in the making of decisions about new courses.

A kind of raffle among universities seems to have succeeded in increasing the number of places for teacher trainers in university departments of education by about 250—the UGC says there has been a good response to its scheme for paying to universities willing to increase their effort in teacher training sums of money which are multiples of £2,500 in each of the last two years of the quinquennium. There has been a similar success with even smaller sums of money as prizes in the provision of 150 extra places for psychiatric social workers, but management education remains a running sore—the two schools at Manchester and London are financed through the Foundation for Management Education, which is in turn dependent on subscriptions from private business and the government, but the UGC now calculates that there will be a rapid growth of its own responsibilities for management education at other universities. Outsiders will marvel that the two systems seem destined for indefinite co-existence.

If the UGC is a little too obviously anxious not to give offence in what it has to say about medical education, there is a robust if modest declaration of more support for marine biology, one of the chief features of which will be the setting up of the Scottish Marine Biology Association at Millport as a common university facility under the Natural Environment Research Council when the present staff moves into its new premises.

On the administration of university finance, the UGC is plainly (and rightly) proud of its scheme to devise a computer model of the university system so as to help with the allocation of funds, but a little nervous in its account of how it has rejected the advice of the Committee of Vice-Chancellors that at the beginning of future financial quinquennia, universities should be told all at once how much they will have to spend. The UGC argues that the first of the five years should be dealt with by means of a provisional settlement, although it does undertake to provide estimates of the likely size of the student population. The committee says that it has taken the opportunity provided by the government decision that local authorities will not in future be expected to help in financing awards to postgraduate students to set up, jointly with the research councils, a Committee on the Co-ordination of Postgraduate Awards.•

NEWS AND VIEWS

Up or Down?

THE assertion of the report of the Royal Commission on Population in 1949 that there would be "a substantial decline in the number of births over the next 15 years" has often been contrasted with the rise in the number of births in Great Britain during the 1950s.

How far has the science of population projection progressed in the 20 years since such estimates were first made? This question must be in the mind of anyone reading the article on recent changes in the birth rate of England and Wales by Eric Thompson, chief statistician of the Research and Intelligence Unit of the Greater London Council, in the current issue of *Family Planning* (**18**, No. 4; 1970). Thompson discusses the decline in the number of births in the past 5 years and the assumptions made about this decline by the Government Actuary in preparing the official population projections for England and Wales to the year 2001. In preparing the projections based on mid-1968, the Registrar-General commented that the post-1964 downward trend seemed to be slowing and was regarded as a temporary aberration in a secular increase. Thompson maintains that this is not so and that recently births have returned to the post-1964 downward trend.

The decline in the number of births since 1964—about 8 per cent in the 5 years to 1969—has been unexpected. Most of the variables which influence the number of births have been moving in ways which would lead to an expectation of increasing births. The number of women aged 20 to 29 increased by 11 per cent between 1964 and 1968 and will increase to 30 per cent over the 1964 figure by the mid-1980s. If the birth rate had remained constant at its 1964 level, this increase in the number of women in the most fertile age group would have led to a total of births in 1969 about 15 per cent greater than that which actually occurred. Age at marriage is decreasing, and because more than 90 per cent of births occur within marriage, other things being equal, this increases the percentage of women in the fertile age groups "at risk" of pregnancy. Changes in the proportion of women married at any particular age will have a short term effect on the birth rate because the range of potential changes is obviously circumscribed at both ends of the age range, more especially at the younger ages, but changes in the mean completed family size of marriages contracted at different times can have a more profound effect on the total of births.

The downward trend in the number of births forced a revaluation of the assumptions about mean completed family size for marriages recently contracted and still to be contracted. In particular, the positive differential in mean completed family size for marriages contracted at an early age (under 20) has been reduced because fertility at early ages has not continued to increase as it did during the past decade. There seems to be a convergence with the decreasing fertility of older ages. Thompson wonders whether the next set of projections should incorporate a more substantial narrowing of the early marriage–late marriage fertility differential, and questions whether it is correct to assume any increase in mean completed family size for current marriages.

It is hard to escape from the fact that the 5 year decline in the number of births is really too short a period on which to base any conclusive arguments. The reduced number of children born during 1964–69 is, however, a fact and must have an effect on the future age structure, and, from about 1984 onwards, on the level of future births. The reduction in the strength of the relationship between early marriage and high fertility seems to be the key to the enigma of the current pattern of number of births. Detailed studies alone will reveal the motivation behind this trend and it is important for assessing the future number of births to discover whether this relationship will soon stabilize somewhere near current levels, or whether the decline will continue until the marriage age differential in completed family size becomes much less marked than at present.

SEYFERT GALAXIES

A Cornucopia of Matter

by our Astronomy Correspondent

SEYFERT galaxies with their extraordinarily bright and unusual nuclei could easily be intermediaries between quasars and normal galaxies. Indeed, part of the evidence is that even superficially normal galaxies such as the Milky Way have a hint of abnormality about them. Yet it is far from settled in which direction the evolution is going. Obviously the more that can be found out about Seyferts the better, and in the current issue of *Astrophysical Journal* Kurt Anderson and Robert Kraft of Lick Observatory present evidence that matter is being ejected from the nucleus of the Seyfert galaxy NGC 4151 (**158**, 859; 1969).

They have shown that the nucleus of NGC 4151 seems to be losing mass at a rate which raises questions about where it is all coming from. Making several reasonable assumptions Anderson and Kraft find that the mass loss is something between 10 and 1,000 solar masses per year, but how nature would provide such an apparently inexhaustible cornucopia of matter, they say, remains decidedly unclear. Conceivably the mass loss of NGC 4151 could pose the same sort of problem as the energetics of the Crab Nebula which should have run down long ago.

The starting point of the study is a coudé spectrogram of the nucleus of the Seyfert obtained in a 5 hour exposure. It shows an absorption feature buried between two emission lines which was first recognized, it seems, by N. U. Mayall in 1934. Anderson and Kraft maintain that the feature between 3869 and 3889 Å is composed of three shell lines of HeI with velocities of -280, -550 and -840 km s^{-1} relative to the corresponding emission line, although there is reason to believe that the -840 km s^{-1} component is distorted by the neighbouring NeIII, Hζ and HeI emission lines so that it ought to be -970 km s^{-1}.

By making assumptions based on other features of the spectrum about the conditions of the clouds or shells of gas which are responsible for the three absorption lines, Anderson and Kraft are able to make estimates of the masses involved. One question is whether the outward flowing mass is moving with the escape velocity. Observations of other Seyfert galaxies suggest that the mass of the nucleus is between 10^8 and 10^{10} solar masses, and if the true value is in the upper half of this interval, 10^9 solar masses or more, probably none of the gas escapes. Yet the spectral lines which would be expected from gas falling back into the nucleus are absent. Assuming then that the observed material is being lost, something between 10 and 1,000 solar masses per year (depending on whether a cloud model or shell model applies) has to be accounted for. Clearly there is a lot to be explained.

STRUCTURE

Conflict in Mercury

by our Solid State Physics Correspondent

THE structure of the low temperature phase of solid mercury has been measured for the first time by X-ray diffraction and found to be different from the form predicted by non-local pseudopotential theory. J. S. Abell, A. G. Crocker and H. W. King (*Phil. Mag.*, **21**, 169; 1970) have produced a crystal sample of γ mercury by straining α mercury at 20 K, and have found four peaks in the diffraction pattern which could not have arisen from the face centred rhombohedral structure proposed by Weaire (*Phil. Mag.*, **18**, 213; 1968).

These results have both theoretical and experimental implications. Although only preliminary, they suggest that even the moderately sophisticated non-local pseudopotential used by Weaire is too inaccurate to distinguish between the two types of structure. The lattice spacings found by Abell *et al.* leave little room for doubt here, for even a generous variation of the axial angle cannot bring the theory into line. Experimentally, the chief difficulty was to strain the sample sufficiently to bring about the transformation to the γ phase and yet avoid fracture. A special jig was built for the purpose. The γ phase may well be thermodynamically stable at low temperatures, but the problem is to find some way of nucleating it which avoids generating too many common interfaces with the remaining α phase, thus producing severe strain in the lattice. The diffraction peaks showed the characteristic broadening of a strained lattice.

A detailed electron microscope study of the cubic form of solid nitrogen has thrown new light on the structure of this phase. J. A. Venables (*Phil. Mag.*, **21**, 147; 1970) has found that the grain boundaries move remarkably easily in α nitrogen, much more so than in oxygen or the rare gases, due partly, it is suggested, to the shape of the nitrogen molecules. Various imperfections were also observed, including dislocation loops and stacking faults. Venables points out that the free energy of stacking faults and twin boundaries may well be very dependent on temperature, implying that these defects might act as nucleation centres for the α–β phase transformation. Barrett suggested last year that small scale diffusion takes place across a large angle boundary in this transition, and Venables hints at the exciting possibility of actually observing this process in the electron microscope.

The *Philosophical Magazine* took on its new mantle as a Europhysics journal this month, but apart from an editorial hint of wider European participation on the board in the near future there was little sign of any new aspect to the journal. Indeed, of the thirty-six authors contributing to this issue, thirty are from Britain, five from North America and one from Africa. Perhaps the forthcoming meeting in Paris of the publications committee of the European Physical Society can inject some life into a policy which, although widely approved, is still felt by many to lack the credibility of a serious project (*Nature*, **224**, 1146; 1969).

MAGNETIC MONOPOLES

Sea Bed Search Sets New Limits

from our Cosmology Correspondent

NEW limits have been imposed on the possible abundance of free magnetic poles after a search for monopoles on the sea bed. In spite of the theoretical view, first proposed by P. A. M. Dirac (*Proc. Roy. Soc.*, A, **133**, 60; 1931), that the existence of free magnetic poles in nature is not only completely within the understanding of the laws of physics but also improves the symmetry of Maxwell's equations, no experimenter has been able to prepare or detect such free poles. The best limits on the existence of free monopoles have recently been reduced by a factor of several thousand by R. L. Fleischer *et al.* (*Phys. Rev.*, **184**, 1393; 1969), who have attempted to detect monopoles originating in cosmic rays using the Earth's oceans as a collecting tank for these energetic particles.

Having been thermalized in the atmosphere and oceans, suitably polarized poles would drift to the ocean bottom where they would be trapped in solid, magnetic material. The particular ferromanganese deposits used, taken from the mid-Atlantic, are particularly suitable because the slow rate at which this sediment was deposited means that a thickness of only 2·5 cm represents a continuous sample over at least 16 million years, while its magnetism will have prevented the escape of any poles. Removing any free poles present by means of a strong magnetic field, and detecting them by extending standard techniques for detecting cosmic ray particles, is, of course, a simple matter, but completely negative results were obtained by Fleischer *et al.* Because of the long time during which the collector—the sea bed—has been in operation, it is clear that the flux of free monopoles arriving at the Earth must be less than $4 \cdot 2 \times 10^{-18}$ cm^{-2} s^{-1}, up to an incident energy of $\sim 2 \times 10^{19}$ eV, a total of less than 1/2 monopole cm^{-2} during the entire age of the Earth.

A search for tracks left by monopoles in natural minerals has proved similarly unrewarding (R. L. Fleischer, P. B. Price and R. T. Woods, *Phys. Rev.*, **184**, 1398; 1969). The only remaining possibility for the existence of monopoles therefore seems to lie in the region of very high mass particles, but this must be a slender chance.

MOON

Mascons may be Plates

by our Astronomy Correspondent

THE mass concentrations beneath the lunar maria which P. M. Muller and W. L. Sjogren reported in 1968 may have a plate-like structure, according to M. F. Kane of the US Geological Survey in the *Journal of Geophysical Research* (**74**, 6579; 1969). This result, which should please people who have been saying that the mascons can be understood in terms of lava flows, was obtained from an analysis of the data for Mare Imbrium. The discovery of mascons came, of course, from detailed tracking of the lunar satellite Orbiter 5, and what Kane has done is to compare the form of the gravity anomaly over Imbrium with that expected for several mascon models.

In their discovery paper, Muller and Sjogren assumed rather arbitrarily a depth of 50 km for the mass concentrations, which meant that the Imbrium gravity anomaly required 20×10^{-6} lunar masses. For a nickel–iron sphere such as an asteroid this would correspond to a diameter of about 100 km, but it raises all sorts of problems about the survival of an object of that size striking the Moon.

Clearly other configurations are possible, and Kane claims that a plate-shaped mascon suits the data better. The problem with a spherical mascon is that although the fit to the data can be improved by bringing it closer to the surface, if the radius is to be kept equal to the depth of the centre the density becomes inordinately high. A suitable mascon, according to Kane, would be a 10 km thick layer at the surface, and if it were 400 km in diameter it need have a density excess of only 1 g cm^{-3} over its surroundings.

Unfortunately although the plate mascon is a good approximation near the centre of the gravity anomaly, it is as unsatisfactory as the spherical mascon elsewhere. Kane suspects that the answer might be found when the topography of the lunar surface is allowed for in the calculations. The ring of mountains around Imbrium, for example, has yet to be taken into account.

It is satisfying that this approach fits nicely with what people have been saying at Houston. Given that the maria were once vast lakes of lava, it seems possible that high density crystals might have sunk to the floor. This kind of structure is seen on the Earth, and density differences of the right size ought to be possible.

The principal implication of Kane's report, however, is that the distinction between a plate mascon and a spherical mascon is clearer in data taken from satellites nearer the surface. In the eighty orbits considered by Muller and Sjogren, the closest approach to the Moon by Orbiter 5 was 100 km. A 30 km orbit would be better for observing mascons. The point is that this kind of relatively simple satellite would give a lot of information about the interior of the Moon which, apart from pickings from deep impact craters, is otherwise inaccessible.

SEA FLOOR SPREADING

Is the Rate Constant ?

from our Geomagnetism Correspondent

THE magnetic evidence in favour of sea floor spreading was almost certainly the most important single geophysical result of the 1960s. The discovery that the pattern of magnetic anomalies normal to mid-oceanic ridges is similar to the polarity–time scale derived from continental rocks was the first significant support for sea floor spreading, and led to the first really convincing tectonic model for the Earth as a whole. But there are still a few details to clear up. For example, the rates of sea floor spreading calculated from almost all of the magnetic anomaly patterns covering the past 3 to 4 million years involve a decrease about two million years ago followed by an increase about 0·9 million years ago. Why should this be ?

Because the two discontinuities in the rate of spreading appear in all profiles from the Atlantic, Pacific and Indian Oceans, it has generally been assumed that they are real—as indeed they may be. The theory of plate tectonics, for example, which implies that all ridges are interrelated suggests that any change in spreading rate should be world-wide. But Emilia and Heinrichs are suspicious (*Science*, **166**, 1267; 1969). They point out that even if all ridges are related, it is unlikely that a universal discontinuity in the rate of spreading would be in the same sense at every ridge. Since this seems to be the case, it is much more likely that some of the accepted relations between the anomalies and the palaeomagnetic scale are incorrect. In this case, all spreading rate curves would contain the same error. They would all tell the same story, but it would be wrong.

The evidence that Emilia and Heinrichs adduce for this hypothesis is rather convincing. The first ocean magnetic anomalies were compared with the continental time scale before the discovery of the Gilsa event, which occurred about 1·65 million years ago. As a result, one particular anomaly was identified with the Olduvai event 1·96 million years ago, a practice which was continued even after the discovery of the Gilsa. But is this the correct thing to do ? It turns out that the spreading rate discontinuity 2 million years ago depends solely on this one identification. If the particular anomaly in question is now identified with the Gilsa event, the rate of spreading between 0·9 and 3·32 million years appears constant.

There is, of course, no *a priori* reason why the rate of spreading should be constant. But not only does it make the solution neater, it also ties up one or two other loose ends. Between the anomaly previously associated with the Olduvai event and the end of the

Gauss normal epoch, two further anomalies, designated *W* and *X*, frequently occur either as a pair close together or as a single anomaly. These have never been identified with any event or events on the continental scale. But if the rate of spreading between 0·9 and 3·32 million years is assumed constant, *W* turns out to have an average age of 1·99 million years, very close to the measured age of the Olduvai (1·96 million years). Anomaly *X*, with an average age of 2·3 million years, still remains unexplained but is less important in the sense that whenever only one anomaly appears in the region it is always *W*.

A further objection to the variable rate spreading interpretation is that the Gilsa event is left without a corresponding ocean magnetic anomaly. The important result, however, is that with the removal of the larger spreading rate discontinuity at 2 million years, the rate of spreading over the whole of the past 3·32 million years can be regarded as constant within experimental error—an altogether simpler solution which has the merit of explaining more of the observations than does variable rate spreading.

COMETS

OAO views Tago-Sato-Kosaka

by our Astronomy Correspondent

INTERESTING data continue to flow down the telemetry channels from the Orbiting Astronomical Observatory (OAO 2) which NASA launched in December 1968. By a happy chance, the brightest comet for several years has now appeared in the sky, and the OAO is being used to secure the first observations of a comet in the ultraviolet. This follows surprising results obtained by the OAO last year and released by the Americans in December (*Nature*, **224**, 1058; 1969) which seem to show that a few galaxies are unexpectedly bright in the ultraviolet.

Ultraviolet light below about 3000 Å is, of course, absorbed in the atmosphere, which among other things makes the important Lyman α line of hydrogen (1216 Å) inaccessible from the ground. With orbiting ultraviolet telescopes now available in the OAO, one of the results should be a better knowledge of the distribution of hydrogen in space.

In this spirit NASA announced last week that Lyman α observations of Tago-Sato-Kosaka by the University of Wisconsin instruments on OAO 2 show that the comet is surrounded by a cloud of hydrogen gas which is said to be comparable in size to the Sun. The hydrogen is presumably being ejected from the comet by solar heating, and will be interpreted in terms of Whipple's "icy-conglomerate" theory. This has it that the nucleus of a comet is made up of mineral particles bound up in a matrix of frozen gases such as water and ammonia—a flying gravel bank, in other words. Some of the gases vaporize when the comet approaches the Sun, to form a cloud known as the coma, but the size of the coma is small compared with the cloud of hydrogen which is now reported.

Following the usual practice, Tago–Sato–Kosaka is named after the three Japanese amateurs who independently discovered it on October 10 last year. (The Japanese have of course become dab hands at spotting undiscovered comets.) By December the comet was visible by eye in the southern hemisphere, moving north, but it is now retreating from the Sun and unlikely to be visible by eye into February. On February 2 it will be in Aries, near Alpha Arietis, at about magnitude five—the naked eye limit.

The Wisconsin instruments acquired Tago–Sato–Kosaka on January 14, and the plan is to follow the hydrogen cloud for as long as possible. The chief experimenter is Dr Arthur D. Code, and the schedule seems to be to observe the comet for 10 per cent of each 100 minute orbit.

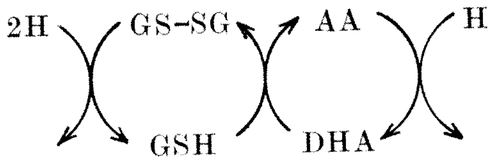
CELL DIVISION

Retine for Inhibition

from our Cytogenetics Correspondent

THE suggestion that dehydroascorbic acid is an inhibitor of cell division is a recent contribution to the discussion of how this process is controlled. Cells of a living organism may be actively dividing or they may be quiescent. How is the transition from one state to the other brought about? And how do cancer cells differ in their regulation of cell division? The task of answering these questions is formidable. One line of attack has been opened up by Szent-Györgyi and his colleagues. They argue that two regulators of division are involved; there is an inhibitor, "retine", and a promoter, "promine" (*Science*, **149**, 34; 1965). Szent-Györgyi's group identified retine as a keto-aldehyde, the simplest member of which is methylglyoxal. Keto-aldehydes are good electron acceptors and inhibit cell division and their effect can be reversed by thiols (molecules with —SH groups) which are good electron donors and so behave as promines (Együd, *Currents Mod. Biol.*, **1**, 14; 1967 and **2**, 128; 1968). These properties led Szent-Györgyi to propose (*Science*, **161**, 988; 1968) that the decision as to whether a cell would divide or not was controlled by the relative balance of retine and promine molecules. Stimuli to cell division, such as wounding of a tissue, cause the removal of retine molecules. In cancer cells it may be that retine is not produced and so the biochemistry of the cell favours cell division.

Much work remains to be done to support such concepts. Now, J. A. Edgar joins the fray with more ideas (*Experientia*, **25**, 1514; 1969). He accepts Szent-Györgyi's scheme but suggests that dehydroascorbic acid (DHA) may be a retine substance. DHA is widely found in animal and plant cells; it is an electron acceptor and is reversibly associated with ascorbic acid (AA). The reaction DHA⇄AA is mediated by glutathione (GSH), an electron donor that for many years has been thought to be implicated with cell division, in the following way



Edgar summarizes a large body of evidence which indicates that the balance between GSH and DHA may control the ability of cells to divide. For example, in plants it has been found that in cells leaving the dormant state (as in seed germination) the amount of DHA decreases while the amount of AA and GSH increases. Furthermore, in tissue induced to grow by the plant hormone indolyl-3-acetic acid a similar state of affairs is found. In animal tumours DHA has been found to be an inhibitor of growth, and some neoplastic cells seem to be deficient in DHA.

Another of Edgar's proposals is that in animal cells a balance of DHA and AA, with AA in excess, could control collagen synthesis, for one step in this process is rate-limited by AA. In this way collagen production would be strictly coordinated with the growth of the tissue.

Some crystal gazers believe that cancer will be controlled by the end of the century, and it may be that electron transfers between retine and promine molecules hold the key to the control of cell division.

NUCLEIC ACIDS

Hybridization and Satellite DNA

from our Cell Biology Correspondent

SINCE 1961 the technique of nucleic acid hybridization has been progressively refined to increase its resolution and range of usefulness, but until recently it has always involved the use of extracted nucleic acids. In theory, at least, if hybridization could be performed with nucleic acids denatured *in situ* this would permit the intracellular localization of specific classes of DNA and RNA. Last year, the rival groups of Gall, Birnstiel and Amaldi devised methods for partially realizing this potential. They all showed that isolated RNA can be made to hybridize with denatured intracellular RNA. Pardue and Gall (*Proc. US Nat. Acad. Sci.*, **64**, 600; 1969) have taken the technique one step further by hybridizing labelled DNA of *Xenopus* oocytes, from which ribosomal DNA had been removed, with denatured DNA of pachytene oocyte chromosomes and nuclei.

With the meiotic cells the labelled DNA was found bound to virtually all the chromosomes, but the experiments with the oocyte interphase nuclei prove the specificity of the hybridization. During oogenesis a cytologically distinct cap appears in the nuclei which contains the amplified ribosomal DNA, about 70 per cent of the total nuclear DNA. Labelled DNA, from which ribosomal DNA has been removed, fails to bind to the cap but binds to the rest of the chromatin. Three other lines of evidence indicate that hybridization is specific: first, cells treated with deoxyribonuclease fail to hybridize with the test DNA; second, alkali denaturation of the cell DNA is essential for binding test DNA; and third, the hybridization can discriminate DNAs of different sources. Mouse satellite DNA will not, for example, hybridize with *Xenopus* DNA, or that of much more closely related species, denatured *in situ*.

Apparently Pardue and Gall have used this new technique to solve a long standing question, the localization of the highly reiterated mouse satellite DNA fraction. The function of this satellite DNA has always been obscure, reducing investigators to suggest, for example, that it may be involved in chromosome "housekeeping", but Pardue and Gall claim that it is localized in the centromeres. It may therefore play a role in chromosome pairing, and this may account for the curious properties of satellite DNA, not least its peculiar base sequence.

It is also clear from Smith's experiments (*J. Mol. Biol.*, **47**, 101; 1970) that chemical properties and intracellular localization are not the only distinguishing features of mouse satellite DNA. Infection of a confluent non-dividing culture of baby mouse cells with polyoma virus results in a cell population with synchronized DNA replication during which enough satellite DNA is replicated before replication of the rest of the DNA is really under way. In Smith's experiments, replication of satellite DNA was completed 11 to 17 hours after infection, while replication of the rest of the DNA began after about 16 hours. Moreover, autoradiographs showed that much of the early replicating DNA ended up in association with the nucleolus. Whether or not early replication of satellite DNA is a characteristic of polyoma infection rather than an inherent cell characteristic remains to be seen. If the first alternative proves to be the case it may have interesting implications for tumour virology, while if the second is correct why is centromeric DNA replicated before the rest of the chromosome ?

TRANSFER RNA

Pieces in the Jigsaw

from our Molecular Biology Correspondent

THE structure of transfer RNA will not, one imagines, be revealed to the impatient public in one blinding flash; rather illumination will come slowly by a process of trial and refinement. The first uncertain steps towards the interpretation of the X-ray patterns of tRNA crystals have already been taken. Groups at two laboratories have almost simultaneously published Patterson syntheses and arrived at essentially similar interpretations. From Wisconsin, Labanauskas *et al.* (*Science*, **166**, 1530; 1969) report analyses of two species, leucine tRNA from *E. coli* and formylmethionine tRNA from yeast.

As prepared, the crystals have respectively a tetragonal and a hexagonal lattice. The data indicate in each case the existence of a long segment of double helix, and the strategy has been to refine the fit progressively by reference to a model derived in an accompanying paper (Connors *et al.*, *ibid.*, 1528), based on low-angle X-ray scattering envelopes. These reflect an asymmetric molecule, and a good fit between the calculated and observed curves can be achieved by making two limbs of the cloverleaf—the terminal stem and the pseudouracil-containing loop—a continuous double helix; this is feasible, because in all of the numerous sequences so far reported there are no intervening unpaired bases at the junction. The two remaining paired segments can then be fitted to lie parallel with the long helix, thus leaving the molecule 85 Å long. The model seems to fit the X-ray diffraction data very well, though clearly other configurations of the two short limbs to fit both the scattering and diffraction data may be possible. Labanauskas *et al.* insist, however, that the evidence for a long helical segment lying along one of the crystal axes is very strong: the introduction of, for example, any deviation from the standard RNA helix dimensions causes the definition in the Patterson synthesis to disintegrate.

A Patterson function has also been constructed by

Kim and Rich (*ibid.*, 1621) for *E. coli* formylmethionine tRNA. This crystal has a different lattice, but again there is a single ridge of density indicating a long asymmetric molecule. From the lattice symmetry it can be deduced that there is a pairwise relation between molecules, the dimer being made up of overlapping monomers, each about 80 Å long. This indicates again that there must be an intramolecular alignment of double helices, and is altogether consistent with the model produced by the Wisconsin group.

Meanwhile, the accumulation of chemical data continues. Some strange results on the effects of modification of pseudouracil residues have now been unequivocally confirmed. Siddiqui *et al.* (*Biochem. Biophys. Res. Commun.*, **38**, 156; 1970) have substantiated that cyanoethylation causes not only loss of acceptor activity, but also a drastic change in conformation, reflected by a diminution in hypochromicity and large broadening of the melting profiles. Moreover, by working with *E. coli* formylmethionine tRNA, which contains only one pseudouracil, and chemically protecting the thiouracil, which is the only other base capable of reacting rapidly with the reagent in question, they have shown that the introduction of a three-carbon chain in the pseudouracil loop destroys the native conformation. This is consistent with the model of Connors *et al.*, in which the loop packs into the large groove of the long helix, most probably with substantial stacking of the bases, this being evidently disrupted when the three-carbon spanner is thrust in.

The search for the functional sites on the tRNA has made relatively little new progress. Cory and Marcker (*Europ. J. Biochem.*, **12**, 177; 1970) determined the sequence of an *E. coli* methionine tRNA in the hope that a comparison with the formylmethionine species might reveal the synthetase recognition site (for both are substrates for the same synthetase), and possibly that of the transformylase. The two sequences turn out, however, to be very different, and no significant regions of homology can be discerned. Abelson *et al.* (*J. Mol. Biol.*, **47**, 15; 1970) have located the single base substitutions in three mutants of tyrosine suppressor tRNA. The acceptor activity is found to be grossly reduced by substitution of an A for a paired G in the helical stem of the anticodon loop. Substitution of A for an unpaired G in the dihydro U loop does not inhibit charging, but interferes with the function after attachment to the ribosome. One cannot yet, of course, exclude an explanation in terms of structural disturbance rather than a bull's-eye on enzyme recognition sites.

ROOT NODULES

Making a Vital Protein

from our Microbiology Correspondent

THE site of synthesis of the haem of leghaemoglobin, the protein necessary for nitrogen fixation in the nodules of leguminous plants, has been traced to the bacterial component of the nodule (J. A. Cutting and H. M. Schulman, *Biochim. Biophys. Acta*, **192**, 486; 1969). Leghaemoglobin has long been recognized as a component of the symbiotic association between legumes and species of the soil bacterium *Rhizobium*, but its function and site of synthesis have remained something of a mystery.

Bergersen's group at the CSIRO in Canberra found that the bacteroids, enlarged forms of the bacteria, which are present in the active nodule, had an electron transport chain similar to that of the free living bacteria except for the lack of cytochrome *a*. The haem protein was suggested to function as an electron carrier in the bacteroid and was implicated in the reduction of nitrogen. The exact function of leghaemoglobin in nitrogen fixation remains to be clarified, but a direct role is clear from many reports of the correlation between its concentration and the fixing capacity of the nodule. Ineffective nodules cannot fix nitrogen and are distinguished by the absence of leghaemoglobin.

The results reported by Cutting and Schulman go a long way towards solving the problem of where the haem is synthesized. Although other investigators had found evidence of a system which synthesizes porphyrin in root nodules, Cutting and Schulman's approach was to fractionate soybean nodules and follow the synthesis of haem by each component. Haem synthesis in unfractionated breis (whole, crushed nodule) and particulate and cytoplasmic fractions was determined according to the degree of incorporation of δ-amino[4-^{14}C] levulinic acid. Compared with the unfractionated breis, the particulate and cytoplasmic fractions accounted for approximately 70–80 per cent and 2–3 per cent of the haem synthesized, while remixing these fractions completely restored the activity of the whole breis. Further analysis of the particulate fraction showed that it lacked plant mitochondrial activity and that bacteroids were the only recognizable particles present.

Finally, Cutting and Schulman demonstrated that a large portion of the haem synthesized by the bacteroids was incorporated into leghaemoglobin, the latter being confined to the plant cytoplasm which encloses the bacteroids. The stimulatory effect of the cytoplasmic fraction on haem synthesis might be due to the provision of haem acceptor molecules which could alleviate feedback inhibition by removing the haem, or to the augmentation of bacteroid enzymes by mitochondrial or soluble enzymes originating in the legume. Thus haem synthesis for leghaemoglobin has been shown to be a function of the bacteroids in the root nodule, and it is likely that one explanation of ineffective nodules is that haem synthesis is defective in the microbial symbiont.

GYNAECOLOGY

Abortions Made Safe

from our Social Medicine Correspondent

A SAFE, effective method of inducing therapeutic abortion by injection of prostaglandin $F_{2\alpha}$ is described in the current *Lancet* (ii, 157; 1970) by S. M. M. Karim and G. M. Filshie. Of fifteen patients in the ninth to twenty-second week of pregnancy who were intravenously infused at the rate of 50 μg of prostaglandin per min, abortion was successful in fourteen cases and complete in thirteen. There were no serious side effects, and no changes in pulse rate or blood pressure were observed. Seven women had diarrhoea, however, and three of these also vomited. All seven responded to treatment.

Thirteen prostaglandins have been isolated from human semen. Although prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ have been used for the induction of natural childbirth, this is the first time a prostaglandin has been used for termination of pregnancy in the first and second trimester. The advantages of the technique are that it is simple to carry out; there is no risk of exposing the uterus to possible trauma from operative procedures; and the risk of infection is minimized. Other advantages of infusion with prostaglandin F_2 are that it brings about a high proportion of complete abortions and seems to be equally effective in both the first and second trimester.

According to Karim and Filshie, after vaginal examination, a needle with stylet was inserted into the amniotic sac of each locally anaesthetized patient and a catheter was passed into the amniotic cavity through the needle, which was then withdrawn. A sterilized, diluted solution of prostaglandin $F_{2\alpha}$ (concentration 50 μg/ml.) was then continuously perfused until abortion was complete. An increase in the muscular tone of the uterus occurred within 1–3 min in each case, followed by regular and rhythmical contractions which resulted in abortion in fourteen cases. In ten women the foetus and placenta were expelled simultaneously, and in three cases the placenta was expelled after a delay of 3–7 h. In each of these women abortion was complete and no further treatment was necessary. There was one case of retained (but detached) placenta; this was removed by evacuation. Pregnancy was terminated in the only woman who failed to abort following perfusion, by intra-amniotic injection of a solution of sodium chloride. After abortion, each woman received 0·5 mg of ergometrine intramuscularly. Loss of blood was less than 50 ml. in thirteen women, and approximately 200 ml. in one woman.

The rate at which prostaglandin $F_{2\alpha}$ had to be infused in order to stimulate the uterus in the first and second trimester (50 μg/min) is much higher than that required to induce labour in women at or near term (4 μg/min). With the former high rate of infusion there is the disadvantage that there seems to be some stimulation of the smooth muscle of the gastro-intestinal tract, leading to diarrhoea and vomiting.

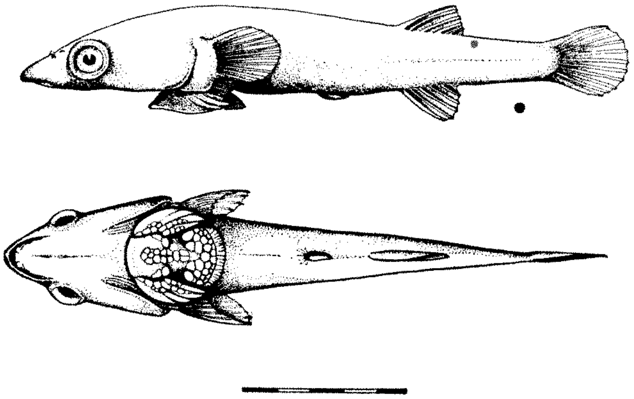
Karim and Filshie modestly say their experience is too limited to enable them to recommend routine use of prostaglandin $F_{2\alpha}$ in the termination of therapeutic pregnancies, but add that their results are very encouraging. As an addendum to their article they point out that they have successfully used another prostaglandin—E_2—at a low rate of perfusion—5 g/min—for inducing abortion in twelve women. None of them had diarrhoea.

ICHTHYOLOGY

More Clingfishes Found

from a Correspondent

CLINGFISHES, recently found on the coasts of South-West Africa and Angola (Penrith, in *Ann. S. Afric. Mus.*, **55**, 123; 1969), are small teleostean fishes which live chiefly in shallow coastal waters, many of them in the intertidal zone. They are particularly well adapted for life in the turbulent waters of the shore, for they possess a drastically modified pelvic fin complex forming a powerful sucker with which they cling to boulders. Although the shore is the stronghold of the group they are not confined to it, and several species are found in coastal waters down to depths of 22 m. Members of the subfamily Gobiesocinae have taken advantage of the sparsity of competition to colonize the smaller, swift flowing streams of the tropical New World and in some places they form an important component of the freshwater fish fauna. A specialized form, *Diademichthys lineatus*, is found in association with the Indo-Pacific echinoderm *Diadema*, living among its poisonous spines.



Eckloniaichthys scylliorhiniceps, a rather rare clingfish, which is one of the three species found on the coasts of South-West Africa and Angola. This fish is always found attached to algae.

Such specialization in a single group of fishes leads to speculation about their evolution. The zoogeography of a relatively small group like the clingfishes with rather restricted habitat requirements also makes them interesting. The problem, however, is that with their habit of insinuating themselves under boulders or in crevices they are often extremely difficult to find even on the shore. This has undoubtedly been reflected in available knowledge of their distribution in little explored regions. The coast of West Africa is a case in point, for in a world revision of the clingfishes, John C. Briggs (*Stanford Ichthyol. Bull.*, **6**; 1955) reported no specimens between Dakar and southern Africa, and only five species in the tropical eastern Atlantic. Collections in the region of Dakar and the Gold Coast, however, produced further specimens, and within 2 years of his revision Briggs was able to describe two previously unrecognized species, one of them a new genus (*Copeia*, No. 3, 204; 1957).

It is not surprising therefore that Penrith has announced the discovery of three species of clingfishes on the coasts of South-West Africa and Angola as a result of intensive collecting by the staff of the South African Museum. She also removes one stumbling block to the better understanding of clingfish distribution by showing that *Apletodon knysnaensis* Smith, 1964 (known from southern Africa only) is identical with *A. pellegrini* (Chabanaud, 1925), which was known from Dakar to Madeira. As in the space of 15 years the known clingfishes of the eastern tropical Atlantic have increased from five to nine species, it becomes apparent that far from being poorly represented there they are present in numbers. This could be cited as an example of the importance of adequate collecting to the progress of zoological studies involving taxonomy, distribution and geography.

Leipzig
Trade Fair

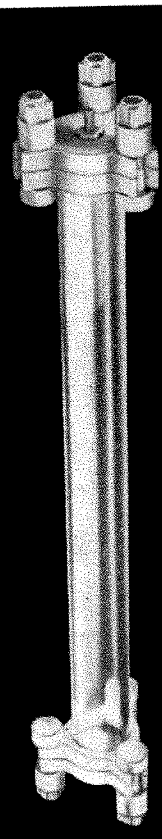
German
Democratic
Republic

Twice yearly centre of trade and technology Leipzig Trade Fair, reflecting the dynamic development of the German Democratic Rebublic, offers the buyer and the technical specialist alike an unmatched opportunity to see the world's latest achievements in design and technology. Leipzig Trade Fair, now over 800 years old, brings the goods of all the world together for all the world to see.

See you in Leipzig!

Leipzig Spring Fair 1970
March 1/March 10
Leipzig Autumn Fair 1970
August 30/September 6

A Visa will be issued on presentation of Fair Card and passport, at diplomatic and consular offices of the GDR, or at the Visa Bureaux on the state border of the GDR.
Further information from
Leipziger Messeamt,
(Dept. L),
DDR-701 Leipzig,
Markt 11-15,
Telex: 51 2294



wright

ACRYLIC
chromatography
columns

versatile inexpensive laboratory columns for chromatographic and gel filtration procedures employing aqueous solvents

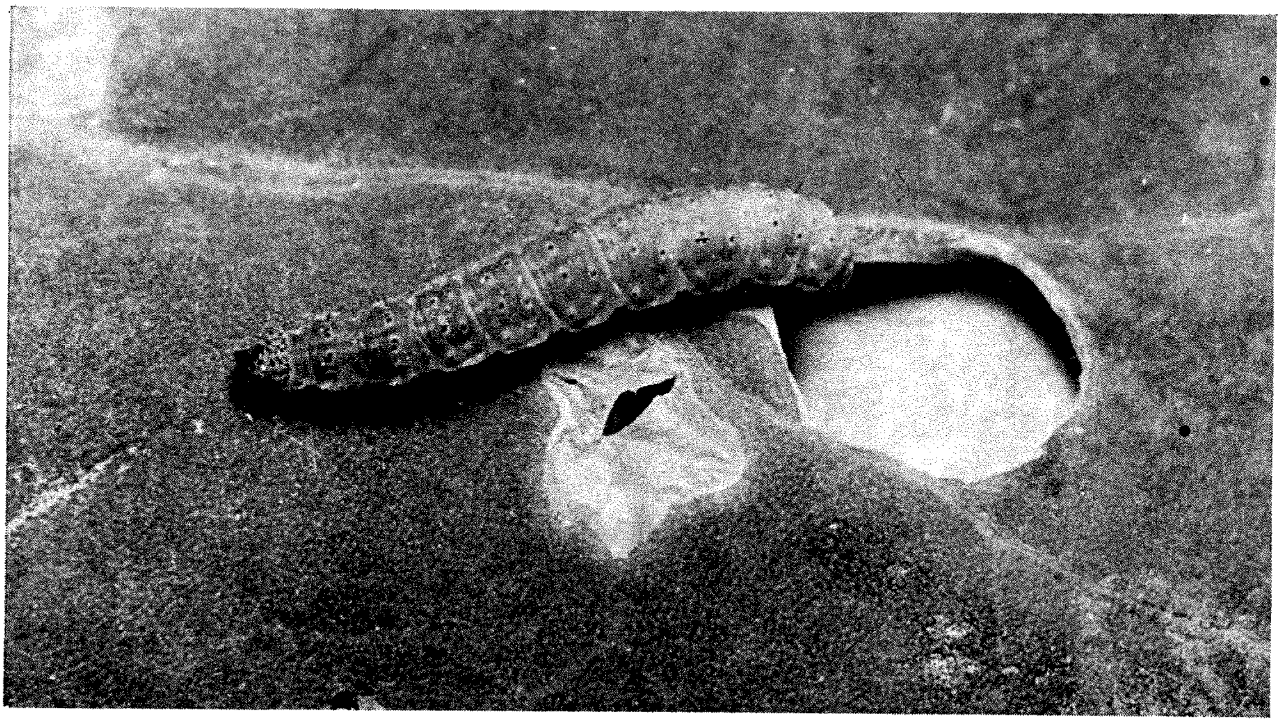
Simple to use—unique patented design in acrylic and nylon with sintered plastics bed support.
Minimal dead space with low-volume connections. Diameters selected to produce cross-sectional area of 2, 4, 8, & 16 sq. cms.
Fixed or adjustable columns with or without jackets.
Standard lengths of 30, 60 and 90 cms.
Columns easily lengthened by joining.
Prompt delivery of standard columns from stock.
Preparative columns with bed volumes up to 50 litres are available to special order

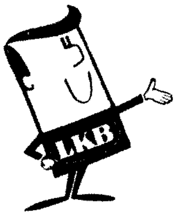
Prices from **£5**

Phone or write for detailed catalogue to Dept. CC1/N

wright SCIENTIFIC LIMITED

3 Lower Road, Kenley, Surrey, CR2 5NH *Tel:* 01-668 4611





Caterpillars, Cabbages and Calories

Measuring the heat effect of a caterpillar eating a cabbage leaf presented no problems for the LKB Batch Microcalorimeter. Carried out as part of an investigation into the effects of insecticides, it proved the incredible sensitivity of the LKB instrument.

Though LKB do not see this as a regular application for the Batch Microcalorimeter, which has been designed primarily for liquid studies, the instrument is capable of measuring heat effects even smaller than those encountered in the "caterpillar experiment". The Batch Microcalorimeter will detect changes as small as one microcalorie.
The LKB Flow Microcalorimeter, which forms part of the system, can measure heat effects as small as one-tenth of a microcalorie per second.
This extreme sensitivity makes the LKB Microcalorimeters powerful analytical tools for studying short or long-term reactions of microscopic organisms such as enzymes and bacteria.
Simple to operate, requiring no special skills, providing fast results.

Write today for our illustrated booklet, which gives information about applications, and operational details of this unique system.

LKB IN THE SERVICE OF SCIENCE

LKB INSTRUMENTS LTD. ● LKB HOUSE ● 232 ADDINGTON ROAD
● S. CROYDON, SURREY. CR2 8YD ● TEL: 01-657-0286

SALES AND SERVICE THROUGHOUT THE WORLD: STOCKHOLM,
WASHINGTON, THE HAGUE, COPENHAGEN, ROME, VIENNA

10700-UK6

British Rail Advanced Passenger Train

by

S. JONES

British Railways Board,
222 Marylebone Road,
London NW1

During the past six years British Rail has carried out a thorough study of the problems of high speed transport. The result is a design incorporating improved acceleration, braking and suspension that makes possible speeds of 150 miles per hour. The first such trains are due to go into service in 1974.

UNLIKE other forms of transport the railways have not benefited greatly from recent scientific progress. Although many attempts have been made, railway speeds much in excess of 100 mph, for example, have been difficult to achieve in acceptable economic terms. This is, of course, not to deny the value of improvements embodied in railways such as the electrified Midland line out of Euston. There are many reasons for this slow progress. The varying pressures of defence needs have played a very important part. And the development of high speed surface transport involves difficulties which are not easy to overcome using a rail network conceived and built well over 100 years ago when conditions were very different. There is, however, a technical difficulty which probably more than any other has inhibited the increase in speeds of railway vehicles. This is a form of dynamic instability arising from the guidance of the railway lines.

Guidance is generally achieved in railways by fixing each pair of wheels solidly on a common axle and by providing the wheels with conical treads, the outer diameters being smaller than the inner diameters. The flanges on the wheels give an additional form of guidance which comes into play when guidance by the treads breaks down, for example, on a sharp curve. The contact between the flange and the rail wheels, which results in slip and wear, is minimized by the proper design of track layout and vehicle suspension. The guiding effect of the treads comes into play when a wheelset moves laterally across a pair of rails from the symmetrical position; the rolling diameter of the wheel further from the central position is then larger than the rolling diameter of the wheel nearer the centre. In consequence, for geometrical reasons, as the wheelset rolls forward it moves laterally back to the central position, overshooting, returning, and executing a sinusoidal motion of constant wavelength.

In practice the motion of the disturbed wheelset generally departs from a uniform sinusoid for several reasons. At low speeds, that is, when the inertial effects can be ignored, the sinusoidal motion rapidly decays to the mean steady state uniform motion of translation along the track because frictional effects absorb the energy of lateral oscillation. At high speeds the inertia of the wheelset calls into play substantial tangential forces at the interface between wheel and rail. These tread forces give rise to tangential creep (that is, incipient slip) which opposes the process of decay in the sinusoidal motion and if large enough can lead to a run-away process of increasing amplitude of oscillation ultimately contained by the flanges striking the edges of the rails. This effect is known as hunting and can be regarded as an instability arising from the feedback process in the guiding mechanism. The mechanism of creep is an essential link in the feedback chain.

This phenomenon of instability has been known for some time. For example, F. W. Carter described it as early as 1922 in his book *Railway Electric Traction*. Further, the general nature of instability arising from feedback in guidance has been extensively analysed in defence work during and after the Second World War. It is only relatively recently, however, that adequate effort and resources have been brought to bear to solve the peculiar difficulties of the railway problem.

Dynamic Study

Some seven years ago British Railways established a small team to make a thorough study of this phenomenon. The difficulties it encountered arose mostly from the application to practical railway vehicles with the many degrees of freedom involved and the gross non-linearities that are present. A linearized small-disturbance analysis was developed which together with analogue and digital computer facilities, small-scale model work on roller rigs, full-scale roller rigs and instrumented test runs on the track has resulted in a body of knowledge making possible prediction of the dynamic behaviour of practical railway vehicles. Further, design criteria have been developed for the design of vehicles capable of running stably at high speeds. A brief outline of this work was presented by A. H. Wickens to the American Society of Mechanical Engineers in December 1968. A series of four-wheel high speed test vehicles has been designed and constructed and run on track at the maximum track speed permissible (100 mph) and on a roller rig at 140 mph. Their dynamic behaviour has been in close agreement with prediction and has established confidence in the theoretical basis of design. Fig. 1 illustrates the latest of this series, High Speed Freight Vehicle, MK.4 (HSFV4).

An important parameter in the design of a railway vehicle is the conicity of the wheel treads (or effective conicity if the wheel treads have a curved profile in the axial plane). High conicity gives good guidance but with a proneness to dynamic instability at low speed. On the other hand, low conicity gives stabler running but poor guidance with frequent flange contact and excessive wear. And however the treads of wheels are first manufactured,

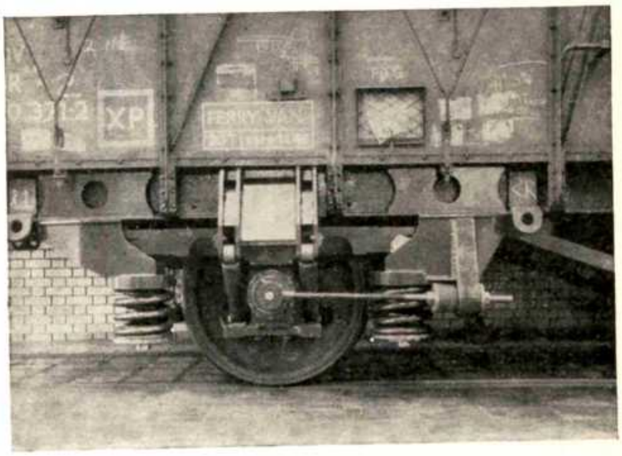


Fig. 1. The latest high speed test vehicle.

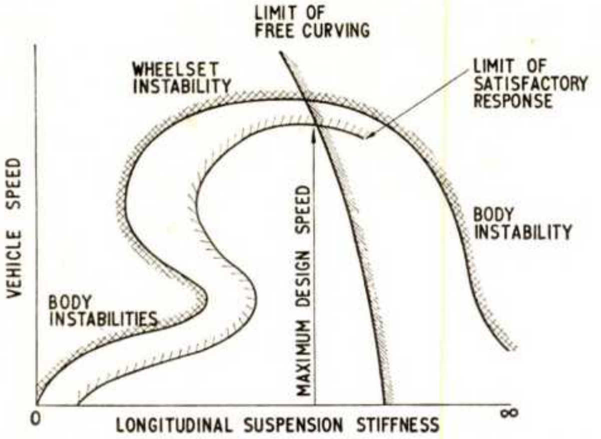


Fig. 2. Variation of stability with the speed of the vehicle and longitudinal suspension stiffness. The numerical values on the two axes will vary with the sophistication of the design. For the advanced passenger train mark I a speed of 200 mph is in the stable region.

they ultimately wear to a common tread shape characterized by a curved profile; additional wear then is to a smaller wheel with a similar profile. The maximum effective conicity of such a tread is high, its actual value then being dependent on the profile of the rail at its point of contact with the wheel. Economic considerations therefore demand a wheel profile sufficiently coned to provide adequate guidance with, if possible, a stable worn profile and a suspension system designed for stable running at all commercially desirable speeds. The HSFV series of vehicles are designed for wheels with such stable worn wheel treads.

The analysis of the dynamics of railway vehicles involves a large number of simultaneous equations and many stiffness, damping and inertial parameters. Stability at high speed is not achieved with a unique solution but by the acceptable choice of several parameters. Fig. 2, for example, illustrates the boundary stabilities as functions of vehicle speed and suspension stiffnesses. Limits are imposed by the need for lateral stability, the need to guide without flange contact around a curve and the need to provide an optimum response to imperfections in the track. These three criteria enclose an area of stable running which it is the objective of the design engineer to achieve.

An important consequence of the analysis of the stability problem of railway vehicles was the realization that the maximum speed of stable running was determined by the vehicle—and in particular its suspension characteristics—and not by the track. This concept leads to the idea that existing good main line track can be used for faster running with little or no change to the existing track provided the vehicles are adequately designed to be stable at these higher speeds. The economic consequences seemed to be so important that the matter was pursued further and so the idea of the advanced passenger train was born.

Advanced Passenger Train

The term "Advanced Passenger Train" is a registered name and relates to trains with certain specific design features. The train, shown in Fig. 3, is "advanced" primarily in the nature of the suspension which can be regarded as a direct descendant of the design philosophy developed in the dynamics studies I have described. The capacity to achieve economically stable running at high speeds presents a new incentive and new dimensions of design freedom. The passenger is rarely interested in speed as such. He is more concerned to reduce his travelling time, and so in a practical transport system it is desirable to increase the average speed by providing high accelerating and retarding characteristics and by the ability to run round bends at the fastest maximum speed. In addition the passenger desires a smooth, quiet and comfortable ride, and amenities and décor in keeping with the growing standards of material comfort available in general to the public. The suspension system must accordingly filter out frequencies particularly objectionable to the human body that arise from irregularities in the track. For economy of costs the design of the vehicle should be such as to reduce to a minimum the need for expensive maintenance.

Dynamic Behaviour

The advanced passenger train consists of an articulated set of eight or so vehicles, the two end cars being provided with motive power and those in between being trailer cars. The powered cars have six wheels and the trailer cars four wheels. The suspension design satisfies the criteria described briefly in the preceding section and the design aim is to provide stable running, in the first instance, of speeds up to 150 mph with a design stretch up to 200 mph (but the train will be operated initially at a maximum speed of 125 mph) and to provide optimum response to track irregularities. For this latter purpose considerable measurements have been made of the roughness of the track and the results expressed statistically in the form of a power spectrum. The suspension is designed as a

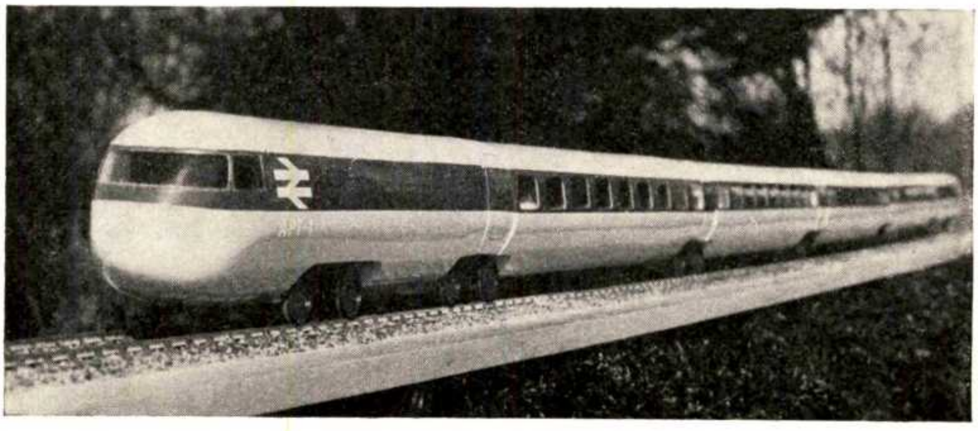


Fig. 3. The advanced passenger train.

mechanical filter to reduce to the minimum those frequencies arising from important track imperfections that are particularly objectionable to the passenger. The coaches are articulated and the suspension is designed to run without flange contact on all curves on the main lines of British railways.

Curving

The important limitation to the speed of a passenger vehicle negotiating a curve is the lateral acceleration imposed on the passengers. It is customary in railways to meet this limitation in part by canting the track on curves. The degree of cant should be sufficient to alleviate most of the lateral acceleration for high speed trains and yet not sufficient to be troublesome for stationary or slow moving trains.

The suspension of the APT is effectively pendular. The concept is not new and the objective is simply to provide a banking—or inward tilting—of the vehicle on curves, to nullify the effects of lateral acceleration on the passenger. In the steady state condition, that is, on a curve of constant radius, a free pendulum satisfies this criterion, but it does not in the transient conditions on a variable radius curve. For this reason, but more importantly to make the maximum use of the relatively small loading gauge of British Rail, the coach is not suspended freely above its centre of gravity but tilted by an active hydraulic suspension system which, sensing lateral accelerations, tilts the coach by the proper amount even in rapidly changing conditions. The effective centre of rotation is chosen so that the cross-sectional area swept out by the swinging coach over $\pm 9°$ is reduced to a minimum.

Acceleration Capability

Rapid acceleration is achieved by a high power-to-weight ratio. The structures of the APT are made of light alloy and as a consequence the structural weight per passenger seat is about half that achieved in conventional railway coaches. In fact it is reduced to a point where further lightening of the structure is prohibited by the need to maintain lateral stability to side winds. The designed axle loads are only about 11 tons each; the unsprung mass is also light. These two factors are of considerable importance in preserving the track from excessive punishment in high speed running.

The motive power is provided by gas turbines and it is proposed to use the automotive gas turbines now being developed by British Leyland. Four turbines, each of 300 nominal horse power, will drive the wheels of each power car. In addition a fifth gas turbine will be provided for domestic power supplies. The gas turbines will be installed so as to facilitate relatively easy removal from the body of the power car for maintenance or replacement.

Braking

The distances between fixed signals beside the tracks in a railway network are determined by the maximum speeds of the trains using the network. This follows from the braking distance that a train can achieve and the relationship between that braking distance and the speed of the train. If a high speed train like the APT is to use existing track and signalling equipment, it must have adequate braking facilities. The increased kinetic energy demands an energy absorption that increases as the square of the speed. The retarding force of the train for a given stopping distance also varies in proportion to the speed, so that the power dissipated by the brake increases as the cube of the speed. The considerable increase on brake requirements as speed increases eliminates the possibility of conventional rim brakes and a form of hydrokinetic brake is being developed to absorb the greater braking power required.

Ultimately, the brake capability is limited by the adhesion between the wheel and the rail. It is believed that the more stable running of the APT will give a greater mean adhesion at high speed than is customary with less stable vehicles. However that may be, development work is in hand to examine the practicability of various ways of increasing the coefficient of adhesion between wheel and rail. A plasma torch is being developed and early results are very promising. It is proposed to use this device, ahead of the driving wheels, to clean the rail surface at the point of contact with the wheel.

Development Programme

The advanced passenger train is being developed by the British Railways Board with the active and practical support of the Ministry of Transport. The conceptual design is now complete and rests on the extensive body of theoretical and experimental knowledge developed during the past six years.

During 1970 laboratory rig work will be undertaken on the power unit, the drive unit, the suspension unit, the braking system and the driver's control system. These units are now being designed in detail. At the same time power and trailer car bodies are being manufactured for an experimental train. It is planned to have the experimental train running on the track by the middle of 1971. This train will be used exclusively for experimental and development work and as the work proceeds the decision will be taken on the final specification and design of two prototype trains which are planned to be introduced into revenue earning service by 1974. The production of further trains will be determined during the progress of the work on the experimental and the prototype trains.

The attraction of the advanced passenger train is that it offers a fuller exploitation of the existing rail network of a good modern railway system with little or no additional investment in track. This is the reason for interest in the possibility of licensing the train abroad. The successful development and commercial exploitation of the train when demonstrated will raise questions of still further development which will then require decision and investment depending on the performance that is sought. The earliest investment decisions would be of a relatively minor kind, for example, the possible need to provide additional safety devices on the rails at points and crossings for the higher speeds and the advantage of smoothing out the sharper curves on main line track. Later decisions would be more important, concerning such matters as level crossings, tunnel entries, the movement of high speed trains through existing stations, new signalling techniques and further improvements to track alignment. From the technical point of view alone, speeds of, say, 300 mph are feasible, but questions of public policy and amenity have to be answered satisfactorily before such speeds can be implemented. It is important, however, to register the fact that in technical terms it seems feasible to contemplate speeds of this order along tracks which are not incompatible with much of the existing track and with many of the existing main line railway stations. This applies, for example, to surface links between air terminals and city centres as well as the longer distances between the main centres of population in Britain.

Diffusion in Embryogenesis

by
FRANCIS CRICK
Medical Research Council
Laboratory of Molecular Biology,
Hills Road, Cambridge

A simple order-of-magnitude calculation suggests that diffusion may be the underlying mechanism in establishing morphogenetic gradients in embryonic development.

> *It has been a great surprise and of considerable importance to find that most embryonic fields seem to involve distances of less than 100 cells, and often less than 50.*
> Professor Lewis Wolpert[1]

WHEN I read this sentence I was delighted because it seemed to confirm some conclusions I had come to on purely theoretical grounds[2]. It is an old idea that "gradients" are involved in embryological development—in fact, C. M. Child[3] in 1941 wrote a whole book on the subject. Many of the gradients to which Child referred seem more likely, in retrospect, to be the results of development rather than its cause. An outsider to embryology has the impression that in recent years gradients have become a dirty word. This is partly because of the failure to isolate unambiguously the molecules involved, whose concentration is presumed to constitute the gradient, and partly because a feeling has grown up that diffusion is not a fast enough mechanism for establishing gradients. In this article I aim to show that this fear is unfounded, and, on the contrary, that the known facts, sparse as they are, fit rather well to a mechanism based on diffusion. The problem can be stated in this way: what is the maximum distance over which a steady concentration gradient could plausibly be set up in the times available during the development of the embryo ?

The obvious model for setting up a simple gradient is illustrated in Fig. 1. At one end of a line of cells one postulates a source—a cell which produces the chemical (which I shall call a morphogen) and maintains it at a constant level. At the other end the extreme cell acts as a sink: that is, it destroys the molecule, holding the concentration at that point to a fixed low level. The morphogen can diffuse from one cell to another along the line of cells. After a time the system approaches a dynamic equilibrium, and it is easy to show that if the effective diffusion constant is everywhere the same, the concentration gradient will be linear.

Of course, real embryological structures will have three dimensions, but if for convenience we restrict ourselves to sheets of cells such as, for example, the insect epidermis[4] or the developing amphibian retina[5] the problem becomes two-dimensional. The source can be considered to be a line of cells (the line being perpendicular to the paper in Fig. 1) and similarly for the sink, thus reducing the problem to one dimension.

It is not difficult to calculate how long it would take to set up such a system, supposing that both the source and the sink are turned on at time zero. Diffusion is a random walk process, and the dimensions of the diffusion constant, D, are L^2T^{-1} (where L is length and T is time). This should be contrasted with a mechanism having a velocity (with dimensions LT^{-1}) as proposed, for example, by Goodwin and Cohen[6]. Because in diffusion the length enters as the square, pure diffusion processes are very rapid over rather short distances (say, the size of a cell) and very slow over long distances (say, the size of an organ).

The concentration approaches its final value asymptotically, so one must have some criterion for deciding whether the gradient at any time is sufficiently close to a straight line. I have arbitrarily taken the gradient to be effectively established when it is everywhere within ΔC of the final value, and chosen ΔC as 1 per cent of C_0 (C_0 is the maximum value at the origin). It would make little difference to the argument if ΔC were considered to have half this value.

The gradient might be set up in various ways. The result in each case can be expressed as

$$t = \frac{A\ (nl)^2}{D}$$

where t = time in seconds to set up the gradient; n = number of cells between source and sink; l = length of each cell, in cm; and D = diffusion constant, in cm^2 s^{-1}. A is a numerical constant, the exact value of which will depend on the way the gradient is developed.

Mathematically the simplest way is to start with zero concentration of the morphogen everywhere at time zero, and thereafter to maintain the source at concentration C_0 and the sink at concentration zero. This gives a value of A of 0·42. Biochemically more realistic models give values only a little larger than this, so a good general value for A would be 0·5. It was pointed out to me by Dr Aaron Klug that, if the initial concentration were uniformly $C_0/2$, the time required is reduced to a little less than one-quarter, and A will have a value of about 0·09. More realistic models of this general sort give values of A of, say, 0·15. The calculations of A were carried out by Mrs Mary Munro.

In what follows, I shall assume that A is 0·5 (the simple mechanism), but it should be remembered that the organism might be able to reduce this to about a third of this value.

The diffusion constant in water for all but the smallest molecules—provided they are roughly spherical—is inversely proportional to their mean radius, to a near approximation. Thus, increasing the molecular weight by a factor of 1,000, from, say, a small organic molecule such as ATP (mol. wt. 507) to a very large protein like polynucleotide polymerase (mol. wt. about $0·5 \times 10^6$) reduces the diffusion constant only by a factor of 10. Now it is reasonable to expect that the morphogen will diffuse rather rapidly, and should be able to pass fairly efficiently from cell to cell. It is also likely to be a rather specific molecule. For these reasons I doubt if morphogens will turn out to be large proteins or common ions[7] like K^+ or Na^+. An obvious choice would be an organic molecule of about the size of, say, cyclic AMP or a steroid. That is, with a molecular weight in the range 300 to 500. The diffusion constant[8] in water (at 20° C) for such a molecule is about 4 or 5×10^{-6} cm² s⁻¹. (The diffusion of salts like NaCl or KCl is about three or four times as fast as this.)

The inside of a cell is very far from being made of water, and one must estimate the effective diffusion constant within a cell. This amounts to estimating the effective viscosity. The cytoplasm being a concentrated mixture of molecules having a large variety of sizes, the relative viscosity will be considerably higher than water at the same temperature. For a small molecule, which can, as it were, slip between many of the other molecules, the effective viscosity is unlikely to be as big as the bulk viscosity of the cytoplasm (wherever that may be). It is difficult to make any precise estimate of the effective diffusion constant, which in any case may vary considerably between different types of cell. Allowing a factor of increase of viscosity of ×6 (corresponding to a sucrose solution 40 per cent by weight), which seems not unreasonable[9], would make the effective diffusion constant about $0{\cdot}8 \times 10^{-6}$ cm² s⁻¹.

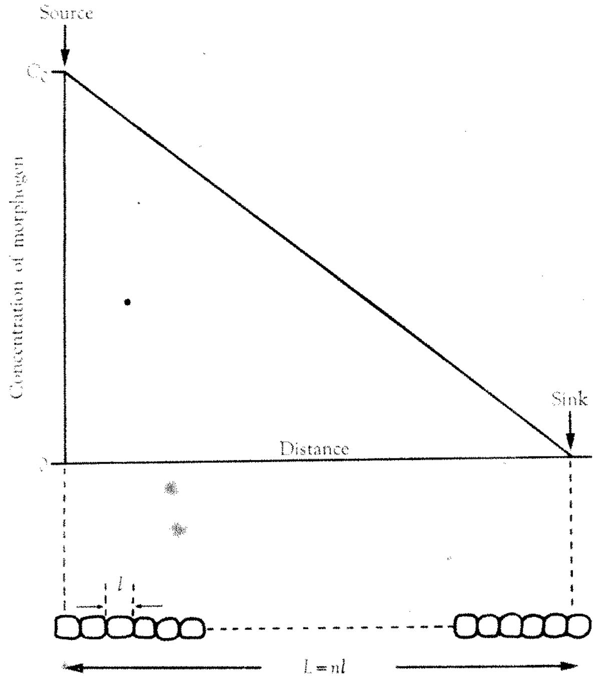


Fig. 1. To illustrate how a source and a sink can produce a linear gradient of concentration. Each cell in the line has length l cm. The distance between source and sink is L cm. Because n is the number of cells in the line, $L = nl$.

How does the morphogen get from cell to cell? It would seem inefficient to make all cell membranes easily permeable to it. To do this it would in any case have to be very small and rather hydrophobic[10]. Moreover, a high general permeability would allow the molecule to escape too easily from the tissue and this would not only disturb the gradient but possibly interfere in other parts of the organism. One is therefore driven to postulate a special mechanism which allows a relatively quick passage of the morphogen from one cell to another in the tissue of interest. Whether this involves tight junctions or other special structures remains to be seen.

Such a mechanism could be facilitated diffusion. This is characterized by being independent of energy, specific (usually stereospecific) and saturatable at high concentrations of the diffusing molecule. The passage of glucose through the membrane of a human red blood cell or an ascites tumour cell is believed to have this character.

At low concentrations (that is, far from saturation) the mechanism can be described by a permeability, P, measured in cm s⁻¹. It is easy to show that the effective diffusion constant, D', for our problem is given by $1/D' = 1/D + 1/Pl$, where l is the length of each cell in the direction of diffusion[11]. Thus, if D is $0{\cdot}8 \times 10^{-6}$ cm² s⁻¹ and $l = 10$ μm (say) we see that if the "resistance" to flow of the morphogen because of permeability between cells was equal to that due to diffusion within a cell, P would have to have the value 8×10^{-4} cm/second. This is a high value, but probably not impossibly high[12]. If we arbitrarily take P as about half this and D as before, we obtain $D' \simeq 0{\cdot}27 \times 10^{-6}$ cm s⁻¹.

We now need an experimental estimate of the time needed to set up a gradient. This is not easy to obtain. Most embryologists would feel that a day is too long. A minute seems far too short. A few hours would seem about right—Wolpert has suggested that between 5 to 10 h is not unreasonable for many of the well-studied cases (ref. 1, page 41). I shall assume a figure of 10^4 s (approximately 3 h), because some time must be allowed for the changes which take place after the gradient is set up.

Combining our formulae we obtain

$$t = An^2l^2\left(\frac{1}{D} + \frac{1}{Pl}\right)$$

and substituting the chosen values: $t = 10^4$ s, $A = 0{\cdot}5$, $l = 10$ μm, $D = 0{\cdot}8 \times 10^{-6}$ cm² s⁻¹, $P = 4 \times 10^{-4}$ cm s⁻¹ we obtain $n \simeq 70$ cells. If l were 30 μm, n would come to a little over thirty cells. Even allowing for the very approximate nature of the calculations, the agreement with the figures given in the quotation at the start of the article is striking. In broad outline what the calculation shows is that, for the times considered, distances or the order of a millimetre (or less) are possible, but distances of a centimetre are too great[13]. Of course, for organisms which develop very rapidly the distances would have to be smaller than a millimetre.

We can take it, then, that assuming the effective viscosity of cytoplasm has not been grossly underestimated, and provided there is a special mechanism to increase the rate of permeability of the morphogen between cells, there are many cases in embryology where the times and distances involved are quite compatible with a mechanism based on diffusion. It is important, however, to make two reservations. There may be special cases, involving setting up gradients quickly over large distances (of the order of several centimetres) which may require other mechanisms, such as the signalling devices suggested by Goodwin and Cohen[6]. Cases of "mushroom growth" (as, for example, the growth of mushroom) are unlikely to be due to diffusion alone. Secondly, in one's enthusiasm for diffusion, it is important to realize that the many other problems remain to be tackled. Even when the gradient has been set up the cell has to recognize it. Because at least in the insect integument the "gradient" appears to impose a polarity on those epidermal cells which become scales and bristles[14] the cells must have some additional mechanism (involving microtubules ?) to do this.

In the case of the amphibian eye the retinal ganglion cells must not only recognize the presumed gradient (so that they know where they are in the retina); each cell must also convey this information to the far end of its growing axon, so that it can make the connexion at the appropriate place on the optic tectum[5]. Moreover, there are likely to be subsidiary mechanisms to guide the growing bundle of nerves along the right path to the tectum.

In two brilliant articles published about ten years ago, Locke[15] showed that the pattern of wrinkles obtained on the adult cuticle of Rhodnius after operations on the earlier larval stages (usually the last larval stage) can only be explained by a "gradient" of some sort, running from one intersegmental membrane to the next, and repeating in successive segments. He showed convincingly that neither mechanical expansion alone, nor polarity alone, could explain the results. Dr Locke kindly sent us the original photographs of some of his material. Mrs Mary Munro and I, together with Dr Peter Lawrence, have attempted to fit these patterns to a pure diffusion model. Although, following Locke's arguments, the observed wrinkles have roughly the expected pattern, they differ in detail from the computed pattern. Moreover, various estimates of the diffusion constant disagree drastically. We are therefore currently exploring a model in which each cell in the epidermis attempts to maintain the concentration of the morphogen within itself to a previously preset level, determined soon after the gradient is first set up. This model, which has only one disposable parameter, is a much better fit with Locke's data. More elaborate hypotheses, within the basic diffusion framework, are also under consideration. It is important, therefore, not to approach these problems with too naïve a model[16].

Finally, one should emphasize that gradients are unlikely to command general acceptance until their biochemical basis is discovered experimentally, and that this may not prove an easy task. Mathematically minded biologists could well object that any theory which has the same mathematical formalism would necessarily fit the observed patterns, and that the agreement between the calculated and observed distances (on the diffusion theory sketched above) may only be coincidental. In spite of these possible objections it is my belief that mechanisms based on diffusion are not only plausible but rather probable. Nature usually has such difficulty evolving elaborate biochemical mechanisms (for example, those used in protein synthesis) that the underlying processes are often rather simple. If this approach serves to make the idea of diffusion gradients respectable to embryologists it will have served its purpose.

I thank my wife for drawing the figure, my colleagues, especially Dr Peter Lawrence and Mrs Mary Munro, for many helpful discussions, and Professors Lewis Wolpert and W. D. Stein for sending me information.

Received January 2, 1970.

[1] Wolpert, L., *J. Theoret. Biol.*, **25**, 1 (1969). This article, entitled "Positional Information and the Spatial Pattern of Cellular Differentiation", should be consulted both for a modern statement of the problem and also for references to earlier work.

[2] The basic idea of this article was presented at a lecture given to students at the Fourth NATO Advanced Study Institute of Molecular Biology in July 1969 at Spetsai.

[3] Child, C. M., *Patterns and Problems of Development* (Chicago University Press, Chicago, 1941).

[4] For a review see Lawrence, P. A., *Adv. Insect Physiol.* (in the press). See especially Stumpf, H., *Roux Arch. Ent. Mech. Org.*, **158**, 315 (1967).

[5] For a review see Gaze, R. M., *Growth and Differentiation, Ann. Rev. Physiol.*, **29**, 59 (1967).

[6] Goodwin, B., and Cohen, M. H., *J. Theoret. Biol.*, **25**, 49 (1969).

[7] The mechanism for forming a source and a sink for ions also presents special problems, whereas for organic molecules rather simple enzymatic processes could do the trick.

[8] The effect of temperature on the viscosity of water does not seriously affect the calculations. Taking the viscosity of water (in arbitrary units) as 1·0 at 20° C, its value at 5° C is about $1\frac{1}{2}$ and at 39° C is close to 2/3.

[9] See, for example, a very ingenious fluorescent method used by Victor W. Burns (*Biochem. Biophys. Res. Commun.*, **37**, 1008; 1969) using a small organic molecule. He obtained a factor of about ×6 for Euglena. The figure for yeast was about twice this.

[10] Lieb, W. R., and Stein, W. D., *Nature*, **224**, 240 (1969).

[11] This assumes that the permeability is fairly evenly distributed over the cell membrane. If it were concentrated in a small patch the effective diffusion would be slower.

[12] For example, P for glucose in ascites cells at 37° C in about $4{\cdot}5 \times 10^{-4}$ cm/s (Kolber, A. R., and LeFevre, P. G., *J. Gen. Physiol.*, **50**, 1907; 1967), or in human red cells at 37° C about 1×10^{-4} cm/s (Millar, D. M., *Biophys. J.*, **5**, 407; 1965). Admittedly these are among the higher values of P known so far. See Stein, W. D., *The Movement of Molecules Across Cell Membranes*, chap. 4 (Academic Press, New York, 1967). It should be remembered that in going from one cell to the next the morphogen may have to cross two cell membranes.

[13] An upper limit can be calculated assuming that the morphogen is an ion, that it diffuses (at 37° C) in the cytoplasm as freely as in water, that the permeability between cells is so high that it does not slow down the process at all, and that the most efficient method is used to set up the gradient. The distance then comes to 1·5 cm, but I feel that this combination of assumptions is quite unrealistic.

[14] Piepho, H., *Naturwissenschaften*, **42**, 22 (1955); Lawrence, P. A., *J. Exp. Biol.*, **44**, 607 (1966).

[15] Locke, M., *J. Exp. Biol.*, **36**, 459 (1959); *J. Exp. Biol.*, **37**, 398 (1960).

[16] The present model could be elaborated by assuming two different morphogens, one having a gradient sloping from left to right, and the other with a gradient from right to left: on this model the position of a cell would be characterized by the ratio of its concentration of the two morphogens. This particular elaboration, however, would not significantly alter any of the arguments given in this article.

Restoration of In-phase Translation by an Unlinked Suppressor of a Frameshift Mutation in *Salmonella typhimurium*

by

JOSEPH YOURNO
SHIGEGI TANEMURA

Biology Department,
Brookhaven National Laboratory,
Upton, New York 11973

When carrying an unlinked suppressor, a frameshift mutant produces small quantities of wild-type protein.

UNTIL recently no frameshift mutations had been observed to respond to unlinked suppressors[1]. Two such frameshifts, both in *Salmonella typhimurium*, have now been described. Riyasaty and Atkins originally reported on the externally suppressible frameshift *tryA91* in the anthranilate synthetase gene of the tryptophan operon[2]. The other such frameshift, *hisD3018* in the histidinol dehydrogenase gene of the histidine operon, was isolated by Oeschger and Hartman after induction with the acridine derivative, ICR–191[3]. These frameshifts probably differ from one another in sign or base content as revealed by reversion response to selected mutagens[2–4]. Examination of histidinol dehydrogenase from several prototrophic double frameshift mutants has shown that the *hisD3018* frameshift is a +1 type, resulting from the inclusion of an extra C residue in mRNA (Fig. 5)[5,6]. Unlinked weak suppressors highly specific for *hisD3018* arise spontaneously and are induced at high frequency by ICR–191[3,4]. All *hisD3018sup* (suppressed mutant) strains examined produce very low levels of electrophoretically normal histidinol dehydrogenase conferring slow growth in the absence of histidine[4]. We present here further genetic evidence for such suppressors. Histidinol dehydrogenase from one *hisD3018sup* strain, produced at about 1 per cent of normal levels by our best calculations, has been purified to homogeneity and found to have the wild-type amino-acid sequence.

Genetic Evidence for Unlinked Suppressors

The *hisD3018* frameshift mutation is located in the operator proximal portion of the *hisD* gene[3]. This site is covered by the extensive deletion *his-2236* but recombines at very low frequency with the deletion *his-646* and at a higher frequency with the deletion *his-712* (Fig. 1, Table 1*A*). The following genetic tests confirm the presence of an unlinked suppressor in *hisD3018sup* isolates.

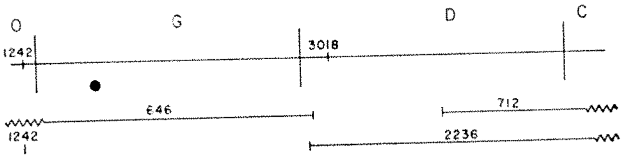


Fig. 1. Map site of the *hisD3018* frameshift. The deletions used for mapping, *hisGD646*, *hisDC2236* and *hisDCBHAFIE712*, are represented below.

All *hisD3018sup* strains, regardless of mutagenic origin, show frequencies of recombination with the aforementioned deletions characteristic of the *hisD3018* site, suggesting that these harbour the parent mutation along with an unlinked suppressor. When crossed into *hisD3018* as recipient these strains transduce the unlinked suppressor gene at high frequency, conferring weak prototrophy on the parent mutant. This is shown for two such strains in Table 1*A*.

A test diagnostic for the presence of suppressors of a mutation is the recovery of the original mutation. We therefore attempted to isolate *hisD3018* recombinants from *hisD3018sup* donors in crosses with the operator constitutive strain *hisO1242* as recipient. Selection was made for *hisO*+ or the closely linked polar *hisD3018* mutation at 43° on histidine-supplemented minimal agar, in which *hisO1242* cannot grow (see ref. 7). One hundred transductants from each cross were isolated and classified nutritionally as *hisO*+, *hisD* or other *his* genotypes, arising through mutation in the *hisO1242* recipient. The purified *hisD* isolates were tested for response to ICR-191 and NG both of which revert *hisD3018*[3,4]. Transductants resembling *hisD3018* were recovered at high frequency only from the *hisD3018sup* donors and the *hisD3018* donor control (Table 2). From each cross, six isolates testing as *hisD3018* were further examined and found to map as *hisD3018* and to be transducible to weak prototrophy at high frequency by a *hisD3018sup* donor (Table 1). Another method has also been used by D. Riddle and J. R. Roth (personal communication) to isolate *his3018* from *hisD3018sup* strains. Thus it is clear that the original *hisD3018* frameshift is maintained in the *hisD3018sup* strains and that prototrophy is the result of unlinked suppressors.

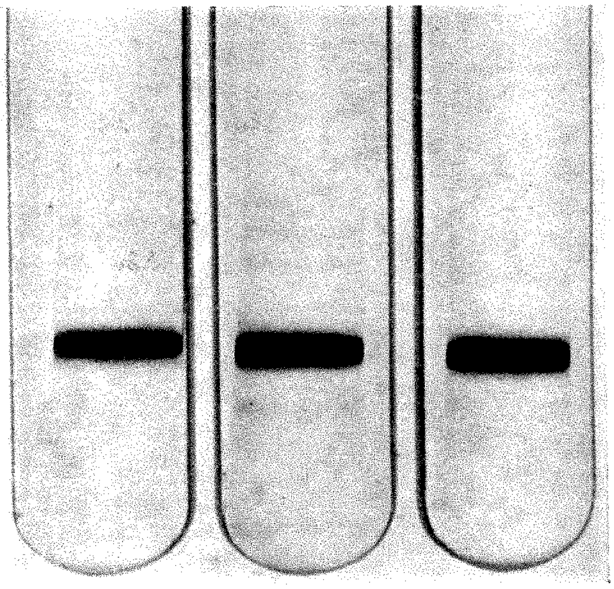


Fig. 2. Polyacrylamide gel electrophoresis of purified TR936 and wild-type histidinol dehydrogenase. The standard *p*H 9·5 system was used. From left to right: wild type, 60 μg; TR936, 60 μg; wild type, 30 μg + TR936, 30 μg.

To examine the specificity of suppression these suppressors were tested as donors with several *hisD* mutants of various types (classified by J. Greeb, J. Atkins and J. Loper; unpublished data) including the frameshifts *3029*, *2252* and *2550*, nonsense *2117*, *2120*, *2131* and *2186*, and missense *451*, *492*, *619* and *1742*. None gave a positive response, suggesting a high degree of specificity of these suppressors toward *hisD3018*.

Analysis of *hisD3018sup* Histidinol Dehydrogenase

The pivotal consideration in any analysis of enzyme produced at very low levels in a mutant cell is that the material isolated does not actually arise through otherwise insignificant background intragenic reversions, particularly if the enzymes of the divergent genotypes are not differentiable. The *hisO1242–hisD3018sup* strain,

Table 1. SUPPRESSOR TEST AND MAPPING OF *hisD3018sup* STRAINS BY TRANSDUCTION RECIPIENT

Donor	Origin	No. of transductants per plate* *hisD3018*	*his-2236*	*his-646*	*his-712*	*leu-428* outside marker control
A. *hisD3018* control	ICR-191	87	0	4	74	275
hisD3018 R1† control	Spontaneous	1,000	750	325	575	72
hisD3018 R20	ICR-191	1,300‡	0	2	22	265
hisD3018 R51	Spontaneous	1,250‡	0	3	29	250
No phage control		121	0	0	0	0
B. *hisO1242–hisD3018* R1† control		1,500	> 2,000 (> 2,000)§			240 (150)
TR936 (*hisO1242–hisD3018sup*) control¶	ICR-191	800	0			250
	Cell wet weight					
TR936 culture 1	6·5 kg	500‡ (210)	0 (0)‖			500 (120)
TR936 culture 2	3·5 kg	400‡ (200)	0 (0)‖			300 (100)
No phage control		89 (52)	0 (0)			0 (0)

* Average for two plates, scored after 72 h incubation at 37° C on double enriched minimal agar plates. Numbers > 300 expressed to nearest 10 per cent.

† R1 is an intragenic reversion to wild-type[4].

‡ > 90 per cent small.

§ All values in parentheses are for a second experiment.

‖ Thirty-four plates tested for culture 1, twenty-one for culture 2.

¶ Grown on double enriched minimal medium.

TR936, constructed by D. Riddle and J. R. Roth, produces derepressed levels of histidinol dehydrogenase on histidine supplemented medium where the growth rate is increased to normal and selection for intragenic revertants is minimized. Cells of TR936 (10 kg wet weight), grown in two 1,000 l. batches on histidine supplemented double enriched medium, were supplied by R. G. Martin. Aliquots of each culture, taken immediately before harvesting, were tested for colony morphology and for transduction characteristics. On agar containing high concentrations of glucose derepressed strains such as *hisO1242* form wrinkled colonies while the highly polar *hisO1242–hisD3018sup* strains form smooth colonies (compare ref. 7). Of a total of more than 10,000 colonies, no wrinkled types formed by *hisD*$^+$ cells (intragenic revertants) were found. Cell counts were not higher on histidine supplemented plates. The level of contamination from reversions to *hisD3018sup*$^+$ (unsuppressed mutant) as well as to *hisD*$^+$ therefore seemed insignificant. Phage prepared from each culture transduced the outside marker control *leu-428* to large colony and *hisD3018* to small colony prototrophy, but produced no transductants with *his-2236* in conditions that should have yielded a total of at least 60,000 colonies with *hisD*$^+$ donors (Table 1*B*). On the other hand, the frequency of transduction of *hisD3018* to prototrophy was only 25 to 50 per cent of normal for TR936 with donor phage from both cultures. We are uncertain of the reason for this behaviour, but the plating tests mentioned above indicate it was not the result of loss of the suppressor gene.

Histidinol dehydrogenase from the TR936 cells was accordingly purified in lots of 1 kg at a time by the standard procedure[8] through the ammonium sulphate precipitation step. The preparations were then passed through either a 'Sephadex *G*-150' column, 10 × 100 cm, or a 'DEAE-Sephadex *A*-50' column, 10 × 25 cm, with the usual buffer systems[5,8]. The concentrated column eluates were combined and passed in alternating fashion through four 'Sephadex *G*-150' and four 'DEAE-Sephadex *A*-50' columns of successively smaller size and the enzyme was crystallized twice (compare ref. 8). TR936 (histidinol dehydrogenase) activity is so low as to be indeterminate in cruder fractions. The specific activity of crude extract enzyme was calculated from the purification data, with the wild-type as a standard, to be approximately 0·0022 units per mg protein. This level is about 1 per cent that of *hisO1242–hisD*$^+$ histidinol dehydrogenase from cells grown in similar conditions (0·26 units per mg protein) (R. G. Martin and J. Y., unpublished observations). The twice crystallized preparation had a specific activity of 11·1 units per mg protein, virtually identical to that of homogeneous wild-type enzyme[8]. We can conclude therefore that histidinol dehydrogenase synthesis in TR936 is about 1 per cent that of the *hisO1242–hisD*$^+$ strain, with the assumption that the histidine-grown TR936 cultures did not contain a sizable fraction of *sup*$^+$ cells. In any event, the level calculated for the total culture is 600-fold that attributable to potential wild-type contaminants, which, as we have shown here, could constitute a maximum of 0·0015 per cent of the cells.

Approximately 28 mg of purified TR936 enzyme was obtained, equivalent to a yield of 30 per cent of the starting activity. This enzyme was indistinguishable from a wild-type control in polyacrylamide gel electrophoresis at *p*H 9·5 (Fig. 2), and in amino-acid composition.

Tryptic peptide T26A of histidinol dehydrogenase is affected by the *hisD3018* frameshift[5]. The wild-type peptide is inseparable from peptide T26B on standard peptide maps. Because (1) the small amounts of TR936 enzyme available precluded examination of peptide T26A by methods more rigorous than peptide mapping, and (2) our working hypothesis was that this enzyme is

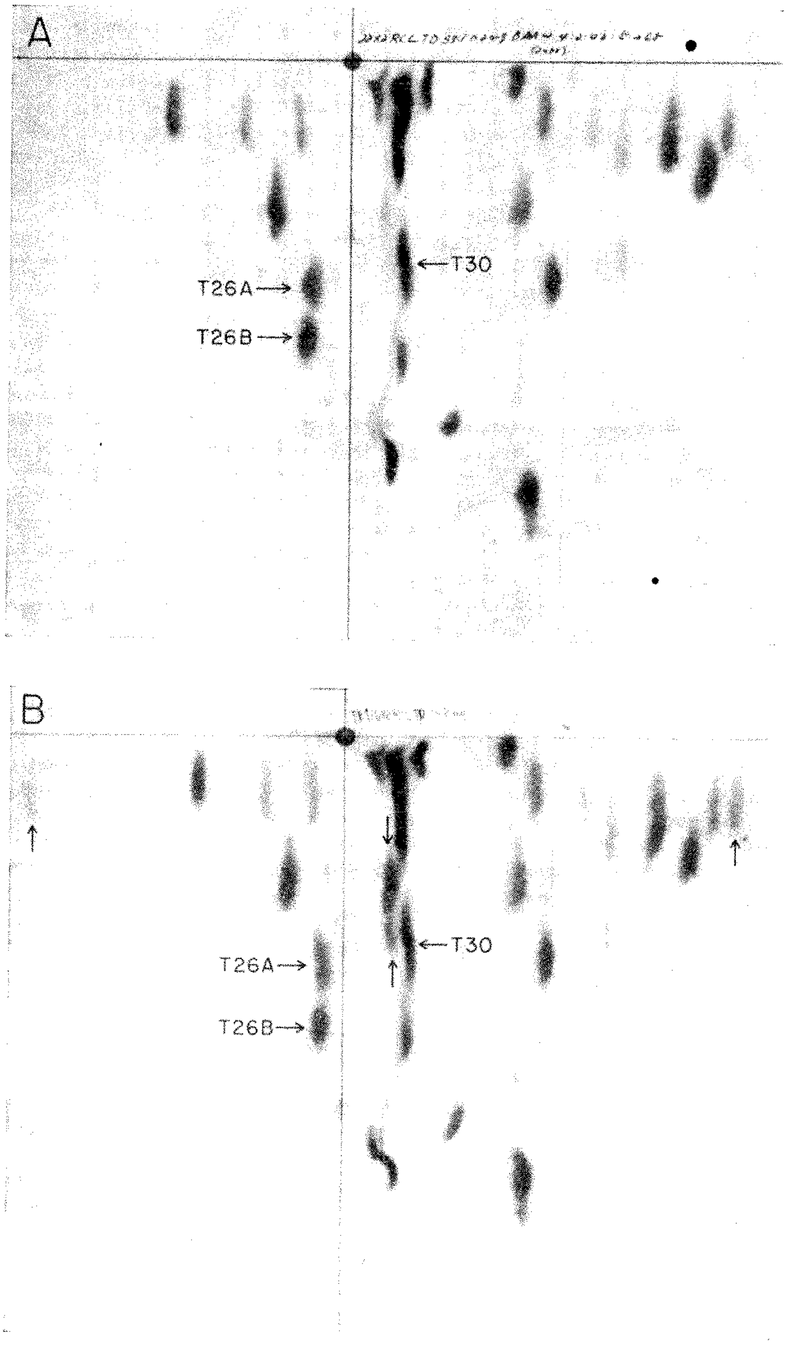


Fig. 3. Tryptic peptide maps of reduced carboxymethylated histidinol dehydrogenase. *A*, Wild type. *B*, TR936. Pertinent peptide spots are indicated by horizontal arrows. Peptides staining more strongly in the TR936 map are indicated by vertical arrows. These are occasionally much more intense in the wild-type as well and therefore seem unrelated to suppression.

Table 2. RECOVERY OF *hisD3018* FROM *hisD3018sup* STRAINS BY TRANSDUCTION INTO *hisO1242*

hisD isolate characteristics	No. of isolates *hisD3018* Control	Donor *hisD3018* R20	 *hisD3018* R51	 *hisD3018* R1* Control
ICR⁺ NG⁺ †	25	21	11	0
ICR⁻ NG⁻	4	3	7	0
ICR⁺ NG⁻	1 ‡	0	2	0
ICR⁻ NG⁺	0	0	2	0
Total *his* isolates	33	39	32	20

* See Table 1 for description.
† +, Responds to mutagen; −, does not respond.
‡ Weak response to ICR-191.

wild-type in amino-acid sequence, we sought a chromatograph solvent which would effectively isolate wild-type T26A in peptide maps. Tryptic peptide maps prepared with butanol–pyridine–acetic acid–water (4 : 2 : 0·2 : 5) proved adequate and revealed that T26A of TR936 was present in the wild-type position (Fig. 3). The combined T26A spots from three peptide maps of TR936 digest, stained with the ninhydrin–collidine reagent[9], were solubilized in pyridine–water (1 : 1), then dried and hydrolysed in 6 M HCl as described previously[5]. The amino-acid composition of T26A from TR936 was indistinguishable from that of a wild-type control prepared in the same manner, even with respect to the levels of contamination observable (Table 3). The wild-type peptide has been shown to have the sequence Val-Thr-Pro-Glu-Glu-Ile-(Gly, Ala_4)-Arg (ref. 5). Tryptic peptide T30 of TR936 N-adjacent to T26A (ref. 10) was also wild-type in map location and amino-acid composition.

Table 3. AMINO-ACID COMPOSITION OF PEPTIDE T26A ISOLATED FROM PEPTIDE MAPS OF TR936 AND WILD-TYPE HISTIDINOL DEHYDROGENASE

	No. of residues per sample Wild-type purified control*	 Wild-type	 TR936
μmoles peptide	0·077	0·075	0·075
Amino-acid			
Val	1·11	0·69 †	0·58 ‡
Thr	1·00	0·96	0·98
Pro	0·93	0·91	0·93
Glu	1·98	2·09	2·06
Ile	0·93	0·97	1·01
Gly	1·05	1·19	1·20
Ala	4·18	4·14	4·00
Arg	1·00	0·93	0·96
(Asp) ‡	—	(0·23)	(0·25)
(Ser)	—	(0·25)	(0·29)
(Leu)	—	(0·28)	(0·30)

* Homogeneous peptide prepared by column chromatography[3].
† The yield of N-terminal valine is reduced because of reaction with the ninhydrin–collidine staining reagent.
‡ Major observable contaminants given in parentheses.

These results strongly suggest that the suppressor gene is capable of restoring small amounts of histidinol dehydrogenase with a wild-type sequence in TR936 cells. To confirm this a method was developed for sequencing the critical section of the protein from T26A. From 12 mg of each tryptic digest, six peptide maps were prepared and stained lightly with 0·025 per cent ninhydrin in ethanol. Peptide T26A was thus detected with virtually no destruction of N-terminal valine, facilitating the subsequent Edman degradation. The pooled peptide, extracted in pyridine–water (1 : 1), was subjected to four steps of the subtractive Edman degradation. For TR936 T26A the sequence Val-Thr-Pro-Glu-(Glu, Ile, Gly, Ala_4)-Arg was unequivocably established for both the TR936 peptide and the wild-type control (Fig. 4). That wild-type glutamic acid residues, rather than glutamines, are present in TR936 T26A is indicated by its normal electrophoretic mobility at *p*H 6·5 (Fig. 3).

The suppressor state of TR936 must therefore involve modification of some component of the translation system so that the frameshifted region of *hisD3018* mRNA, containing an extra C residue, is translated in phase, producing an intact polypeptide chain of normal sequence (Fig. 5). Previous studies on missense suppression[11,12] and nonsense suppression[13,15] have established altered tRNA as the suppressor agent. Fink *et al.*[7] have shown that ICR compounds can induce unlinked suppressors of nonsense mutations in *S. typhimurium* and have proposed an addition–deletion mechanism at the level of tRNA. A similar suggestion has been made for ICR-induced nonsense suppressors in yeast[16]. Riyasaty and Atkins (ref. 2) have alluded to the possibility of suppression by tRNA in their study of the suppressible frameshift, *tryA91*. Although other mechanisms, such as those based on re-initiation of translation in a compensatory phase, may obtain in other systems[2,5], our results for *hisD3018* favour a model of low efficiency in-phase reading of the +1 frameshift by a suppressor tRNA. One appealing possibility is a suppressor tRNA with a quadruplet anticodon[5]. The fact that ICR-191 induced the *hisD3018* + 1 frameshift and is very effective in inducing unlinked suppressors[4] is consistent with such a mechanism. To produce wild-type enzyme this could occur at three possible quadruplet codon sites: (1) ACCC as threonine, (2) CCCU as proline, (3) UGA$^{A}_{G}$ as glutamic acid (Fig. 5*A*). The quadruplet anticodon for proline or threonine could originate in repair or replication of the tRNA gene by the same slippage mechanism[17] in a DNA segment of repeating GC pairs considered to have generated the *hisD3018* frameshift[5]. The *tryA91* frameshift may be a −1 type[2]. In terms of this model, suppression of *tryA91* could be mediated by a

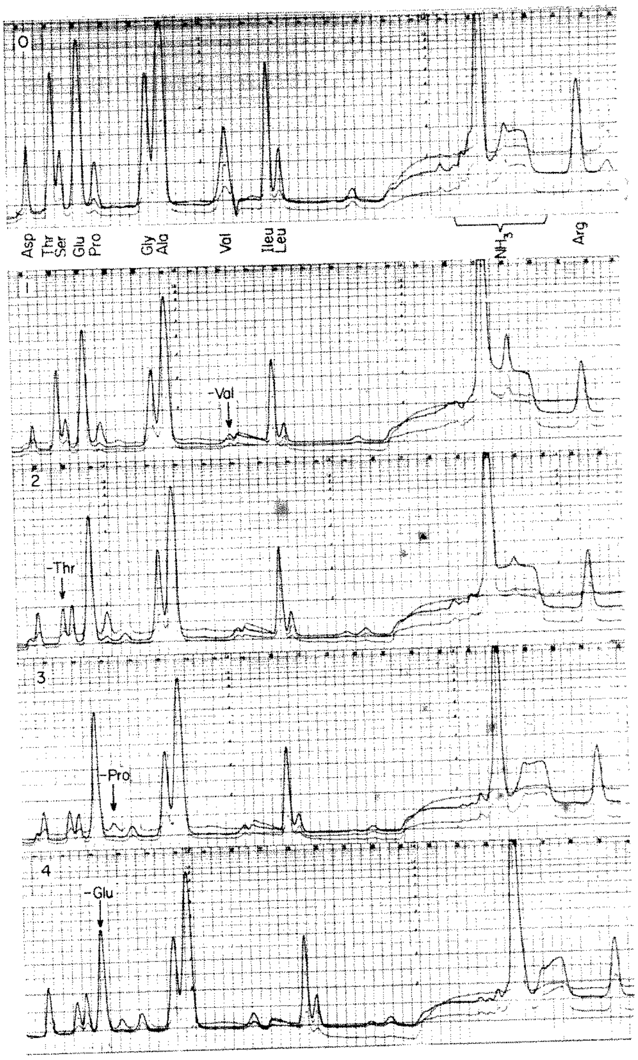


Fig. 4. Amino-acid composition and partial sequence of TR936 peptide T26A by Edman degradation. Shown are amino-acid analyser chromatograms of hydrolysates. The 570 nm line, and the 440 nm line for proline only, have been traced with Indian ink. Smaller peaks are accentuated on the logarithmic scale. 0, undegraded peptide 0·075 μmole analysed. See Table 3 for amino-acid composition. 1, 2, 3, 4, peptide after successive steps of the Edman degradation, about 0·040 μmoles analysed per step. The wild-type T26A control gave virtually identical results. See text for further details.

tRNA with a doublet anticodon. NG, and to a lesser extent ICR-191, induce unlinked suppressors of *tryA91*[2]. Both of these mutagens can delete single base pairs from DNA[3,5]. Another possibility is a suppressor tRNA with out of phase pairing properties imparted by alterations in tertiary structure induced by addition or deletion. Through distortion or steric hindrance such a tRNA might skip a base or slip when pairing with mRNA and thus correct the +1 frameshift of *hisD3018* (Fig. 5*B*). Given "wobble" pairing[18] at the third and fourth base pairs in codon–anticodon recognition, the number of possible *hisD3018* suppressor tRNA species is large.

In Fig. 5 it can be seen that a polar UGA codon[19,20] is generated in the +1 phase immediately after the frame-shifted segment of *hisD3018* mRNA. The UGA codon might well enhance the level of *hisD3018* suppression by impeding normal translation of mRNA in the +1 phase at the frameshifting site, thus allowing an inefficient suppressor tRNA to compete effectively in codon pairing.

Although there is less precedent, other components of the translation system, such as the ribosome, could be the site of suppression. Unlike many base substitution mutations[21] and typical of frameshifts[1], *hisD3018* is not phenotypically curable by streptomycin[3]. These observations, however, do not rule out a different suppressor site or mechanism at the ribosomal level

We thank our colleagues D. Riddle and J. R. Roth, who prepared strain TR936, and R. G. Martin, who provided large quantities of TR936 cells. We also thank J. R. Roth and P. E. Hartman for a critical reading of this manuscript. The research was carried out under the auspices of the US Atomic Energy Commission.

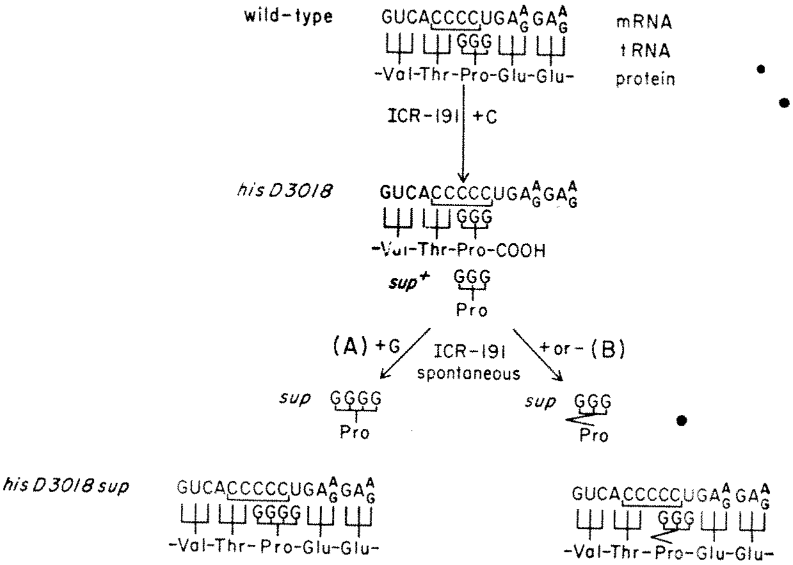


Fig. 5. Possible modes of *hisD3018* suppression. Aminoacyl-tRNAs are diagrammatically shown paired to their respective mRNA codons. The frameshifting properties of *hisD3018* result from the inclusion of an extra C residue in mRNA somewhere within the bracketed area. The chain-terminating codon, UGA, dictating formation of a polypeptide fragment with C-terminal proline, is generated in the +1 phase immediately after the frameshifting site in *hisD3018*. Suppression may occur as follows: *A*, a proline suppressor tRNA with the quadruplet anticodon GGGG restores in phase translation by pairing with the quadruplet CCCU; *B*, a conformationally altered proline suppressor tRNA with the normal triplet anticodon GGG skips a nucleotide or slips when pairing with the quadruplet CCCU.

Received November 24, 1969; revised January 9, 1970.

[1] Whitfield, H. J., Martin, R. G., and Ames, B. N., *J. Mol. Biol.*, **21**, 335 (1966).

[2] Riyasaty, S., and Atkins, J. F., *J. Mol. Biol.*, **34**, 541 (1968).

[3] Oeschger, N., and Hartman, P. E., *J. Bact.* (in the press).

[4] Yourno, J., Barr, D., and Tanemura, S., *J. Bact.*, **100**, 453 (1969).

[5] Yourno, J., and Heath, S., *J. Bact.*, **100**, 460 (1969).

[6] Tanemura, S., and Yourno, J., *J. Mol. Biol.*, **46**, 459 (1970).

[7] Fink, G. R., Klopotowski, T., and Ames, B. N., *J. Mol. Biol.*, **30**, 81 (1967).

[8] Yourno, J., and Ino, I., *J. Biol. Chem.*, **243**, 3273 (1968).

[9] Canfield, R. E., and Anfinsen, C. B., in *The Proteins* (edit. by Neurath, H.), **1**, 319 (Academic Press, New York, 1963).

[10] Yourno, J., *J. Mol. Biol.* (in the press).

[11] Carbon, J., Berg, P., and Yanofsky, C., *Proc. US Nat. Acad. Sci.*, **56**, 764 (1966).

[12] Gupta, N. K., and Khorana, H. G., *Proc. US Nat. Acad. Sci.*, **56**, 772 (1966).

[13] Capecchi, M. R., and Gussin, G. N., *Science*, **149**, 416 (1965).

[14] Englehardt, D. L., Webster, R. E., Wilhelm, R. C., and Zinder, N. D., *Proc. US Nat. Acad. Sci.*, **54**, 1791 (1965).

[15] Goodman, H. M., Ableson, J., Landy, A., Brenner, S., and Smith, J. D., *Nature*, **217**, 1019 (1968).

[16] Magni, G. E., von Borstel, R. C., and Steinberg, C., *J. Mol. Biol.*, **16**, 568 (1966).

[17] Streisinger, G., Okada, Y., Emrich, J., Newton, J., Tsugita, A., Terghaghi, E., and Inouye, M., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 77 (1966).

[18] Crick, F. H. C., *J. Mol. Biol.*, **19**, 548 (1966).

[19] Brenner, S., Barnett, L., Katz, E. R., and Crick, F. H. C., *Nature*, **213**, 449 (1967).

[20] Weigert, M. G., Lanka, E., and Garen, A., *J. Mol. Biol.*, **21**, 335 (1966).

[21] Gorini, L., Jacoby, G. A., and Breckenridge, L., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 657 (1966).

Eye Dominance in the Visual Cortex

by

COLIN BLAKEMORE*
JOHN D. PETTIGREW†

Neurosensory Laboratory,
School of Optometry,
University of California,
Berkeley, California 94720

The projection from the contralateral eye dominates the cat's visual cortex. Binocular neurones in any small region have their receptive fields more widely scattered over the ipsilateral retina than over the contralateral. Stereoscopic vision may depend on this difference in the two projections.

As far as we know, the fibres in the optic nerves of all primitive mammals cross over, or decussate, completely at the optic chiasma[1]. Each eye projects its information entirely to the opposite hemisphere of the brain. But in more advanced mammals with frontal eyes and therefore a considerable binocular visual field, a certain proportion of the retina on the temporal side sends its optic nerve fibres into the optic tract on the same side, and therefore to the ipsilateral hemisphere[2].

This process seems to go hand in hand with the development of binocular vision[3] and so furnishes the clue to why partial decussation occurs, for in an animal with frontal eyes the temporal retina of one eye is viewing the same region of space as the nasal retina of the other. Thus each hemisphere is concerned with information from the opposite half of the visual world, whether it comes from one eye in animals with lateral eyes or from part of both eyes.

---

* Present address: Physiological Laboratory, University of Cambridge.
† Present address: The Royal Prince Alfred Hospital, Camperdown 2050, NSW, Australia.

We are here concerned with the nature of the inputs from the two eyes to the striate area of the cat, a binocular animal whose brain has been intensively studied in recent years. How are signals in the primitive fibres from the contralateral eye combined with those in the phylogenetically newer projection from the ipsilateral eye ?

We shall discuss the results of neurophysiological investigations of single neurones which receive an input from both eyes. More than 80 per cent of the units in area 17 of the cat's cortex can be activated by a linear target (a bright slit, black bar or black–white edge) presented to either eye[4,5]. We have recorded action potentials from them with tungsten-in-glass microelectrodes and plotted their receptive fields by moving visual images across a screen 114 cm from the eyes. Image quality was preserved by corneal contact lenses, 3 mm artificial pupils and correcting lenses. The cat was anaesthetized by artificial respiration with a nitrous oxide/oxygen mixture and was paralysed by intravenous infusion of gallamine triethiodide (Flaxedil) and *d*-tubocurarine. The latter procedure, along with mechanical fixation of the eyes to metal rings, kept the eyes very stable. The projections of the optic disks on the screen were periodically plotted to ensure that no eye movement occurred. Our methods have been described in detail elsewhere[5,6].

Eye Preference of Single Cortical Neurones

We confirmed Hubel and Wiesel's observation[4] that binocularly driven units are often more effectively activated by a stimulus to one of the eyes than by a stimulus to the other, and that the contralateral eye is more often dominant. Fig. 1 illustrates responses from a unit that would fall into Hubel and Wiesel's eye-dominance group 2 and the histogram of Fig. 2 shows the distribution of eye-dominance among 228 cortical neurones from eighteen cats. We have excluded from the analysis 28 monocular units that were recorded in the white matter under the cortex. They had the fast action potential waveforms typical of nerve fibres and the receptive field properties of lateral geniculate cells[7]. We presume that they were axons in the optic radiation. We have also ignored a number of cortical units that were lost before receptive fields of both eyes were thoroughly explored. The eye-dominance groups of Hubel and Wiesel are explained in the legend to Fig. 2 and it is clear that almost twice as many cells are dominated contralaterally as are ipsilaterally.

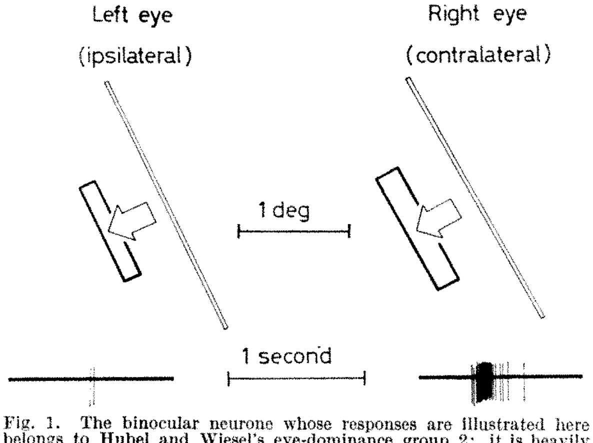


Fig. 1. The binocular neurone whose responses are illustrated here belongs to Hubel and Wiesel's eye-dominance group 2: it is heavily dominated by the input from the contralateral eye. The receptive fields for the left and right eyes are shown as rectangles on the left and right, respectively. Below each field is an oscilloscope record of the action potentials typically evoked from the neurone by a single, slow sweep of a thin bright slit across the receptive field in that eye. Clearly the unit is far more strongly activated by a stimulus to the right (contralateral) eye than by one to the left.

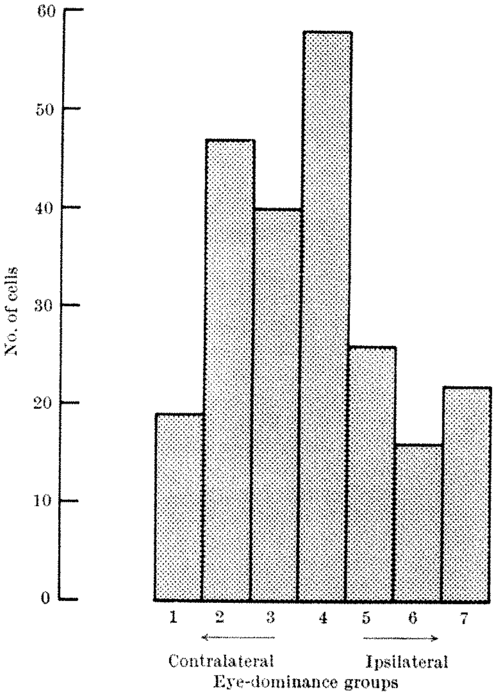


Fig. 2. This histogram shows the distribution of eye-dominance amongst 228 binocular cortical neurones. The group classification is as follows. Group 1: the neurone can only be influenced directly through the contralateral eye; 2: the contralateral eye is much more effective than the ipsilateral; 3: the contralateral eye is slightly more effective than the ipsilateral; 4: both eyes are equally effective; 5: the ipsilateral eye is slightly more effective than the contralateral; 6: the ipsilateral eye is much more effective than the contralateral; 7: the neurone can only be influenced directly through the ipsilateral eye; 106 of these units were therefore contralaterally dominated and 64 ipsilaterally dominated.

Each hemisphere, then, receives a somewhat greater excitatory input from the opposite eye than from the ipsilateral retina. It is noteworthy that the group classification of eye-dominance caters mainly for excitatory influences. The proportion of monocular neurones is even smaller if more subtle inhibitory effects are included in the analysis[8].

Retinal Disparity and the Two Projections

An interesting property of the cat's binocular neurones is that they do not necessarily have their receptive fields on precisely corresponding points in the two retinae. The horizontal disparity of the receptive fields varies over several degrees from cell to cell[5,6,9]. In effect, the trigger feature of a binocular cortical unit is an orientated target placed at such a position in the visual field, and at such a distance from the eyes, that its image falls on the receptive field in both eyes: and the optimal distance differs from cell to cell.

We now have a little more information about the anatomical and perhaps the phylogenetic origin of these disparities.

The first hint comes from Nikara, Bishop and Pettigrew's observation[9] that, for any small recording site in the cortex, the receptive fields tend to cover a wider area of retina in the ipsilateral eye than in the contralateral. We also noticed this during our experiments with H. B. Barlow[5] and decided to examine the point in more detail.

In each cat we made one or more penetrations into a tiny craniotomy which only exposed 3 or 4 mm^2 of cortical surface. We plotted the receptive fields of each binocular unit separately in the two eyes. They were invariably separated by a few inches on the screen because we had manipulated the eyes to make the visual axes divergent. We satisfied ourselves that if an image of the appropriate orientation was projected approximately on to the centres of the two fields and they were simultaneously moved back

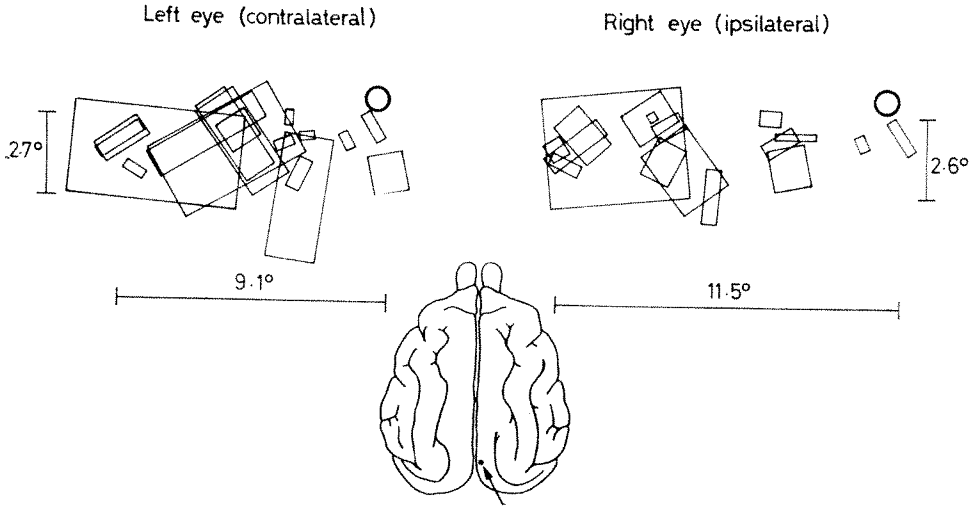


Fig. 3. Five penetrations were made, close together, into area 17 of cat 11 at the point shown on the sketch of the brain. All the reliably plotted receptive fields of the binocular neurones in the penetrations are shown above, those on the left for the left eye and on the right for the right eye. The arrays of fields have been rotated slightly to correct for twisting of the eyes during the experimental preparation. Near each array a circle marks the approximate position of the area centralis for that eye. Because the electrode was in the right hemisphere, the left eye is contralateral, and the receptive fields in that eye are less widely scattered horizontally than in the right eye.

and forth across the fields, the unit gave a facilitated binocular response[5,10].

We took photographs of the slit-like entrance pupils in the unanaesthetized cat and again after all the preparation. So, in spite of the slight twisting inevitably introduced by suturing the eyes to metal rings, we knew the true horizontal and vertical on the screen for each eye. We were therefore able to measure the horizontal and vertical dimensions of the patch of retina covered in the two eyes by the receptive fields.

Fig. 3 shows the results for one cat. In this case, five penetrations were made in the same region of area 17 in the right hemisphere, shown in the sketch of the brain. The receptive fields for all the binocular neurones are illustrated as rectangles: the array on the left is for the left eye and that on the right for the right eye. (This kind of receptive field plot has been called a minimum response field, or simply a response field, and the method and reliability of plotting have already been described[5,6,9].) The arrays have been rotated to correct for slight torsion in the two eyes. The circle near each set of fields marks the approximate projection of the area centralis in each eye. Clearly, for these seventeen binocular neurones the receptive fields are more widely scattered over the retina of the ipsilateral (right) eye than they are over the contralateral. In fact, the centres of the fields cover 11·5° horizontally and 2·6° vertically in the right eye, 9·1° horizontally and 2·7° vertically in the left.

Usually the horizontal spread of receptive field centres was a few degrees greater in the ipsilateral eye than in the contralateral, though there was no consistent difference in the vertical scatters. For some penetrations, particularly in the representation of the peripheral visual field, which is greatly compressed in its cortical map[4], the receptive fields from units in a small cortical region covered a large retinal area. Even in those cases the spread was usually a few degrees wider on the ipsilateral retina.

Figs. 4 and 5 analyse the results for 138 reliably plotted binocular neurones from fifteen craniotomies in twelve cats. The horizontal and vertical separations of the most extreme receptive field centres in every sample were measured, as in Fig. 3, and each point on the graph of Fig. 4 plots the spread in the ipsilateral eye against that in the contralateral, for one craniotomy. Solid circles show the horizontal spread, open circles the vertical. The continuous line with a slope of 1 is the expected relationship if the area of retina covered by the fields is identical in the two eyes. Clearly in only two cases was the horizontal range of receptive fields a little greater in the contralateral eye than in the ipsilateral. This is significantly different from a chance discrepancy (P = 0·008 : two-tailed binomial test).

The interrupted lines in Fig. 4 are the best fitting (least squares) straight lines through the solid and open circles. The line for the vertical spread is close to the continuous line but that through the horizontal data points is displaced upwards by about 2°.

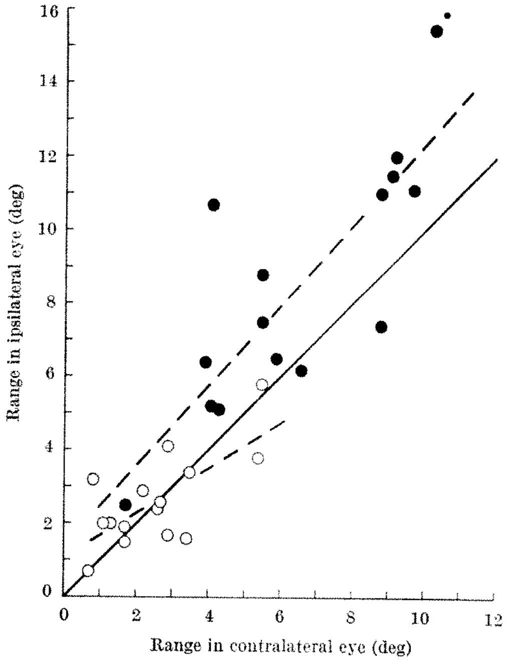


Fig. 4. Each point on this graph represents the angular separation of the centres of the most extreme receptive fields for the binocular units recorded in a single craniotomy. The smallest sample had four cells, the largest had twenty-one. The solid circles are for the horizontal spread of receptive field centres, and the open circles show the vertical spread. The range on the ipsilateral retina is plotted against that on the contralateral, for each sample. If the spread was always the same in the two eyes, all the points should lie along the solid line with a slope of 1. If the spread is greater in the ipsilateral eye the point will lie above this line. In these fifteen samples the horizontal scatter was greater in the ipsilateral eye for thirteen, but the vertical scatter for only seven. The upper interrupted line is the regression line through the solid circles and the lower one is that for the open circles.

Fig. 5 shows that the inequality in the horizontal range of receptive fields for the two eyes entails a genuine difference in scatter, and is not simply the result of one or two greatly misplaced fields on the ipsilateral retina. For in this second graph we have plotted not merely the separations of the extreme receptive fields in the samples, but the standard deviations of the distributions of position for all the receptive fields in each sample. Again, the scatter in the ipsilateral eye is plotted against that in the contralateral, and the solid and open circles refer to the standard deviations of the horizontal positions (angles of azimuth) and vertical positions (angles of elevation), respectively, of the centres of the receptive fields. See how this method confirms the fact that the fields are horizontally more broadly scattered across the ipsilateral retina, for twelve of the fifteen solid circles lie above the line with a slope of 1 ($P = 0{\cdot}036$: two-tailed binomial test). The vertical dispersion is, however, very similar in the two eyes: seven of the open circles are below the line and eight above.

We conclude from our experiments that the retinotopic mapping of the ipsilateral eye on the cortex is less precisely laid down than that of the contralateral eye. Indeed, it seems to be the apparent lack of anatomical specificity in the ipsilateral input which creates the range of horizontal disparities. Imagine a group of binocular cells in a small cortical region. The fibres arriving at this part of the cortex from the opposite eye represent a fairly well defined area of retina, but the phylogenetically younger input from the same side is, somehow, not so precisely localized. The anatomical diffusion in the ipsilateral retinotopology would, then, automatically lead to the formation of binocular neurones connected to a variety of points in the ipsilateral eye for each spot in the contralateral.

In the newborn kitten the vertical spread of receptive fields might also be greater in the ipsilateral eye and hence the range of vertical disparity might be as large as that of horizontal. Binocular cells representing large vertical disparities would almost never be simultaneously stimulated through both eyes in normal viewing conditions and, through lack of use, they might lose one or both inputs[11] and become functionless. Thus, in time, the anisotropy of horizontal and vertical disparity would appear.

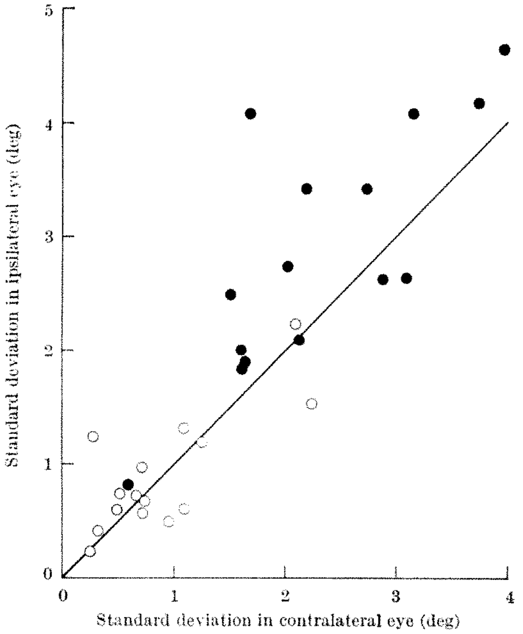


Fig. 5. The scatter on the two retinae for the samples of binocular units analysed in Fig. 4 is treated rather differently in this graph. The horizontal and vertical positions of the centre of every receptive field in the sample were measured, and each point plots the standard deviation of the distribution of locations in the ipsilateral eye against that in the contralateral eye for all the units in a single sample. Solid circles represent the horizontal scatter and open circles the vertical. The continuous line again has a slope of 1, the expected relationship if the dispersion were identical in the two eyes.

Binocular Nature of Cortical Columns

Another clue to how binocular messages combine comes from Blakemore[12], who investigated the organization of binocular neurones within the columns of the visual cortex. Hubel and Wiesel[4] have described how these blocks or sheets of cortical tissue contain cells that all have the same optimum target orientation, and it turns out that these orientation columns fall into two classes according to the arrangement of their binocular cells. Some of them, termed depth columns, have almost identical arrays of receptive fields in the two eyes and consequently the disparity is virtually the same for all units in the column. The column as a whole is looking at objects of some orientation at a certain distance from the eyes.

On the other hand, there are certain so-called direction columns in which all the receptive fields are superimposed on the retina contralateral to the hemisphere containing the column, but are horizontally scattered in the ipsilateral eye. The disparity therefore varies enormously from cell to cell, and the column as a whole is viewing objects of one orientation falling along a single oculocentric visual direction based on the contralateral eye.

If certain higher-order neurones in each column receive messages from many of the lower-order cells, as suggested by Hubel and Wiesel[4], then such a "complex" cell would, in a depth column, represent an object at one distance from the eyes but over some range of position in the visual field. In a direction column the "complex" unit would signal the oculocentric position of an object in the contralateral eye, regardless of its depth in space.

In these direction columns, then, the topographic representation of the contralateral eye is more exactly specified than that of the ipsilateral input, just as in any patch of cortical tissue, containing many columns.

A picture thus emerges of the visual cortex of the cat dominated by the traditional crossed visual input. The intruding optic fibres from the eye on the same side are usually less effective in exciting the neurones and not so precisely projected on the cortex. But it is this very element of difference in the two projections which creates the disparities of binocular cells and gives them their ability to encode the distances of objects in space. A system has developed for using the disparity information and the neurones have been organized into functional columns. Within many of these columns it is still the contralateral eye which dominates and determines the arrangement.

It is therefore the dispersion of the phylogenetically younger ipsilateral input which supplies the evolutionary advantage of frontal eyes, namely, the cue for discriminating the relative distances of objects from the eyes.

We thank Professor H. B. Barlow for help and for financial support from a US Public Health Service grant. C. B. was sponsored by a research fellowship from Fight For Sight, Inc., New York.

Received October 3; revised December 16, 1969.

[1] Walls, G. L., *The Vertebrate Eye and its Adaptive Radiation*, 319 (Hafner, New York, 1963).
[2] Wollaston, W. H., *Phil. Trans. Roy. Soc.*, **114**, 222 (1824).
[3] Gudden, B. von, *Albrecht v. Graefes Arch. Ophthal.*, **25**, 237 (1879).
[4] Hubel, D. H., and Wiesel, T. N., *J. Physiol.*, **160**, 106 (1962).
[5] Barlow, H. B., Blakemore, C., and Pettigrew, J. D., *J. Physiol.*, **193**, 327 (1967).
[6] Blakemore, C., *J. Physiol.*, **205**, 471 (1969).
[7] Hubel, D. H., and Wiesel, T. N., *J. Physiol.*, **155**, 385 (1961).
[8] Henry, G. H., Bishop, P. O., and Coombs, J. S., *Vision Res.*, **9**, 1289 (1969).
[9] Nikara, T., Bishop, P. O., and Pettigrew, J. D., *Exp. Brain Res.*, **6**, 353 (1968).
[10] Pettigrew, J. D., Nikara, T., and Bishop, P. O., *Exp. Brain Res.*, **6**, 391 (1968).
[11] Hubel, D. H., and Wiesel, T. N., *J. Neurophysiol.*, **28**, 1041 (1965).
[12] Blakemore, C., *Binocular Interaction in Animals and Man* (PhD thesis, University of California, Berkeley, California, 1968).

Low Mode Coherent Synchrotron Radiation and Pulsar Phenomena

by
BERNARD J. EASTLUND
Division of Research,
US Atomic Energy Commission

Information on the properties and distributions of the charges responsible for pulsar emission can be deduced from an appraisal of the coherent synchrotron radiation from an oblique rotator model.

THIS article is an extension of my original calculations on the oblique rotator–coherent synchrotron radiation model for pulsar r.f. emission[1,2] and a comparison with observations. In this model the knife-like angular pattern of coherent synchrotron radiation is converted into a time dependent pulse by rotation of the star (Fig. 1).

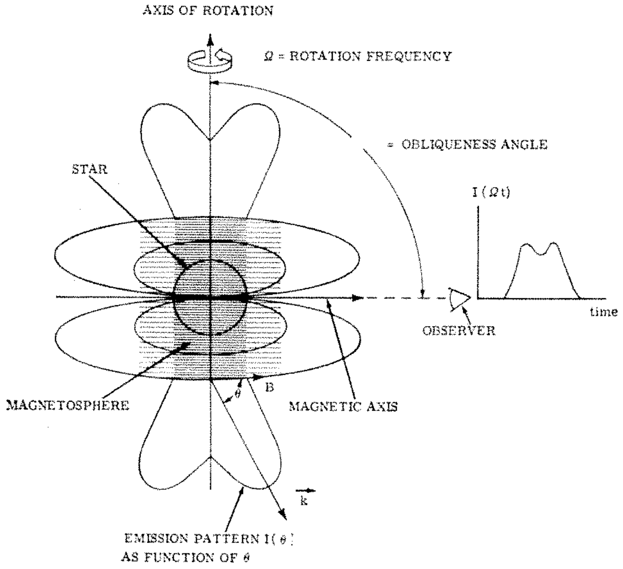


Fig. 1. The oblique rotator–coherent synchrotron radiation model for pulsar emission.

The comparison with observations determines (*a*) a lower limit for the average energy of the radiating charges ($E > 100$ MeV), (*b*) the average magnetic field in the radiation region (10–100 G), and (*c*) the particle Larmor radius (on the order of kilometres). A refined model is then found to lead to an explanation of (*a*) the fine structure, (*b*) repetitive phenomena on a kilocycle time scale, (*c*) the absence of even–odd pulse effects for obliqueness angles in which two pulses should be emitted per rotation, and (*d*) the appearance of a double peak on averaged signals when single pulses appear on a pulse to pulse basis.

The Calculation

The synchrotron radiation intensity as a function of mode, *m*, and angle θ (the angle perpendicular to the plane of the particle orbit) was shown in ref. 1 to be

$$P_m = \frac{e^2 m^2 \omega_L^2}{8\pi\varepsilon_0 c}\left[\left(\frac{\cos\theta - \beta_{\parallel}}{\sin\theta}\right)^2 \mathrm{J}_m^2\,(m\beta_{\perp}\sin\theta) + \beta_{\perp}^2\,\mathrm{J}_m'^2\,(m\beta_{\perp}\sin\theta)\right] \quad (1)$$

where ω_L is the Larmor angular frequency, $\beta_{\parallel} = v_{\parallel}/c$, and $\beta_{\perp} = v_{\perp}/c$. The polarization of this radiation, in cartesian coordinates, was shown to be

$$E_x = -A\,\frac{\cos\theta}{\sin\theta}\,(\cos\theta - \beta_{\parallel})\,\mathrm{J}_m(x)$$

$$E_y = -A\,i\beta_{\perp}\,\frac{\mathrm{d}\,\mathrm{J}_m(x)}{\mathrm{d}x} \quad (2)$$

$$E_z = A\,(\cos\theta - \beta_{\parallel})\,\mathrm{J}_m(x)$$

where $x = m\beta_{\perp}\sin\theta$ and $A = (e^2\omega_L^2 m^2/8\pi^2\varepsilon_0 c)^{1/2}$.

The present calculations evaluate the equations up to mode number 950 (the limit of the Bessel function subroutine). Transformation from angular dependence to time dependence was made as follows

$$P_m(\theta) = P_m\left(\frac{2\pi}{\tau}\,t\right) \quad (3)$$

where τ is the pulsar repetition rate. Such a transformation corresponds to the assumption that the repetition rate is equal to the rotation period. Twice the experimental period is also used in calculations for Table 1. It is assumed that $\beta_{\parallel} = 0$. (Further work is in progress to study the effect of $\beta_{\parallel} \neq 0$.)

Table 1. PARAMETERS FOR TWO PULSARS

	Case	Period (s)	r.f. measured (MHz)	Mode No. calculated	f_0 (kHz)	B (G)	r_L (m)
CP 1133	B	1·187	111·5	710	162	11·5	290
CP 1133	A	2·376	111·5	4,800	24	1·72	1,980
NP 0527	B	3·745	320	135	2,360	165	20
NP 0527	A	7·490	320	880	364	25·8	132

Pulse shapes as a function of mode number are presented in Figs. 2 and 3 for pulsar CP 1133 ($\tau = 1{\cdot}187$ s). Fig. 2 is a set of pulses calculated for a charged particle energy of 5 MeV ($\gamma = ||$ where $\gamma = (1-\beta_{\perp}^2)^{-1/2}$). Note the transition from a double peak at low mode numbers to a single peak at high mode numbers. Pulse shapes for an energy of 100 MeV are plotted in Fig. 3 ($\gamma \approx 200$). The double pulse persists up to the limit of the calculations.

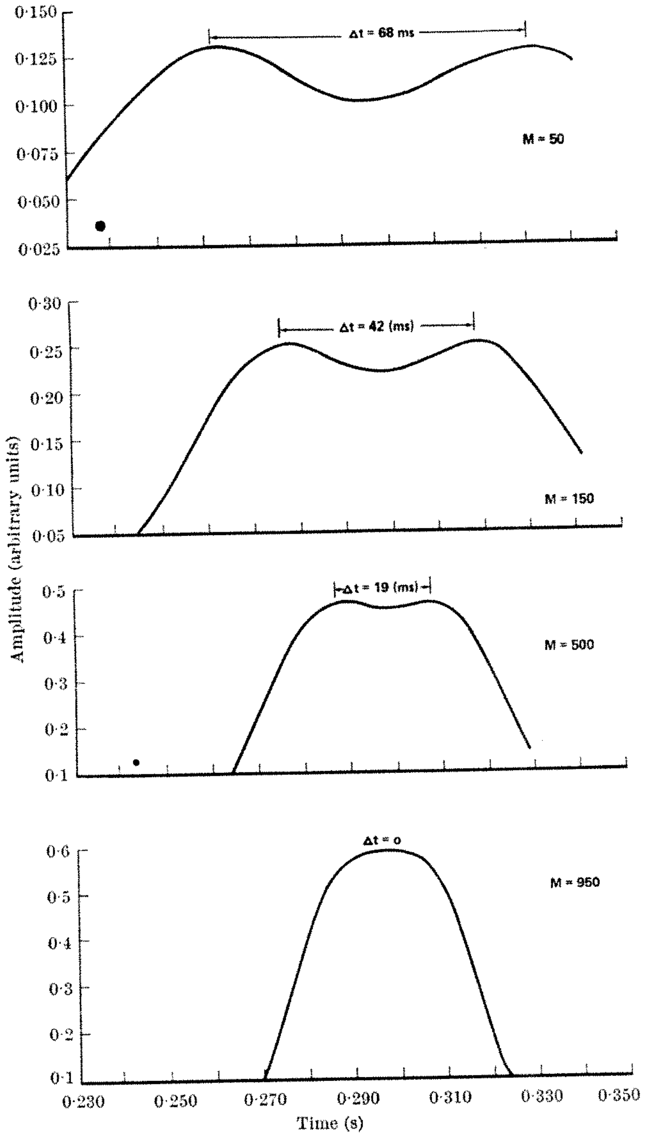


Fig. 2. Predicted pulse shapes (CP 1133) as a function of synchrotron mode number with rotation period, τ, equal to 1·187 s. Charged particle energy is 5 MeV.

The frequency dependence (synchrotron mode number) of the peak to peak pulse widths is presented in Fig. 4 with charged particle energy as a parameter. As the particle energy increases, the transition from double to single peaks is seen to move to higher mode numbers. When the particle energy becomes greater than 100 MeV the frequency dependence approaches a constant slope. Thus one might expect the calculated slope to be found in more than one pulsar (as indeed is the case).

This frequency behaviour of pulse shapes can easily explain why some pulsars exhibit frequency dependent pulse widths and others do not. Clark and Smith, who have used a coherent synchrotron radiation model, use the standard approximation for synchrotron angular emission to relate pulse widths to the energy of the radiating particles[3]. The standard expression is[4]

$$\gamma \cong \frac{1}{\Delta\theta} \tag{4}$$

($\Delta\theta$ represents one-half the total angle). This expression can be derived from equation (1) for large γ and values of m greater than γ^3. The present calculations are for mode numbers less than 1,000 and show both the width and the shape of the polar diagram to be frequency dependent. Note that when the transition from a double to a single peak occurs (for high mode numbers) the pulse width does approach that predicted by equation (4). For example, at 5 MeV, equation (4) predicts a pulse width of 34 ms for CP 1133. At mode 950 (see Fig. 2) the single peak is 40 ms wide at its half power point. The width of the single peak is found to be independent of mode number for modes greater than 800 at 5 MeV.

The curves in Fig. 4 can be easily transformed for comparison with other pulsars by the following expression:

$$\Delta t_2 = \tau_2 \, \Delta t_1 / 1{\cdot}187 \tag{5}$$

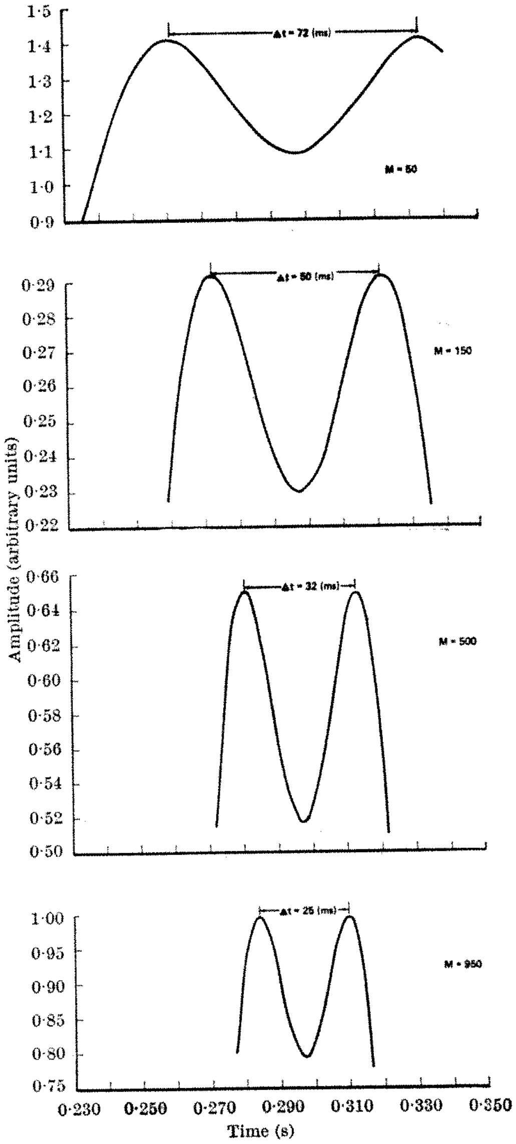


Fig. 3. ulse shapes as a function of mode number, with $\tau = 1{\cdot}187$ s, for particle energy of 100 MeV.

where Δt_1 = peak to peak time separation in Fig. 4, τ_2 = period of pulsar to be studied, and Δt_2 = peak to peak time separation of the new pulsar.

Representative plots of predicted polarization for CP 1133 are presented in Figs. 5 and 6. Fig. 5 is a plot of E_z, which could represent the circular component of a received signal. It is found that, for a given mode number, the separation between the peaks in polarization is greater than the peak to peak separation of the signal amplitude. The linear, E_x and E_y components are plotted in Fig. 6. Measurements of polarization for CP 1133 and NP 0527 have not been reported. Clark and Smith[3] have measured the polarization of signals from CP 0238 and have found effects similar to those shown in Figs. 5 and 6.

Comparison with Experiment

Average pulse shapes as a function of frequency have been reported by Craft and Comella[5] for CP 1133 and by Zeissig and Richards[6] for NP 0527. Ekers and Moffet[7] have reported both individual pulse and average data for CP 1133. The calculated frequency dependence of peak to peak time separation is plotted in Fig. 4. The data for CP 1133 and NP 0527 are found to have a constant slope; thus the data were compared with the calculations for electron energy $E > 100$ MeV.

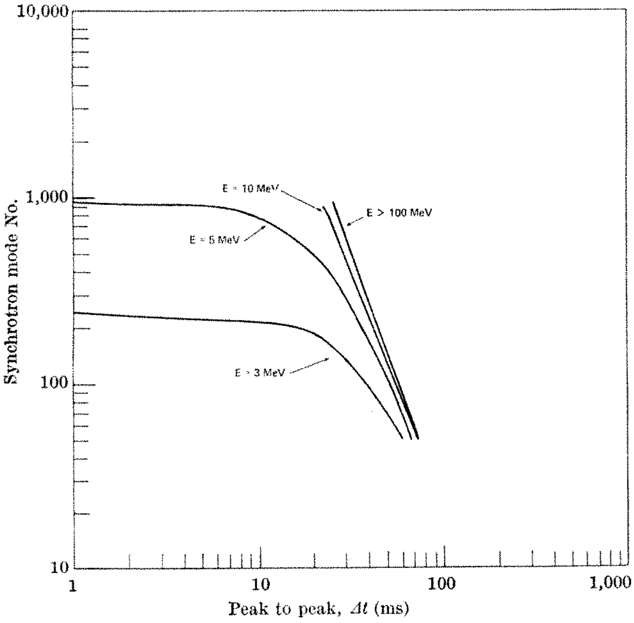


Fig. 4. Peak to peak pulse separation, Δt (for double peaked pulses), as a function of synchrotron mode number with charged particle energy as a parameter ($\tau = 1{\cdot}187$ s). The particles were assumed to be electrons. If protons are to be considered, protons with a value of γ that corresponds to the equivalent electron γ should be chosen.

The data were normalized in mode number by (*a*) choosing the cleanest average pulse with the best signal to noise ratio and (*b*) setting the observed frequency of that pulse equal to the mode number that corresponds theoretically to the measured pulse width.

Pulsar CP 1133 is compared with the calculations in Fig. 7. The data between 40·12 MHz and 430 MHz are average pulse data from Craft and Comella[5]. The point at 2,295 MHz is an individual pulse from Ekers and Moffet[7]. The computations were only performed up to mode 950. Fig. 4, however, seems to indicate that the slope of the $E > 100$ MeV line is constant until the double peak behaviour ceases (see the 5 MeV curve in Fig. 4). Thus the solid line in Fig. 7 has been extended by a dashed line to approximate the behaviour of the Δt dependence for energetic particles at mode number > 950. It is interesting to note that both individual pulse data and average pulse data agree well with the calculations over a factor of 50 in frequency. (Smith[8] has determined that individual pulses from pulsar CP 0328 do not show frequency dependent pulse widths over a frequency range from 151 to 1,420 MHz. The individual and average pulse data from CP 1133 indicate that this is not the case for all pulsars. The model proposed by Smith may be applicable to CP 0328 but does not seem to apply in the case of CP 1133.) The actual average pulse shape for the received signal at 111·5 MHz is compared with the predicted pulse shape for mode 700 in Fig. 8.

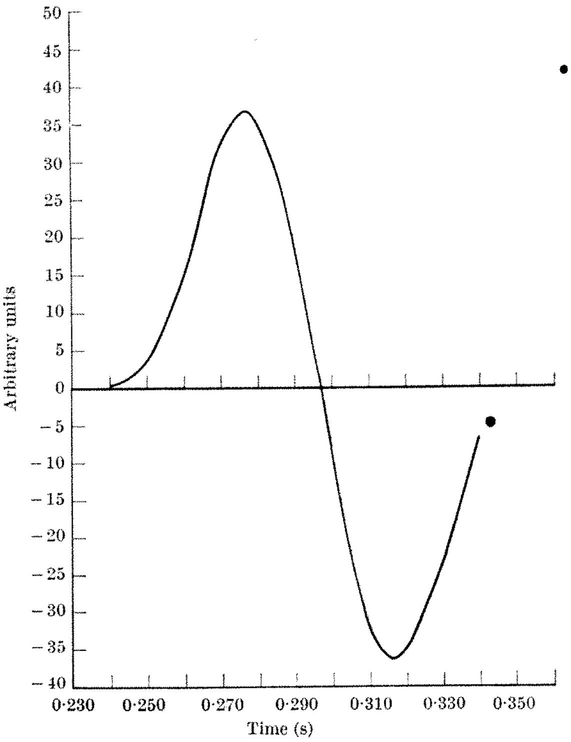


Fig. 5. The circular component (E_z in equation (2)) of the predicted polarization at 96 MHz for CP 1133.

Finally, the average pulse data from NP 0527 are compared with the calculations in Fig. 9. As indicated by theory, the slope indicated for frequency pulse widths of particles with $E > 100$ MeV seems to be a phenomenon present in more than one pulsar.

Charge Orbital Parameters

Further information concerning the properties of the radiating charges and their orbital characteristics can be

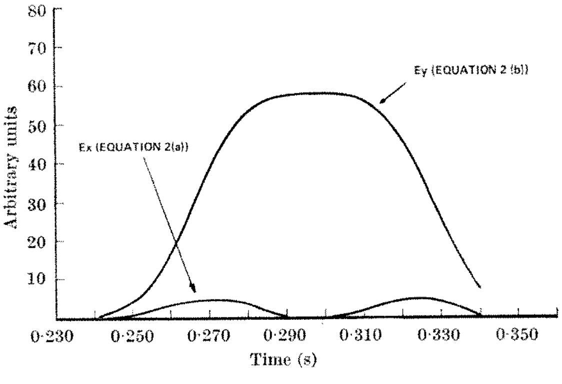


Fig. 6. The linear components (E_x and E_y in equation (2)) of the predicted polarization at 96 MHz for CP 1133.

deduced from the data. These properties are (*a*) the average particle energy, (*b*) the fundamental synchrotron frequency, (*c*) the average magnetic field in the radiating region, and (*d*) the Larmor radius of the particle orbits. The sign of the charges (electrons, protons or higher Z atoms) cannot be deduced from the analysis as the theoretical emission patterns are independent of the charge.

The comparison of pulse width versus frequency indicates that the average electron energy is greater than 100 MeV ($\gamma \simeq 200$). The corresponding proton energy would be greater than $1 \cdot 8 \times 10^{11}$ eV. If a pulsar can be found in which the transition from a double peak to a single peak can be measured, then the actual particle energy could be determined.

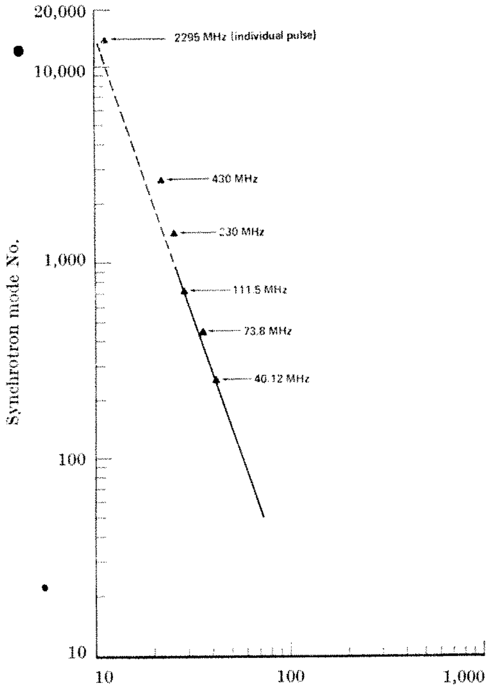


Fig. 7. Frequency dependent pulse width data for CP 1133 (▲) compared with the calculations. The normalizing mode number was found by matching the theoretical pulse width value with the measured value at 111·5 MHz. ——, Equation (1), $E > 100$ MeV.

The normalization procedure that related mode number to measured frequency also gives the fundamental Larmor frequency, f_0, of the radiating charges. This fundamental is equal to the measured frequency divided by its corresponding mode number. The equation for f_0 is

$$f_0 = \frac{eB}{2\pi\gamma m_0 c} \tag{6}$$

For large values of γ, the Larmor radius, r_{L}, is related to f_0 as follows

$$f_0 = \frac{c}{r_{\mathrm{L}}} \tag{7}$$

Table 1 shows the values of f_0, B and r_{L} obtained in this manner for pulsars CP 1133 and NP 0527. One pulse per rotation period was assumed in the calculations for Figs. 7 and 8 (that is, the measured period was used in equation (3)). As two pulses per period are also possible (case A of ref. 1), the parameters f_0, B and r_{L} were also determined for $\tau_2 = 2\tau$ and plotted in Table 1 for each pulsar.

A Refined Model

The large Larmor orbits deduced here are of the order of kilometres and are thus comparable with the size of neutron stars[9]. This suggests to me that the bunches of high energy ($\gamma > 200$) charged particles responsible for the r.f. pulse may actually be orbiting the magnetosphere. The r.f. emission might thus originate from a train of charge bunches with each bunch having dimensions of the order of the largest wavelength of the emitted radiation (Fig. 10). The observed average pulse shape (Fig. 8) would then represent the envelope of a succession of very sharp r.f. bursts.

The following should be considered: (1) The means of production of charges with values of $\gamma > 200$. (2) The means by which the charges could enter an orbit encircling the magnetosphere. (3) Possible mechanism for bunching of orbital relativistic charged particles.

Several mechanisms have been proposed for the production of ultrarelativistic charged particles at both the surface of a rotating neutron star with a magnetosphere (unpublished work of Goldreich) and in the near-field region of such a spinning object[10]. Only a small fraction of such particles would need to be trapped to account for the r.f. emission. (Gunn and Ostriker show that the r.f. pulse itself represents only 10^{-7} of the total magnetic dipole energy that can be released by a neutron star with an oblique magnetic field[10].)

The means by which such particles could orbit the star is not clear, but stable or quasi-stable orbits would certainly exist[11], and either direct particle collisions or instability mechanisms could deflect particles into such orbits.

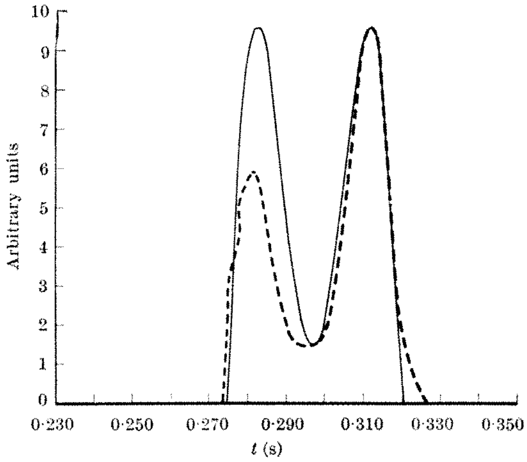


Fig. 8. Comparison of the measured average pulse shape at 111·5 MHz with synchrotron mode number 700. – – –, Data (CP 1133); ——, equation (1).

Once in orbit, relativistic charges (electron or proton) would be subject to the negative mass instability[12]. This instability is prevalent in circulating charged particle beams in accelerators and storage rings. According to ref. 12, "the instability can arise whenever the frequency of rotation of the particles is a decreasing function of energy—as, for example, with a relativistic electron beam in a uniform magnetic guide field. In such cases, an accelerating force on the particles results in a decrease in the angular velocity, so it is not hard to see that electrostatic space-charge forces arising from some small perturbation in the beam charge density would then result in motion towards a region of increased density, thus leading to a progressive enhancement in the beam bunching". This instability has been observed in the Astron device at the Lawrence Radiation Laboratory[13]. A ring of 3·8 MeV electrons at current levels of the order of 100 A is observed to bunch azimuthally. Theoretically predicted growth rates were found to agree well with observation.

The negative mass instability is an example of a natural means by which a sufficient number of electrons can be aligned in phase and in space to produce coherent synchrotron radiation. Various factors can influence the size of the bunch and the length of time over which a given bunch or bunches can maintain alignment. Two mechanisms which destroy the alignment come to mind. First, the bunch can lose energy via the synchrotron emission and thus be more susceptible to space charge spreadings. Second, the synchrotron emission could result in an energy spread of particles in the bunch, and violate conditions necessary for the instability.

The growth of bunches and their decay can lead to a wide variety of pulse shapes, depending on the time scales involved. For example, if the decay rate is slow compared with the pulse width, one could expect to see a complete double pulse for some pulses as is observed in CP 1133 (ref. 7).

If there are no bunches sufficiently aligned to produce coherence, then pulses would not be observed. If the decay rate is shorter than a pulse width, then only a portion of a double pulse would be observed. All such behaviour is denoted in Fig. 3 of ref. 7.

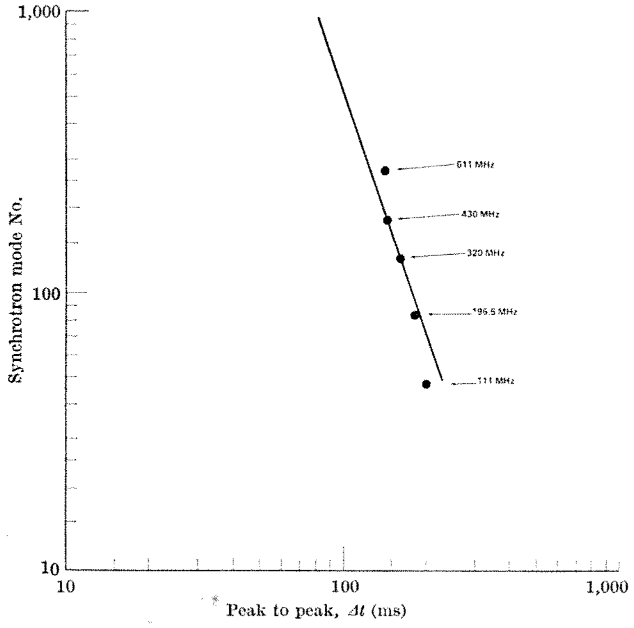


Fig. 9. Frequency dependent pulse width data for NP 0527 (●) compared with the calculations[6]. The normalizing frequency was chosen at 320 MHz. ——, Equation (1), $E > 100$ MeV.

Thus, individual pulses can have an erratic behaviour, sometimes appearing as single peaks or double peaks, while the average pulses have a shape more characteristic of the calculated synchrotron pattern. The refined model suggested here can lead to an explanation of some of the other rather erratic pulse to pulse behaviour that has been observed. Fine structure (sharp microsecond pulses within the main envelope)[14] could be generated by emission from many individual bunches during the time in which the synchrotron emission pattern is aligned with the observer. For example, at a value of f_0 of 162 kHz one bunch would orbit the magnetosphere 8,100 times during one 50 ms r.f. burst (CP 1133). One might expect to observe periodic kilocycle effects in the fine structure that would result from the growth and decay of individual bunches.

The growth and decay times of individual bunches of charged particles could be longer than the time between pulses. If so, then the rapid orbiting behaviour described could account for the absence of pronounced even–odd pulse effects for obliqueness angles in which two pulses are emitted per rotation of the source star. This can happen because the same coherently emitting bunches would be orbiting the star during both pulses. As long as the bunch size remains the same, the amplitude of the r.f. bursts should be approximately equal. (To avoid confusion, it should be noted that while the phenomenon resulting in the bunching is referred to as an "instability" its effect is to align charged particles in bunches of finite dimensions for finite time periods, that is, times much greater than the cyclotron period.)

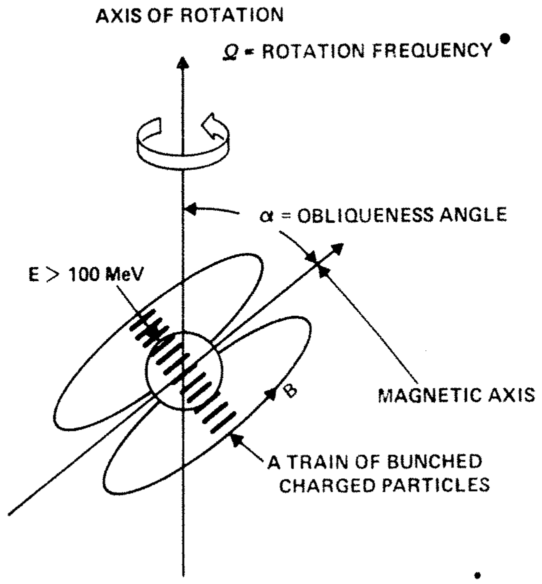

AXIS OF ROTATION
Ω = ROTATION FREQUENCY
α = OBLIQUENESS ANGLE
E > 100 MeV
MAGNETIC AXIS
B
A TRAIN OF BUNCHED CHARGED PARTICLES

Fig. 10. A refined pulsar model. One or more (growing or decaying) charged particle bunches are hypothesized to be in orbits encircling the neutron star's magnetosphere.

If the model is realistic, and if a negative mass instability is present, then the mass of the charged particle could be determined from the growth rates of the instability. This could allow direct detection of cosmic rays by study of their emission patterns. At energies as great as those deduced from the data, optical, X-ray and possibly even gamma ray emission are possible. (The r.f. power spectrum would still be similar to that described in Fig. 2 of ref. 1, however.)

Extension of the calculations to (*a*) higher mode numbers, (*b*) finite $\beta_{\parallel}$ and (*c*) arbitrary obliqueness angles[15] is under way.

Received September 12; revised December 13, 1969.

[1] Eastlund, B. J., *Nature*, **220**, 1293 (1968).
[2] Maran, S. P., and Cameron, A. G. W., *Physics Today* (August 1968).
[3] Clark, R. R., and Smith, F. G., *Nature*, **221**, 724 (1969).
[4] Bekefi, G., *Radiation Processes in Plasma* (John Wiley and Sons, New York, 1966).
[5] Craft, H. D., and Comella, J. M., *Nature*, **220**, 676 (1968).
[6] Zeissig, G. A., and Richards, D. W., *Nature*, **222**, 151 (1969).
[7] Ekers, R. D., and Moffet, A. T., *Nature*, **220**, 756 (1968).
[8] Smith, F. G., *Nature*, **223**, 934 (1969).
[9] Wheeler, J. A., *Annual Review of Astronomy and Astrophysics*, vol. IV (Annual Reviews, Inc., of Palo Alto, California, 1966).
[10] Gunn, J. E., and Ostriker, J. P., *Nature*, **221**, 455 (1969).
[11] Bano's, A., Uribe, H., and Lifshitz, J., *Rev. Mod. Phys.*, **11**, 137 (1939).
[12] Briggs, R. J., and Niel, V. K., *Plasma Phys.*, **9**, 209 (1967).
[13] Christofilos, N. C., *Prog. Nuclear Energy, Series XI*, **1** (Pergamon Press, London, 1958).
[14] Ekers, R. D., and Moffet, A. T., *Nature*, **220**, 756 (1969).
[15] Papagiannis, M. D., *Nature*, **222**, 1261 (1969).

LETTERS TO THE EDITOR

PHYSICAL SCIENCES

High Resolution Observations of Solar Microwave Bursts

At Toyokawa Observatory we have been mapping the brightness and polarization distribution across the Sun at intervals of 10 s by a quick scanning system installed at the 8 cm radio interferometer since the end of August 1968 (ref. 1). The base line of the 32 element adding interferometer is 213·28 m or 2,666 wavelengths, which corresponds to a half-power beam width of 1·1 min of arc. This letter describes two examples of bursts with double structure which have been observed during the past eight months.

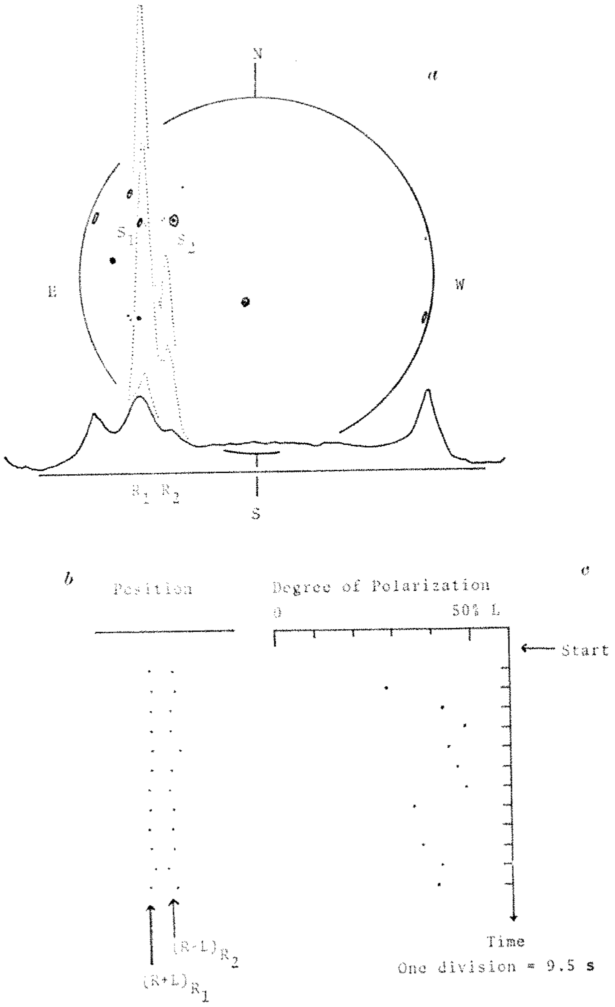


Fig. 1. *a*, One-dimensional brightness distribution at 3·75 GHz and the sunspot sketch (Mitaka) on January 18, 1969. R_1 and R_2 represent the burst sources and S_1 and S_2 are the corresponding sunspots respectively. *b*, The time variation of the position of sources R_1 on the $(R+L)$ channel and R_2 on the $(R-L)$ channel. *c*, The time variation of the degree of polarization of R_2.

Table 1. RADIO AND OPTICAL DATA OF THE BURST AND ASSOCIATED FLARE ON JANUARY 18, 1969

Radio burst

Date (Jan.)	Freq. (MHz)	Starting time (UT)	Time of max. (UT)	Duration (min)	Type	Max. flux density (10^{-22} W m^{-2} Hz^{-1}) (peak)
18	9400	0201	0201·7	3	S	190
	3750	0201	0201·7	4	S	108
	2000	0201	0201·8	6	S	178
	1000	0201	0201·9	8	S	135

Solar flare

Date (Jan.)	Observatory	Starting time (UT)	Time of max. (UT)	Duration (min)	Location (Lat.)	Location (Mer. dist.)	Importance
18	Mani*	0119	0123	25	N14	E46	1B
	Hale†	0120	0124	55	N17	E41	1N
	Hale		0202				
	Mani	0152	0159D‡	7D	N16	E43	1N

* Manila Observatory.

† Haleakala Observatory.

‡ Greater than.

On January 18, 1969, at 0200.5 UT, an impulsive burst of the simple type was observed. Fig. 1*a* shows drift curves of the Sun before the burst (solid line) and at different stages of the burst (dotted lines) together with a sunspot sketch from Tokyo Astronomical Observatory (Mitaka). There were two radio sources, R_1 and R_2, with R_2 appearing about 10 s after R_1.

The results of single frequency observations and H_α solar flare patrols are summarized in Table 1. The position of R_1 may certainly be identified with that of the flare, but there are no detailed flare records for R_2, and all we can say is that it may be located at or near the sunspot S_2.

R_1 was unpolarized, but R_2 showed 40–50 per cent left-handed circular polarization (Fig. 1*c*). The positions of the sources R_1 and R_2 were stationary during the burst on the channel of the sum of left-handed (L) and right-handed (R) circular components. On the $(R-L)$ component channel, on the other hand, the source R_2 showed a definite movement of roughly 1 min of arc in 10 s near the maximum stage of activity (Fig. 1*b*). In our polarization measurements we do not observe all the Stokes parameters, so we cannot discriminate polarized components from randomly polarized components. It can be shown that the apparent motion of the source of the polarized component may have to be multiplied by the factor $2P/(1+P)$, where P denotes the degree of polarization. In this case the factor is 0·6. Then the motion of the radio source R_2 is estimated to have been 0·6 min of arc in 10 s, that is, $\sim 2{,}500$ km s^{-1}.

What is most striking about the January 18 event is the parallelism of the activities in both radio sources from just before the maximum and later on (Fig. 2). If our identification of the radio sources is correct, the distance between both sources is $\sim 1{\cdot}6\times10^5$ km. We conclude that the time delay of radioactivities in the two sources spaced by $\sim 1{\cdot}6\times10^5$ km was less than a few seconds.

From drift-scan observations at 9·4 GHz and of single frequency observations at 9·4, 3·75, 2 and 1 GHz it is possible to separate the activities in each source by the time delay of their start. The estimated polarization character of each source during the burst activity is shown in Table 2. Each source had an opposite sense of polarization and the sense of circular polarization reversed with frequency around 3·75 GHz for the source R_1 and between 2 and 1 GHz for the source R_2.

On December 24, 1968, weak and complex radioactivity started at 0154 UT and an impulsive burst of simple type

Table 2. POLARIZATION CHARACTER OF THE JANUARY 18 BURST

Frequency (MHz)	Radio sources R_1	Radio sources R_2
9400	R	~0
3750	~0	L
2000	L	L
1000	L	R
	● or ~0	R

occurred in succession. A composite picture of drift curves and a sunspot sketch similar to Fig. 1*a* is shown in Fig. 3*a*, where dotted lines and a broken line indicate brightness distributions of the sources at different phases of the burst activity. The associated flare was observed at Mitaka and is illustrated in Fig. 3*a* as F.

This event is quite similar to the January 18 event in that there were two radio sources R_1 and R_2 corresponding to a burst; that the source R_1 preceded the source R_2 by one drift curve, namely, by 10 s; and that the source R_1 was unpolarized whereas the source R_2 was left-handed circularly polarized.

In details, however, there were some differences. Neither source moved in the (R − L) channel or the (R + L) channel, and the radioactivity in both sources was not paralleled as in the sources of January 18, 1969. The enhancement rise time was definitely different in both sources (Fig. 4). The polarization of each source is shown in Table 3. At 2 and 1 GHz we cannot separate the components corresponding to each source by the polarimeter observations, as we have done in the case of the January 18 event. As Table 3 shows, the reversal with frequency is not clear in this case.

Table 3. POLARIZATION CHARACTER OF THE DECEMBER 24 BURST

Frequency (MHz)	Radio sources R_1	R_2
9400	L	~0
3750	~0	L

The most interesting point with respect to the burst of December 24, 1968, is that, although there was a flare of importance 2F associated with the radio source R_1, none was observed for the burst source R_2. But we may suspect that the radio burst took place somewhere in the neighbourhood of the sunspot S_2 without any flare activity or even a subflare.

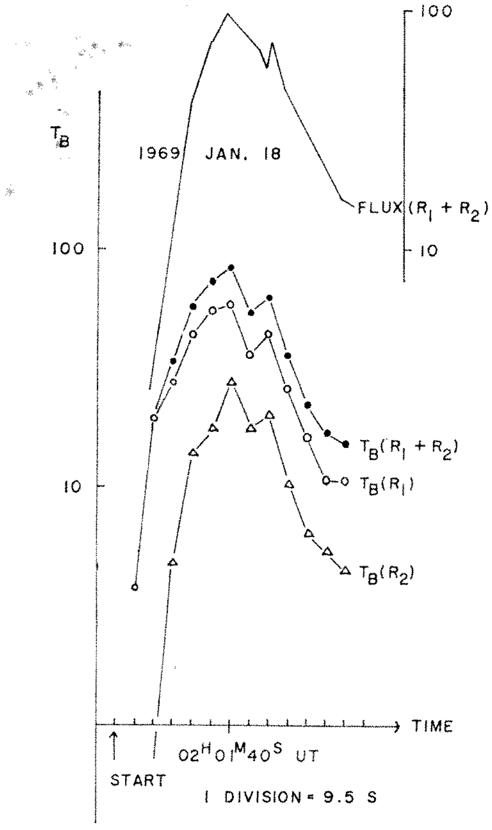


Fig. 2. From upper to lower; time variations of the flux density in units of 10^{-22} W m^{-2} Hz^{-1} with the scale on the right hand side, the sum of the brightnesses of both sources, the brightness of R_1, and the brightness of R_2 in arbitrary units.

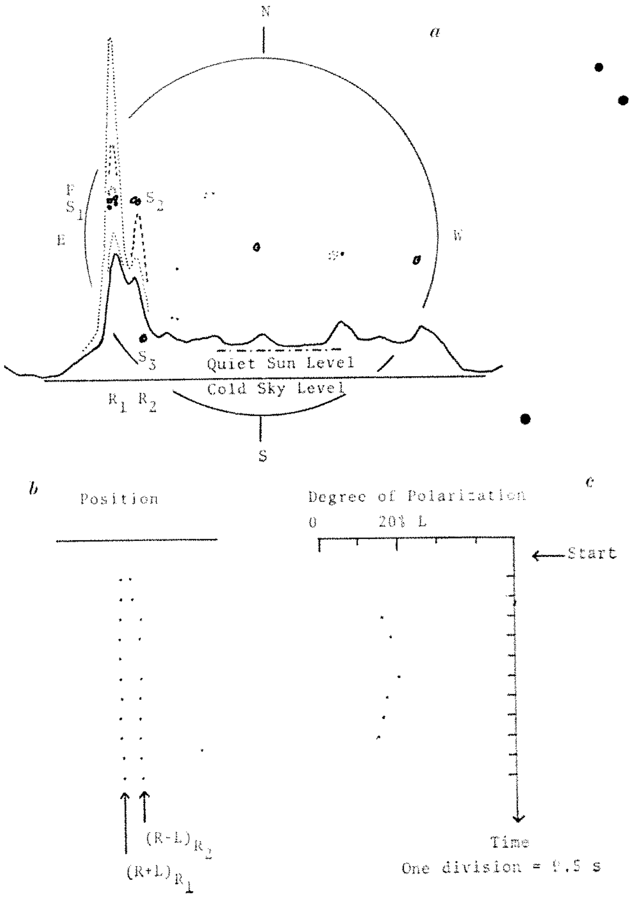


Fig. 3. *a*, One-dimensional brightness distribution of the Sun at 3·75 GHz and the sunspot sketch (Mitaka) on December 24, 1968. R_1 and R_2 represent the burst sources and S_1 and S_2 are the corresponding sunspots respectively. S_3 has nothing to do with the present activity. F is the flare associated with R_1. *b*, The time variation of the position of the sources R_1 on the (R + L) channel and R_2 on the (R − L) channel. *c*, The time variation of the degree of polarization of R_2.

The time delay of the start of radio enhancement observed clearly for two separated sources in both cases suggests a disturbance which triggered the microwave burst propagated from one source to another at $\sim 1{\cdot}5 \times 10^4$ km s^{-1}. But there is no known disturbance with such a velocity. Here we consider as possible mechanisms hydromagnetic shock waves and high energy particles.

If we take an electron density of 10^8 cm^{-3} and a magnetic field strength of 70 oersted for the active region, the Alfvén wave velocity may be $1{\cdot}5 \times 10^4$ km s^{-1}. Thus as in the case of Moreton wave phenomena it may be possible to consider triggering due to hydromagnetic waves[2,3].

In the case of high energy particles, it may be that high energy electrons radiating microwave emission, probably by magnetobremsstrahlung, in the first source, transferred to the second source and caused secondary emission. In this case, however, the emission mechanism suggested by Takakura and Kai[4], by which they tried to explain the spectrum and the duration of microwave bursts as due to the magnetobremsstrahlung by electrons with an energy of hundreds of keV, has to be extended to account for the rather slow propagation of electrons along the magnetic field lines between the radioactive centres.

We are not in a position to conclude which model is correct, but the difference of rise time for the burst of December 24 may not be accounted for easily by the particle model. On the other hand, the fact that there was only radioactivity in the region of R_2 for the event of December 24, and that there was a parallelism between both sources for the event of January 18, may be in

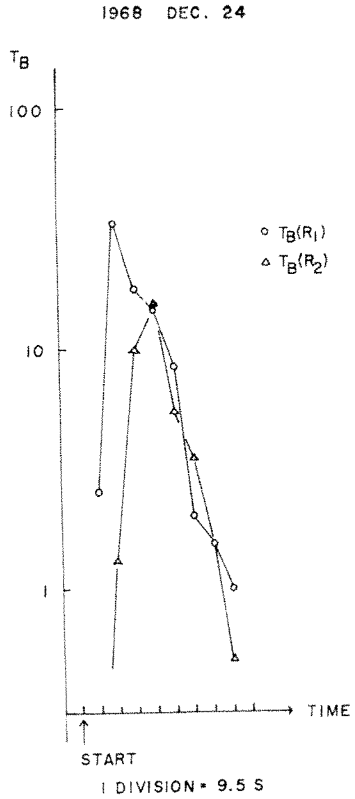


Fig. 4. Time variations of the brightness of the sources R_1 and R_2 in arbitrary units.

favour of the particle model, because they suggest radiation from common high energy electrons. We suggest as a working model that hydromagnetic shock waves accelerate electrons which emit the microwave radiation and heat the magnetic flux tubes between both sources, and that these hot flux tubes may become good channels for the propagation of high energy electrons.

The sense reversal of polarization with frequency was originally found by Kakinuma[5,6], and since then various theories have been proposed[5,7-11]. We have pointed out another possibility in which a bipolar source with opposite senses of polarity may give rise to a sense reversal if the sources have a different radio spectrum[12]. It is clear from Table 2, however, that both the propagation condition and the spectrum of each source took part in the observed polarization character of the January 18 burst. Thus the problem of the polarization characteristics of solar radio bursts due to the propagation condition is still unsolved.

There are several observations of motion of the sources of solar radio bursts[13,14] in which a polarization centre moved at the beginning of the second phase of an intense burst. We have inferred that this movement is caused by a motion of the magnetic field. But for movement as rapid as that observed during the impulsive burst on January 18, we have to consider the possibility of motion of the radiating centre as a whole.

We thank the Division of Solar Physics of Tokyo Astronomical Observatory for the optical data, and the staff of our laboratory for their help.

HARUO TANAKA
SHINZO ÉNOMÉ

Research Institute of Atmospherics,
Nagoya University,
Toyokawa,
Aichi, Japan.

Received June 6; revised December 29, 1969.

[1] Tanaka, H., Kakinuma, T., Énomé, S., Torii, C., Tsukiji, Y., and Kobayashi, S., *Proc. Res. Inst. Atmospherics Nagoya Univ.*, **16**, 113 (1969).
[2] Meyer, F., *IAU Symp. No. 35, Structure and Development of Solar Active Regions* (edit. by Kiepenheuer, K. O.), 485 (D. Reidel Publ. Co., 1968).
[3] Uchida, Y., *Solar Phys.*, **4**, 30 (1968).
[4] Takakura, T., and Kai, K., *Publ. Astr. Soc. Japan*, **18**, 57 (1966).
[5] Kakinuma, T., *Proc. Res. Inst. Atmospherics Nagoya Univ.*, **5**, 71 (1958).
[6] Tanaka, H., and Kakinuma, T., *Paris Symp. Radio Astronomy* (edit. by Bracewell, R. N.), 215 (Stanford Univ. Press, 1959).
[7] Takakura, T., *Publ. Astron. Soc. Japan*, **12**, 325 (1960).
[8] Takakura, T., *Publ. Astron. Soc. Japan*, **12**, 352 (1960).
[9] Takakura, T., *Publ. Astron. Soc. Japan*, **16**, 230 (1964).
[10] Cohen, M. H., *Astrophys. J.*, **131**, 664 (1960).
[11] Cohen, M. H., *Astrophys. J.*, **133**, 978 (1961).
[12] Tanaka, H., Kakinuma, T., and Énomé, S., *Proc. Res. Inst. Atmospherics Nagoya Univ.*, **14**, 23 (1967).
[13] Tanaka, H., and Kakinuma, T., *Intern. Conf. Cosmic Rays and the Earth Storm, J. Phys. Soc. Japan*, **17**, Suppl. A-II, 211 (1962).
[14] Énomé, S., Kakinuma, T., and Tanaka, H., *Solar Phys.*, **6**, 428 (1969).

Pulsar Periods and Rapid Changes in the Terrestrial Rotation Rate: a Reply

Maran and Ögelman[1] have called attention to the influence on observed pulsar periods of a changing rotation period of the Earth. We think that their estimates are not entirely correct. They have not properly distinguished between the time rate of period change $\Delta P/P = \dot{P}$, and the uncertainty of a period determination $\delta P/P$. They give an estimate of the absolute uncertainty in the determination of the dimensionless quantity $\dot{P}$ and interpret this number as relative uncertainty for the period determination. (Note that $\Delta P/P$ is not uniquely defined. In ref. 2 it is a dimensionless quantity and equals $\dot{P}$, that is, the time derivate of the period, or the relative change of period per period. In ref. 3 it has, apparently, the dimension of a time and equals $P\dot{P}$, or the absolute change of period per period.)

It is necessary to distinguish between an absolute period change and a rate of period change. The basic observed quantity for any period determination is the pulse arrival time. If the length of a day changes by a fixed amount, then the apparent period during rising or setting time of the pulsar changes, due to a changed Doppler effect, by a fixed amount. A fixed period change has a linearly accumulating effect on the arrival times, and can therefore be measured with a precision that increases linearly with time. On the other hand, a continuous period change by a fixed rate which seems to be an intrinsic property of some pulsars has a linearly accumulating effect on the period, and can therefore be measured with a precision that increases with the square of time.

If one observes over a period which totals $m+1$ pulses the period is given by $P = T/m = (t(m+1) - t(1))/m$, where t is the arrival time. The uncertainty is $2\tau/m$, where τ is the accuracy in the timing of the first and the last pulse. τ contains a systematic part, τ_{sy}, due to clock errors, long-term changes in the instrument, and so on, as well as a statistical contribution τ_{st} which is roughly proportional to $n^{-1/2}$, where n is the number of pulses observed. The relative accuracy for the period determination is therefore

$$A = \delta P/P = 2\tau/T \qquad (1)$$

In order to find a period change, one has to subdivide the sample into at least two groups. A period change within the time $\sim T/2$ can be determined with roughly the accuracy (relative to the period)

$$B = \delta(\Delta P)_{T/2}/P = 2 \times 2\tau/(T/2) \qquad (2)$$

as long as the observations cover about uniformly the whole interval T. The period change per period is related to the period change in the time T by $(\Delta P)_P = (\Delta P)_T P/T$.

The rate of period change is therefore given with an absolute accuracy of

$$C = \delta\dot{P} = \delta(\Delta P)_P/P = (\delta(\Delta P)_{T/2}/P) \times P/(T/2) \qquad (3)$$

We apply these formulae to the example given in ref. 1. They assume daily observing periods of $T = 10^4$ s $= 2{\cdot}8$ h each, with $P = 0{\cdot}1$ s, $\tau = 10^{-4}$ s. For the sake of simplicity, let us assume that τ is independent of n, the total number of pulses observed. For one day's observations, we find that A (2·8 h) $= 2 \times 10^{-8}$, B ($2 \times 1{\cdot}4$ h) $= 8 \times 10^{-8}$ and C ($2 \times 1{\cdot}4$ h) $= 1{\cdot}6 \times 10^{-12}$. For two days' observations, separated by one month, we find that A (30 days) $=$ 8×10^{-11}, B ($2 \times 2{\cdot}8$ h; 30 days) $= 2$ A (2·8 h) $= 4 \times 10^{-8}$ and C ($2 \times 2{\cdot}8$ h; 30 days) $= 1{\cdot}6 \times 10^{-15}$. For thirty consecutive days' observations, we find that A (30 days) $=$ 8×10^{-11} as in the previous case, while B (2×15 days) $=$ 3×10^{-10} and C (2×15 days) $= 2{\cdot}5 \times 10^{-17}$.

In order to compare our estimates with actual measurements, we mention the Crab pulsar[4] and the Vela pulsar[2]. Within several weeks, their periods P were determined with a relative accuracy of 10^{-10} and 10^{-11} while their rates of period change $\dot{P}$ were determined with an absolute accuracy of 8×10^{-18} and 2×10^{-19}.

In ref. 1, the numbers A (2·8 h) and C ($2 \times 2{\cdot}8$ h; 30 days) were given. The latter number was compared with the apparent pulsar–period change of about 8×10^{-15} P due to a change in Doppler effect from a change of $\sim 0{\cdot}5$ ms in the length of a day. One should rather use B (2×15 days) for the comparison. On the other hand, if this change of period occurs within 15 days, the mean rate of period change $\dot{P}$ is about 6×10^{-22}. This number should be compared with C (2×15 days).

The easiest way to see that the effect simply cannot be detected is the following: when the day has been longer by 0·5 ms for 15 days, then the accumulated effect on the pulse arrival time at the rising time of the pulsar is only about 10 ns while the assumed timing precision for a combination of ~ 4 pulses is about 400 μs. The three methods of analysing the problem in terms of either arrival times, or periods, or rates of period change are equivalent, however. The ratios of accuracy to expected effect are the same in each case, that is

$$\frac{400\ \mu\text{s}}{10\ \text{ns}} = \frac{B\,(2 \times 15\ \text{days})}{8 \times 10^{-15}} = \frac{C\,(2 \times 15\ \text{days})}{6 \times 10^{-22}}$$

We conclude that even with better timed and more extended pulsar measurements, there is little hope to detect changes in the length of a day smaller than about 100 ms.

I thank R. Manchester and J. Sutton for discussions. This work was supported by the European Space Research Organization under a NASA International University Fellowship in Space Science. The National Radio Astronomy Observatory is operated by Associated Universities, Inc., under contract with the US National Science Foundation.

JÖRG PFLEIDERER*

National Radio Astronomy Observatory,
Green Bank, West Virginia.

Received November 24, 1969.

* On leave from Bonn University.

[1] Maran, S. P., and Ögelman, H., *Nature*, **224**, 349 (1969).
[2] Reichley, P. E., and Downs, G. S., *Nature*, **222**, 229 (1969).
[3] Cole, T. W., *Nature*, **223**, 487 (1969).
[4] Boynton, P. E., Groth, E. J., III, Partridge, R. B., and Wilkinson, D. T., *Astrophys. J. Lett.*, **157**, L197 (1969).

On the Connexion between Pulsars and Supernovae

I REPORT here some preliminary results of a statistical study of the spatial distribution of pulsars in the galaxy which have direct relevance to the pulsar–supernova hypothesis and to certain features in the observed distribution of pulsar galactic latitudes. I present evidence that most pulsars may have been formed in Type II supernova explosions and initially possessed extremely high velocities, of order 1,000 km s^{-1}. On this basis, four new pulsar–supernova identifications are suggested.

Some time ago Large *et al.*[1,2] reported that the pulsars tended to cluster a few degrees south of the galactic plane, while Davidson and Terzian[3] noted a complete absence of pulsars between $b^{\rm II} = +6°$ and $+25°$. Since that time, the former effect has persisted, suggesting that it is real, but a couple of new pulsars (MP 1706, JP 2021) have been found in the underpopulated band, suggesting that the latter effect was due to observational selection.

A natural explanation of the apparent clustering of pulsars south of the galactic plane arises if we are willing to assume that most pulsars found to date are nearby (within 1 kpc) and possess a plane of maximum density that is coincident with the plane of maximum density of local young optical Population I objects. The pulsar distance estimates of Prentice and ter Haar[4,5] and others certainly support the assumption of nearness, while $\dot{P}/P$ measurements[6–8] yield typical pulsar lifetimes of 10^7 yr, which is young. Now Blaauw *et al.*[9] conclude that the Sun lies at a height $z_0 = 22 \pm 2$ pc above the mean plane defined by the local distribution of OB stars and cepheids. Letting z' be the height in pc above this plane our assumption is that the pulsar density $\rho(z')$ is maximum at $z' = 0$. A reasonable fit to the data is then

$$\rho(z') = \rho_0 \exp(-|z'|/110) \qquad (1)$$

We found for the mean height 110 ± 20 pc, which agrees with Mills's[10] figure of 110 pc. A simple calculation now yields that $\rho(b^{\rm II})$, the distribution with galactic latitude, $b^{\rm II}$, of all pulsars counted within a sphere of radius R centred on the Sun, has a sharp maximum at $b^{\rm II}_{\rm max}$ given by

$$\sin(b^{\rm II}_{\rm max}) = -2z_0/R$$

Substituting $z_0 = 22$ pc and $R = 1{,}000$ pc we find $b^{\rm II}_{\rm max} = -2{\cdot}5°$ which is in good agreement with the value $-2{\cdot}5 \pm 1°$ deduced from the observational data.

We now turn to the more important question regarding the pulsar–supernova hypothesis, that is that pulsars are created in supernova explosions. The reason why it is important to investigate this hypothesis is that Gold[11] suggested that pulsars would be rotating neutron stars, for which there are also strong indications based on the magnitude of the pulsar period. On the other hand, Cameron's recent considerations[12] have shown that the previously generally accepted connexion between neutron stars and supernovae explosions[13] meets with grave theoretical difficulties[14,15]. It should be mentioned that so far two, but no more, pulsars have been successfully associated with supernovae.

Using the pulsar distances of Prentice and ter Haar[5], the galactic spatial distribution of forty-two pulsars has been compared with observational data on local distributions of hot OB stars, O to B5 open galactic clusters and associations, O to B5 supergiants and "runaway" stars, and supernova remnants. The following facts emerge.

(i) Setting aside known selection effects due to instrumental limitation (for example, field-of-view and dispersion measure restriction), the distribution in galactic longitude and distance in the plane is essentially random and isotropic. In particular, there is no suggestion of aggregation along the "optical" spiral arms of the galaxy[16–18]; this differs from the conclusions reached by Davidson and Terzian[3] and by Mills[10].

(ii) Within 1 kpc of the Sun there is no significant correlation between the positions of pulsars and those of the hot O stars, O to B5 supergiants and O to B5 "runaway" stars.

(iii) Of the sixteen known supernova remnants within about 2 kpc that are catalogued by Poveda and Woltjer[19], and forty-two pulsars under investigation, there were nine instances (see Table 1) where a pulsar lay within 6° of a remnant site, while the distance estimates for pulsars and supernova remnants were compatible. The chance that this event should arise on a null pulsar–supernova association hypothesis was estimated to be less than 0·005. For the remaining supernova remnants and pulsars there was very little positional coincidence.

Table 1. PULSAR–SUPERNOVA ASSOCIATIONS

Supernova remnant	Pulsar	θ/d	r	t_p (10^4 yr)	t_s (10^4 yr)	Association indicated
Crab Nebula	NP 0532	0 ?	1·3	0 ?	(0·1)	Yes
Crab Nebula	NP 0527	16	1·2	6	(0·1)	No
Sh 147	NP 0527	1·7	1·3	24	25	Yes
Vela X	PSR 0833–45	0·26	0·8	1	1	Yes
Vela X	MP 0835	2·4	0·8	10	1	No ?
Vela Y	MP 0835	2·0	0·3 ?	18	7	Yes ? ?
P 1439–62	MP 1449	3·5	> 0·4	25	4	Yes ?
W 28	PSR 1749	11	0·8	25	0·3	No
HB 21	JP 2111	2·8	1·3	23	6	Yes ?

What conclusions can be drawn from this ? We note first that the mean pulsar height $<|z|> = 110$ pc (see equation (1)) is significantly higher than the value 57 pc appropriate to galactic clusters[17], the value 76 pc to all local O to B5 stars[20], and the value 64 pc found for supernova remnants, thought to be mostly Type II, having $|z| < 300$ pc (ref. 19). It is, however, comparable with that of the O to B5 "runaway" stars ($<|z|> \sim 100$ pc, ref. 22), which have been studied in great detail by Blaauw[21]. Because the mean height of pulsars with $|z| < 300$ pc is still greater than 100 pc, we cannot attribute the high $<|z|>$ to the Type I supernovae which Poveda and Woltjer found deficient in our galaxy. The OB "runaway" stars are known to have acquired a high z in their short lifetime ($\sim 10^7$ yr) by virtue of their very high velocities, typically 100 km s^{-1}. Thus because the pulsars are probably also young, we may infer that they too, perhaps, possess very high velocities. In view of Blaauw's suggestion that runaway stars originate in the supernova explosion of a binary companion, we consider it not unreasonable to assume that the kinematic properties of pulsars and "runaway" OB stars bear a resemblance, and we shall assume that the pulsars move with these high velocities unimpeded by the deceleration processes in the interstellar medium which slow down the supernova remnants. A suggestion which seems attractive is that the pulsar leaves an asymmetric Type II supernova explosion with a velocity of the order of 1,000 km s^{-1}.

As Poveda and Woltjer have shown that the supernova remnants clearly delineate the local "optical" spiral arms, (i) also implies that, if the pulsar–supernova hypothesis is correct, the pulsars possess high speeds. As the distance between arms is approximately 2 kpc, the pulsars need a minimum average speed of 100 km s^{-1} in order to lose all identity of origin within a lifetime of 10^7 yr.

The final piece of evidence (iii) allows us to accept the hypothesis of the pulsar–supernova association at a very high level of significance. It is, at the same time, consistent with the hypothesis of intrinsically high pulsar velocities. These two hypotheses, in fact, go hand in hand, because a minimum speed of 200 km s^{-1} is necessary to explain the complete absence of pulsars inside the older supernova remnant shells—the oldest known remnants have ages of about 2×10^5 yr and radii of about 40 pc (ref. 19).

The test case of our arguments is the young pulsar PSR 0833–45 discovered by Large *et al.*[23] inside the Type II supernova remnant Vela X. Taking the distance estimate[24] of 500 pc, and age estimate[25] 1×10^4 yr, the observed angular separation between PSR 0833–45 and the centre of Vela X implies a minimum velocity of separation of 500 km s^{-1}. This means that the pulsar should leave Vela X after not greater a period than 3×10^4 yr, which is a time well inside the observational lifetime of the remnant.

Accordingly, there is very good reason to hope that some of the nine instances mentioned under (iii) are real pulsar–supernova associations. To proceed a little further the ages of the remnants and independently those of the suspected pulsars were computed using the PSR 0833–45—Vela X identification as a yardstick. The results are shown in Table 1. In the table, θ/d is the ratio of pulsar–supernova angular separation to supernova angular diameter and r the ratio of the estimated[5] pulsar to catalogued[19] supernova distance. The pulsar age t_p was computed by extrapolating θ back to 0 with a transverse pulsar velocity of 500 km s^{-1} and is subject to a large uncertainty, principally because we have no knowledge of the velocity along the line of sight. The supernova age was computed from the formula $t_s \propto R_s^{5/2}$ (ref. 22, p. 170) normalized against Vela X, R_s being the remnant radius. The only physical justification we have for making a uniform calibration is that, excepting the Crab Nebula, all of the supernova remnants listed are probably of the same type, that is, Type II (ref. 22, pp. 141, 160).

I want to make a few comments on the results of Table 1.

(i) The Crab supernova, apart from being Type I, is too young for a time t_s to be defined. Also, it is so young that if NP 0532 had been lying outside the remnant, we would have had difficulty in associating it with the Crab supernova.

(ii) It seems to us that NP 0527 should not be associated with the Crab supernova, but rather with Sh 147. It is interesting that the pulsar with the longest period would then be associated with the oldest supernova remnant, although the age of Sh 147 is probably only about 250,000 yr, which is much shorter than the suggested lifetimes of about 10^7 yr for old pulsars.

(iii) If one accepts Milne's[24] suggestion that Vela Y and Vela X are both parts of the same supernova remnant at a distance of about 500 pc, the value of r for MP 0835 becomes 0·8 and t_p to Vela Y, 7×10^4 yr.

(iv) The distance of MP 1449 is only a lower limit. It is interesting to note that MP 1449 is the pulsar with the third shortest known period. From Goldreich's theory[26] one obtains a lifetime of about 40,000 yr which agrees well with $t_s \sim 4 \times 10^4$ yr.

I thank Dr D. ter Haar for reading the manuscript and for helpful suggestions, and other members of the Department of Theoretical Physics for discussions. I also thank both the Royal Commission for the Exhibition of 1851 and Wolfson College, Oxford, for generous support.

A. J. R. PRENTICE

Department of Theoretical Physics,
University of Oxford,
12 Parks Road,
Oxford OX1 3PQ.

Received December 16, 1969.

[1] Large, M. I., Vaughan, A. E., and Wielebinski, R., *Nature*, **220**, 753 (1968).
[2] Wielebinski, R., Vaughan, A. E., and Large, M. I., *Nature*, **221**, 47 (1969).
[3] Davidson, K., and Terzian, Y., *Astron. J.*, **74**, 849 (1969).
[4] Prentice, A. J. R., and ter Haar, D., *Nature*, **222**, 964 (1969).
[5] Prentice, A. J. R., and ter Haar, D., *Mon. Not. Roy. Astron. Soc.*, **146**, 423 (1969).
[6] Davies, J. G., Hunt, G. C., and Smith, F. G., *Nature*, **221**, 27 (1969).
[7] Cole, T. W., *Nature*, **223**, 487 (1969).
[8] Ostriker, J. P., and Gunn, J. E., *Nature*, **223**, 813 (1969).
[9] Blaauw, A., Gum, C. S., Pawsey, J. L., and Westerhout, G., *Mon. Not. Roy. Astron. Soc.*, **121**, 123 (1960).
[10] Mills, B. Y., *Nature*, **224**, 504 (1969).
[11] Gold, T., *Nature*, **218**, 731 (1968).
[12] Cameron, A. G. W., *Comments Astrophys. Space Phys.*, **1**, 172 (1969).
[13] Colgate, S. A., and White, R., *Astrophys. J.*, **143**, 626 (1966).
[14] Arnett, W. D., *Nature*, **222**, 359 (1969).
[15] Arnett, W. D., *Astrophys. Space Sci.*, **5**, 180 (1969).
[16] Morgan, W. W., Osterbrock, D. E., and Sharpless, S. L., *Astron. J.*, **57**, 3 (1952).
[17] Becker, W., *Z. Astrophys.*, **57**, 117 (1963); **58**, 202 (1964).
[18] Courtès, G., Georgelin, Y. P., Georgelin, Y. M., and Monnet, G., *Astrophys. Lett.*, **4**, 129 (1969).
[19] Poveda, A., and Woltjer, L., *Astron. J.*, **73**, 65 (1968).
[20] Kurochkin, N. E., *Sov. Astron. AJ*, **35**, 74 (1958).
[21] Blaauw, A., *Bull. Astron. Inst. Netherlands*, **15**, 265 (1961).
[22] Shklovsky, I. S., *Supernovae*, 67 (Wiley, 1968).
[23] Large, M. I., Vaughan, A. E., and Mills, B. Y., *Nature*, **220**, 340 (1968).
[24] Milne, D. K., *Austral. J. Phys.*, **21**, 201 (1968).
[25] Radhakrishnan, V., Cooke, D. J., Komesaroff, M. M., and Morris, D., *Nature*, **221**, 443 (1969).
[26] Goldreich, P., *Proc. Astron. Soc. Austral.*, **1**, 227 (1969).

Variations of Linear Polarization in Extragalactic Radio Sources

SINCE mid-1965 the linear polarizations of the stronger extragalactic variable radio sources have been monitored at 8 GHz using the University of Michigan 85-foot paraboloid equipped with a rotating, linearly polarized feed horn. Strong thermal and non-thermal sources were measured each observing day to determine the stability of the instrumental polarization. Five sources, 3C 120, 3C 273, 3C 279, 3C 345 and 3C 454·3, have exhibited significant changes in polarization, and three sources, 3C 84, NRAO 150 and 3C 446, are suspected of having variable polarizations. Preliminary observational results for several of these sources have been reported previously[1,2].

The general characteristics of the observed variations in polarization are as follows: (1) They do not mimic the variations in the total flux densities; (2) they occasionally have time scales as short as one week, which are much less than the time scales of the variations in the total flux densities of these sources; (3) their amplitudes are as large as 5–10 per cent of the amplitudes of the variations in total flux density; (4) large, rapid changes in polarization have been observed only when the total flux density was increasing or had reached a maximum; (5) variations in both the position angle of the plane of polarization and in the degree of polarization have been observed.

I wish to point out that these characteristics of the observed variations in polarization are consistent with expanding source type models (for example, ref. 3) and do not require any special source geometry except that the magnetic fields in these sources be relatively well ordered. In these models the low frequency cut-off in flux is produced by synchrotron self-absorption. As the source expands, the total absorption depth through the source decreases and the flux reaches a maximum at any frequency ν shortly after τ_ν (the absorption depth averaged over all polarizations) reaches unity in the source. Two processes which cause variations in polarization and which apparently have not been reported before occur as a result of this decrease of τ_ν with time.

Process I is the change with τ_ν of the intrinsic polarization of a synchrotron source. From the expressions for the emission and absorption coefficients of the synchrotron mechanism[4], it may be shown that when $\tau_\nu \gg 1$ the intrinsic degree of linear polarization is $3/(6\gamma+13)$ (ref. 5) with the plane of polarization parallel to the magnetic field. γ is the negative exponent in the electron energy-density power-law relation. This state of polarization is different from the case which is usually discussed of $\tau_\nu \ll 1$ when the intrinsic plane of polarization is perpendicular to the field, and the intrinsic degree of polarization is $(3\gamma+3)/(3\gamma+7)$. Studies of the variable sources 3C 120 (ref. 6) and 3C 273 (ref. 7) indicate that $\gamma \sim 1{\cdot}5$ for these sources. For this value of γ the intrinsic degree of polarization changes rapidly with τ_ν over the range $0{\cdot}5 \lesssim \tau_\nu \lesssim 10$ and equals zero (where the plane of polarization changes by 90 degrees) at $\tau_\nu \sim 5$. Thus even in the unrealistic case that the magnetic field was oriented in the same direction throughout the emitting region, an expanding source would exhibit variations in polarization as the source passes through the phase where $10 \gtrsim \tau_\nu \gtrsim 0{\cdot}5$. This phase occurs immediately before the expanding source reaches maximum flux output at the frequency ν.

Process II arises when the orientation of the magnetic field is not constant throughout the emitting region of the source. The observed polarization of a source is the average of the contributions of all polarized emitting regions within the source and thus depends upon the absorption depth of each region. As τ_ν decreases with time the contribution to the observed polarized radiation of emitting regions situated deep within the source increases. The observed polarization will then change because the states of polarization of different regions within the source are not the same. The rate of change of the observed polarization depends on the amplitude of $d\tau_\nu/dt$ and would generally be largest when $\tau_\nu \gtrsim 1$. Relatively rapid changes in polarization produced by this process do not require the magnetic field in the source to have small scale structure.

Both processes I and II are only important for $\tau_\nu \gtrsim 1$ and do not produce appreciable variations when $\tau_\nu \gg 1$ or $\tau_\nu \ll 1$. Thus the fact that large, rapid changes in polarization have been observed only during periods when $\tau_\nu \gtrsim 1$ (according to the expanding source model) strongly suggests that one or both of these processes are the chief cause of the observed variations in linear polarization. Neither process depends on changes in the structure of the radiating region (aside from overall expansion) or on any particular source configuration in order to produce polarization variations. Also the relatively well ordered structure of the magnetic field required to account for the large amplitudes of the polarization variations is not inconsistent with the rapid variations being produced by these processes. A detailed description of the observational results and the interpretation of the observed polarization variations is in preparation.

I thank W. A. Dent for pointing out that the plane of polarization of an opaque synchrotron source is parallel to the magnetic field, and F. T. Haddock for many discussions. This work was supported in part by the US Office of Naval Research and by the US National Science Foundation.

HUGH D. ALLER

Radio Astronomy Observatory,
University of Michigan.

Received November 24, 1969.

[1] Aller, H. D., and Haddock, F. T., *Astrophys. J.*, **147**, 833 (1967).

[2] Aller, H. D., and Haddock, F. T., *Relativistic Astrophysics* (edit. by Cameron, A. G. W., and Maran, S. P.) (Gordon and Breach, in the press).

[3] Laan, H. Van der, *Nature*, **211**, 1131 (1966).

[4] LeRoux, E., *Ann. d'Astrophys.*, **24**, 71 (1961).

[5] Pacholczyk, A. G., and Swihart, T. L., *Astrophys. J.*, **150**, 647 (1967).

[6] Kellermann, K. I., and Pauliny-Toth, I. I. K., *Astrophys. J. Lett.*, **152**, L169 (1968).

[7] Dent, W. A., *Astrophys. J. Lett.*, **153**, L29 (1968).

Primordial Synthesis of Superheavy Nuclei in the Galaxy

ASTROPHYSICAL evidence in favour of the thesis that an appreciable amount of the heavy elements was produced in a giant explosion in the nucleus of the galaxy about 7×10^9 yr ago has been given by Unsöld[1]. Cosmochemical arguments could support Ambartsumyan's concept[2] of the presence of massive bulks of prestellar matter in the nuclei of the galaxies. The latter concept has now received further support from the calculations of Saakyan and Mnatsakanyan[3], who have reported on the possibility of hydrostatic equilibrium of superdense matter bulks of arbitrary mass in the framework of a slightly modified general relativity theory with variable gravitational constant G.

Synthesis of heavy elements in exploding massive bulks of prestellar matter may go preferentially through some kind of neutron capture process, similar in principle to those going on in stars. This synthesis occurs predominantly in the external layers of the body, where the density of matter drops below a subnuclear value of 10^{13} g cm^{-3} and the matter exists in the so-called "neA" phase (neutrons, electrons, nuclei). Synthesis of nuclei up to uranium in such high density conditions (without specifying whether this occurs in stars or in other objects) has been considered by Amiet and Zeh[4]. The shift of the valley of beta-stability of nuclei in the (Z,N) plane towards the N-axis at higher densities made it possible to bypass the α-instability region. At the time when these results were reported there were still no explicit results on the possible island of stability near $Z = 114$, and it was not necessary to extend the neutron capture tracks up to that region.

There has been much theoretical work on the region near the doubly closed shell nucleus $^{298}114$, and it was found that the nuclide $^{294}110$ might be the best-fitted candidate for survival (see ref. 5, which contains a list of references on the properties of superheavy nuclei). A half-life exceeding 2×10^8 yr cannot be excluded *a priori*. Experimental searches for this and closely related elements are in progress; there are certain indications in favour of extinct superheavy elements in meteorites. The evidence comes from isotopic anomalies in the Xe and Kr composition[6]. We have to deal here with the effect of symmetric fission. It may be added that in the region from tellurium to rare earths we find some characteristic abundance irregularities: several odd isotopes of even elements (such as Xe, Sm, Gd, Dy) are more abundant than their even neighbours. These more abundant isotopes are unshielded from the side of proton-deficient nuclides, and thus their relative overabundance may be regarded as a mere consequence of fission product β⁻-decay chains from the fission of superheavy elements. This indirect evidence for extinct superheavy elements makes the search for the last relics from an island of quasi-stability more promising.

In such a search we must be guided by some ideas resulting from the way in which we understand the mechanism of synthesis. In addition to the probably single event of synthesis in the explosion in the galactic core, the continuous mechanism of production in supernova explosions should be taken into account. A lower estimate of the production of about 10^{32} nuclei of the element 110 per second in the galaxy by the mechanism of supernovae explosions was obtained[7]. It would be interesting to estimate the production in the explosion of the galactic core; it is possible, in principle, to have here a larger time scale and a much higher neutron flux. Because the prestellar matter bulks are relativistic objects, detailed results should be obtained within the theory of relativity which will determine the time scale of the process. Such calculations will be made in the future, and it is possible now to remark only that their results should be compared with observational abundances of the superheavy elements and with such abundance peculiarities as those discussed. It is possible that observational results on the abundance of the element 110 would help to decide whether Unsöld[1] was right in accentuating the role of explosions in galactic nuclei for the synthesis of heavy elements.

B. KUCHOWICZ

Department of Radiochemistry,
University of Warsaw,
Warsaw, Poland.

Received July 7, 1969.

[1] Unsöld, A., *Z. Phys.*, **202**, 13 (1967).

[2] Ambartsumyan, V. A., in *La Structure et l'Evolution de l'Univers* (edit. by Stoops) (Bruxelles, 1958).

[3] Saakyan, G. S., and Mnatsakanyan, M. A., *Astrofizika* (in Russian; in the press).

[4] Amlet, J. P., and Zeh, H. D., *Phys. Lett.*, **25B**, 305 (1967); *Z. Phys.*, **217**, 485 (1968).

[5] Nilsson, S. G., Thompson, S. G., and Tsang, C. F., *Phys. Lett.*, **28B**, 458 (1969).

[6] Dakowski, M., *Inst. Nucl. Res. (Warsaw) Report No. 1031/IA/PL* (1968).

[7] Kowalski, M., and Kuchowicz, B., *Phys. Lett.*, **308**, 79 (1969).

Two New Plasma Instabilities in the Solar Wind

IN this communication we consider the left-hand mode in a hot plasma, propagating in the direction of the average magnetic field, and for frequencies near the proton gyrofrequency we find two new instabilities. The first is driven by strong electron thermal anisotropy and the second by strong proton thermal anisotropy in the presence of electron anisotropy. We believe that the first of these new instabilities may have important consequences for the development of the solar wind. This mode is similar to the low-frequency firehose instability but it involves only the electron anisotropy. The wavelength is short, and the destabilizing centrifugal force on the electrons is able to overwhelm the cyclotron damping due to the resonant protons. If this instability occurs in the solar wind, it results in an additional coupling between the electrons and protons, with the result that it can contribute to the proton heating.

Considering a hydrogen plasma, Stix's dispersion relation[1] for circularly polarized electromagnetic waves propagating in the direction of the average magnetic field, B_0, may be written in the form

$$k^2 v_A^2 + \Omega_e \Omega_p A_e + \Omega_p^2 A_p = i(\Omega_e \Omega_p / v_e) F_0(\alpha_{\mp e}) \times$$

$$\left[\frac{\omega}{k} - v_e A_e \alpha_{\mp e}\right] + i(\Omega_p^2 / v_p) F_0(\alpha_{\pm p}) \left[\frac{\omega}{k} - v_p A_p \alpha_{\pm p}\right] \qquad (1)$$

Our calculations are in the rest system of the plasma; the results in other systems are simply Doppler shifted in the usual way. The subscripts p and e refer to the protons and electrons respectively. v_p is the thermal velocity of the protons, based on their temperature parallel to the magnetic field: $v_p = (2KT_{\parallel}^{(p)}/m_p)^{1/2}$. Their thermal anisotropy is given by $A_p = 1 - T_{\perp}^{(p)}/T_{\parallel}^{(p)}$. The proton gyrofrequency is $\Omega_p = qB_0/m_p c$ where q is the proton charge. $\alpha_{\pm p}$ is defined by

$$\alpha_{\pm p} = \frac{\omega \pm \Omega_p}{k v_p} \qquad (2)$$

The quantity $v_A = (B_0^2/4\pi n m_p)^{1/2}$ is the Alfvén speed, where n is the proton concentration. Analogous definitions hold for the electrons, and we define $\Omega_e = (m_p/m_e)\Omega_p > 0$. As usual, ω, k and c are the angular frequency (assumed complex), the wave number (assumed real), and the speed of light, respectively; all wave quantities are assumed to vary as $\exp(ikx - i\omega t)$. F_0 is the plasma dispersion function

$$F_0(s) = -(i/\pi^{1/2}) P \int_{-\infty}^{+\infty} \frac{e^{-z^2}\,dz}{z - s} + \pi^{1/2}\,\mathrm{sgn}\,(k) e^{-s^2} \qquad (3)$$

We consider the left-hand mode, taking the lower sign in equation (1). For frequencies near the proton gyrofrequency, equation (2) suggests that we should seek solutions for which the approximations

$$\alpha_{-p} \ll 1; \;\; \alpha_{+e}^2 \approx \left(\frac{\Omega_e}{k v_e}\right)^2 \gg 1 \qquad (4)$$

are valid. Expanding F_0 accordingly[1] yields for the protons

$$F_0(\alpha_{-p}) \cong \pi^{1/2}\,\mathrm{sgn}\,(k) \exp(-\alpha_{-p}^2) + 2i\alpha_{-p}(1 - 2\alpha_{-p}^2/3 + \ldots) \qquad (5)$$

and for the electrons

$$F_0(\alpha_{+e}) \simeq i\alpha_{+e}^{-1}(1 + 1/2\alpha_{+e}^2 + \ldots) \qquad (6)$$

From equations (1), (5) and (6) we then obtain

$$k^2(1 + \xi_e) = \frac{\Omega_p^2}{v_A^2} \left\{ -(1 + A_p) + \frac{i\pi^{1/2}}{kR_p} - \alpha_{-p}\left[\left(kR_p + \frac{2}{kR_p}\right) - i\pi^{1/2}\,\mathrm{sgn}\,(k)(1 - A_p)\right]\right\} \qquad (7)$$

where $R_p = v_p/\Omega_p$ is the proton gyroradius, and ξ_e represents the electron anisotropy

$$\xi_e = 4\pi nK(T_\perp^{(e)} - T_\parallel^{(e)})/B_0^2 \tag{8}$$

We note that $|\xi_e|$ can differ significantly from zero even for small temperature anisotropies, if $4\pi nKT_\parallel^{(e)} \gg B_0^2$; such a situation prevails in the solar wind. In deriving equation (7) we have consistently kept terms only to the first order in α_{-p}.

Equation (7) may be solved for $R_e(\alpha_{-p})$ and $\mathrm{Im}(\alpha_{-p})$. The results are complicated, however, and we confine our attention to cases where

$$k^2R_p^2 \gg 1 \tag{9}$$

We find that consistent solutions are obtained for this case. Inequality (9) must, however, be considered in the context of inequality (4*b*). Taken together, they require

$$\frac{m_p}{m_e} \; \frac{T_p}{T_e} \gg k^2R_p^2 \gg 1 \tag{10}$$

If T_e/T_p is less than about 10 (as is the case in the solar wind, for example), we expect that $k^2R_p^2$ will be able to satisfy both requirements in (10) simultaneously.

From equation (7) one obtains for the real part ω_r (assumed positive) and the imaginary part ω_i of the frequency

$$\frac{\omega_r}{\Omega_p} + A_p \cong -k^2R_p^2(v_A^2/v_p^2)(1+\xi_e) \tag{11}$$

$$\frac{\omega_i}{\Omega_p} \cong (\pi^{1/2}/k|R_p)\left[A_p + \frac{\omega_r}{\Omega_p}(1-A_p)\right] \tag{12}$$

Equations (11) and (12) are the desired solutions of the dispersion relation, and we distinguish two cases.

First (case I) let us suppose that $T_\perp^{(p)}/T_\parallel^{(p)}$ is small enough for

$$\frac{\omega_r}{\Omega_p} + A_p > 0 \tag{13}$$

This will be the usual case in the solar wind. Then equation (11) tells us that we must require

$$1+\xi_e < 0 \tag{14}$$

for a solution, because k is real. But we have assumed $k^2R_p^2$ to be large, and from equation (11) this usually further requires

$$|1+\xi_e| \ll 1 \tag{15}$$

We thus find that the existence of this wave depends critically on the perpendicular electron temperature being sufficiently smaller than the parallel temperature so that (14) holds, but that there is a limit to how much smaller it can be if one of our assumptions is not to break down.

The wave propagates much more slowly than the Alfvén velocity, in virtue of the largeness of k.

ω_i can be written in terms of ω_r as follows

$$\frac{\omega_i}{\Omega_p} \cong \pi^{1/2}(v_A/v_p)\left[A_p + \frac{\omega_r}{\Omega_p}(1-A_p)\right] \left(A_p + \frac{\omega_r}{\Omega_p}\right)^{-1/2}|1+\xi_e|^{1/2} \tag{16}$$

The waves always grow (that is, ω_i is always positive) if the basic condition (13) is fulfilled. We thus suggest that (14), that is, the condition for the existence of the waves, represents the real instability criterion, and that the waves are therefore driven by the electron anisotropy.

This instability is thus similar to the low frequency firehose instability[2,3] but, in contrast, the proton anisotropy does not appear in the present instability criterion because we have assumed frequencies in the vicinity of the proton gyrofrequency. The wave is destabilized by the centrifugal force felt by the electrons as they move along the perturbed magnetic field. The centrifugal force increases with increasing k, and, for the large k values assumed here, overwhelms the damping effect due to the resonant ions, which decreases like k^{-1}. We expect, in fact, that the growth rate of the instability can be larger than that of the low frequency firehose, and this instability may therefore be of greater significance.

Do these waves exist in the solar plasma? Direct measurements of the electron thermal anisotropy are unreliable. Parker[4] has pointed out in a recent review, however, that direct measurements of high frequency ($>10^{-2}$ Hz) fluctuations in the solar wind suggest the existence of an internal instability. The firehose is favoured, but Parker argues that the electrons must contribute substantially to its excitation, because the protons cannot drive the firehose alone. This implies that ξ_e must be close to -1. Whether ξ_e is in fact less than -1 cannot at present be decided, but this is a viable possibility.

The presence of the unstable left-hand mode in the interplanetary plasma may have important consequences. Because the instability is driven by the electron anisotropy, energy will go from the electrons into the wave. But the wave is resonant with the protons, and we expect the wave to give up some energy to the perpendicular motion of the protons. The result will be a (partial) transfer of thermal energy from the electrons to the protons. Now, recent work by Burlaga and Ogilvie[5] indicates that the two-fluid solar wind model of Hartle and Sturrock[6] represents an extremely quiet situation, and that the solar wind typically exhibits proton temperatures and bulk velocities which are higher than those calculated by Hartle and Sturrock. This implies that some heating of the protons usually occurs. There is also an indication that the electron temperatures calculated by Sturrock and Hartle are somewhat higher than those observed, although it is not clear whether the difference is significant. We believe that the transfer of energy from electrons to protons may provide a suitable heating mechanism for the solar wind protons. Furthermore, the energy goes into the motion of the protons perpendicular to the magnetic field, which may help to explain why, in the solar wind, $T_\perp$ is not as small as might be expected on theoretical grounds[7]. We plan to discuss these points in detail in future publications.

Referring once again to equation (11), consider the situation where $T_\perp^{(p)}/T_\parallel^{(p)}$ is sufficiently large (case II) that

$$\frac{\omega_r}{\Omega_p} + A_p < 0 \tag{17}$$

Then an allowed solution for (11) exists only if

$$1+\xi_e > 0 \tag{18}$$

The wave number k can then be found from equation (11); we see that if $k^2R_p^2$ is to be large, according to the assumption, we will usually require

$$0 < 1+\xi_e \ll 1 \tag{19}$$

This wave exists in the absence of electron anisotropy, but some anisotropy is usually necessary to satisfy the assumption of large k. This situation contrasts with the previous case where electron anisotropy was necessary for the existence of the wave.

The propagation speed is again much less than the Alfvén speed.

The growth rate is the same as in case I, equation (16), with the exception that $(A_p+\omega_r/\Omega_p)$ must be replaced by its modulus. The instability criterion is

$$A_p + \frac{\omega_r}{\Omega_p}(1-A_p) > 0 \qquad (20)$$

subject to inequality (17). These requirements are plotted simultaneously in Fig. 1.

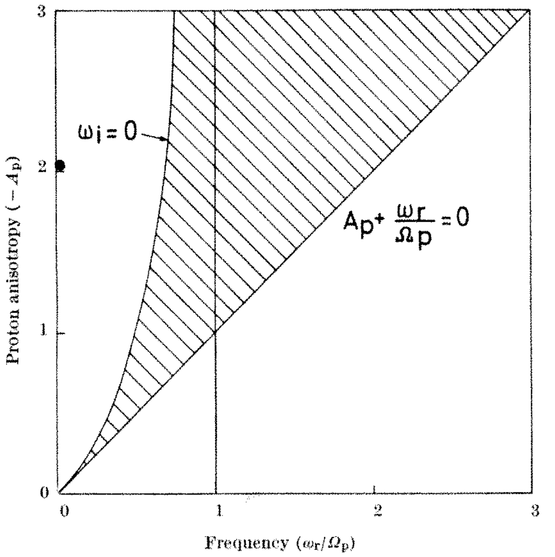


Fig. 1. Plot of the proton temperature anisotropy, $-A_p$, against frequency, for case II. The hatched area represents the region for wave growth (upper curve) subject to the requirement defining case II, $A_p+\omega_r/\Omega_p<0$.

This instability is driven by perpendicular proton temperatures sufficiently in excess of the parallel temperatures. We expect that it will be important primarily for laboratory plasmas, although it may occur in the solar wind in local regions where $T_\perp^{(p)} > T_\parallel^{(p)}$.

J. V. H. thanks the German Bundesministerium für wissenschaftliche Forschung for a fellowship grant, and Professor R. Lüst for hospitality.

JOSEPH V. HOLLWEG
H. J. VÖLK

Max-Planck-Institut für Physik und Astrophysik,
Institut für Extraterrestrische Physik,
8046 Garching bei München,
Germany.

Received September 11; revised November 28, 1969.

[1] Stix, T. H., *The Theory of Plasma Waves* (McGraw-Hill, New York, 1962).
[2] Parker, E. N., *Phys. Rev.*, **109**, 1874 (1958).
[3] Kennel, C. F., and Scarf, F. L., *J. Geophys. Res.*, **73**, 6149 (1968).
[4] Parker, E. N., *Space Sci. Rev.*, **9**, 325 (1969).
[5] Burlaga, L. F., and Ogilvie, P. A., *Astrophys. J.* (in the press).
[6] Hartle, R. E., and Sturrock, P. A., *Astrophys. J.*, **151**, 1155 (1968).
[7] Scarf, F. L., Wolfe, J. H., and Silva, R. W., *J. Geophys. Res.*, **72**, 993 (1967).

Identification of the ν_3 NO_2 Band in the Solar Spectrum observed from a Balloon Borne Spectrometer

SINCE the detection of HNO_3 in the atmosphere[1], it has been expected that oxides of nitrogen, such as NO_2, should also be present. In fact, in a previous paper by some of us[1] a tentative identification of NO_2 absorption was made. The most intense vibration–rotation band of NO_2 is the ν_3 fundamental, centred at 1,618 cm^{-1}, near the centre of the ν_2 H_2O band.

In this region, the solar spectrum[2] as observed from a balloon borne spectrometer at float altitude (30 km), while the Sun was setting, reveals a series of weak lines which occur among the well known H_2O lines. For example, a number of weak lines are observed on both sides of the normally isolated $1_{-1}-1_1$ H_2O line at 1616·8 cm^{-1}. Many more lines can be seen in this region, and on closer examination a faint band envelope can be observed centred near 1,618 cm^{-1}. These additional lines also show some absorption in high altitude spectra measured at zenith angles smaller than those obtained during the sunset.

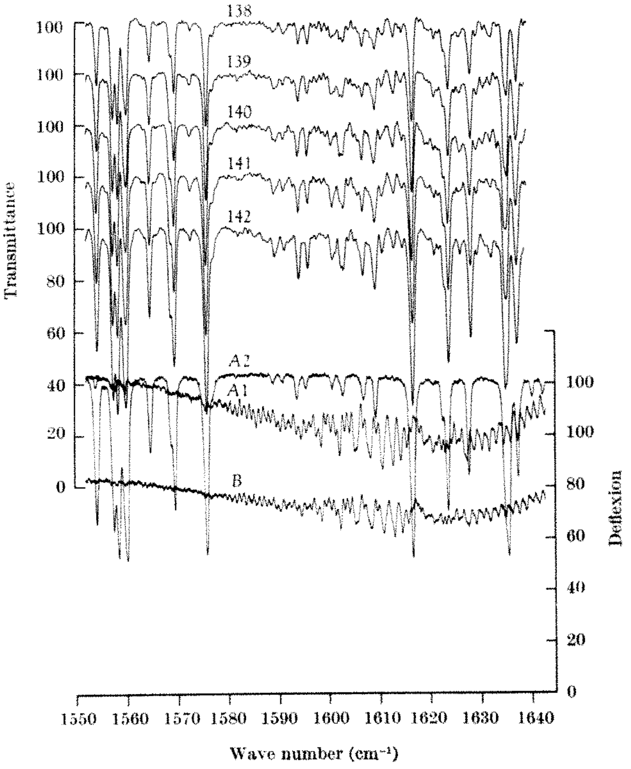


Fig. 1. Identification of the ν_3 NO_2 band. Records 138–142: sunset balloon spectra (reproduced from ref. 2). *A*, Spectrum of NO_2 obtained from reagent grade red fuming nitric acid supplied by Baker Co. (curve 1) and the corresponding portion of the ν_2 H_2O spectrum (curve 2). *B*, Spectrum of CP grade NO_2 from Matheson Co., at a pressure of 2·5 mm Hg.

Laboratory spectra of the ν_3 region of NO_2 have been taken under a resolution of ~0·5 cm^{-1}, which is similar to the resolution obtained during the balloon flight. The corresponding portion of the ν_2 H_2O spectrum was recorded for comparison.

The laboratory spectra were obtained on a double-beam Beckman infrared spectrophotometer, model IR7, using a 10 cm glass absorption cell with AgCl windows, at 40° C. A Kel-F halocarbon oil manometer was used for pressure measurements.

The results are shown in Fig. 1, in which the relevant portion of the sunset solar spectrum is reproduced from ref. 2, along with two laboratory spectra of the ν_3 NO_2 band. In sample *A*, curve 1 represents absorption by NO_2 vapour generated from reagent-grade red fuming nitric acid, supplied by J. T. Baker Chemical Co. This sample shows traces of H_2O lines probably due to residual H_2O vapour used to record the ν_2 H_2O lines (curve 2). Sample *B* is CP grade NO_2 from the Matheson Co. The two spectra agree qualitatively with that of Arakawa and Nielsen[3].

The NO_2 and H_2O line complexes were checked against the solar spectrum, and the NO_2 lines were found to match, both in position and intensity, the line structure which is additional to the H_2O lines. The centre of the NO_2 band envelope is masked by the $1_{-1}-1_1$ H_2O line, but the NO_2 line structure is clearly identified. The weak line at 1,574 cm^{-1} is probably due to ν_2 HDO.

This research was supported by the Advanced Research Projects Agency and was monitored by the Air Force Cambridge Research Laboratories.

A. GOLDMAN
D. G. MURCRAY
F. H. MURCRAY
W. J. WILLIAMS

Department of Physics,
University of Denver,
Denver,
Colorado 80210.

F. S. BONOMO

Department of Chemistry,
University of Denver,
Denver,
Colorado 80210.

Received January 2, 1970.

[1] Murcray, D. G., Kyle, T. G., Murcray, F. H., and Williams, W. J., *Nature*, **218**, 78 (1968).

[2] Murcray, D. G., Murcray, F. H., Williams, W. J., Kyle, T. G., and Goldman, A., *Appl. Opt.* (in the press).

[3] Arakawa, E. T., and Nielsen, A. H., *J. Mol. Spectr.*, **2**, 413 (1958).

Crystal Chemistry of the Plagionite Group

LEAD forms a large number of complex sulphosalts. At least twelve Pb–As and nineteen Pb–Sb sulphosalt minerals are known[1] and, although the Pb–As minerals have received considerable study, no structure for a Pb–Sb sulphosalt has been completely determined. Curiously, minerals in the two systems exhibit little similarity in either composition or lattice constants in spite of the fact that isomorphism and even complete solid solubility are found for a number of arseno- and antimonian sulphosalts. This is undoubtedly due to the rather different sizes of As and Sb coordination groups relative to the dimensions of a Pb–S polyhedron.

Table 1. THE PLAGIONITE GROUP

		a (Å)	*b* (Å)	*c* (Å)	*β*	Ref.
Fülöppite	$Pb_3Sb_8S_{15}$	13·39	11·69	16·91	94° 41′	2
Plagionite	$Pb_5Sb_8S_{17}$	13·4857	11·8656	19·9834	107° 10′	Present work
Heteromorphite	$Pb_7Sb_8S_{19}$	—	—	—	—	*
Semseyite	$Pb_9Sb_8S_{21}$	13·64	12·01	24·57	105° 49′	4

* Crystals of heteromorphite are extremely rare and are invariably distorted. No accurate X-ray or morphological data exist. The mineral was placed in the plagionite group by Spencer[3] on the basis of its composition and approximate goniometric measurements.

Minerals of the plagionite group (Table 1) form a particularly interesting series. No As analogues occur and the known members of this group form an apparent homologous series, $Pb_{3+2n}Sb_8S_{15+2n}$ with $n=0$ to 3. Two lattice constants remain invariant throughout the group, while c increases uniformly with n. The probable space group for all of the phases is $C\ 2/c$ and $Z=4$. We are investigating the crystal structures of these minerals and we have completed a study of plagionite. An analysis of semseyite is in progress. We wish to present a preliminary description of the plagionite structure; its features suggest the probable relation between the structures of the other members of this family.

A plagionite crystal from Wolfsberg, Harz, Germany (Harvard Museum Catalogue No. 11391), was ground into a sphere of 0·054 mm radius ($\mu r=6{\cdot}51$ for Cu$K\alpha$ radiation). Precession photographs identified the diffraction symbol as $2/m\ C\ -/c$, which permits Cc and $C\ 2/c$ as space groups. The morphology of the mineral suggested the centric option and this was subsequently confirmed by statistical tests. Least-squares analysis of measurements obtained from back-reflexion Weissenberg films provided the lattice constants presented in Table 1. A complete three-dimensional set of diffracted intensities was obtained with the aid of an equi-inclination Weissenberg diffractometer equipped with a proportional counter; 2,953 of the 3,579 independent reflexions available in the Cu$K\alpha$ sphere were accessible with the instrument used. The intensities were very weak, the maximum peak height of any reflexion recorded being less than 500 counts/s, and as a result 651 of the accessible reflexions were undetectable.

The structure of plagionite was solved by the symbolic addition procedure. A total of 633 signs were determined at probability levels greater than 0·989 from among the 950 largest ($E\geq 0{\cdot}83$) normalized structure factors. An E-map revealed locations for all metal atoms. Positions for sulphur atoms were suggested by this map, but their location was deferred to subsequent electron density maps based on signs computed from the metal atom positions. We have refined the structure by least-squares techniques to a disagreement index of 18 per cent.

Table 2. ATOMIC POSITIONS AND TEMPERATURE FACTORS IN PLAGIONITE (STANDARD DEVIATIONS IN PARENTHESES)

Atom	*x*	*y*	*z*	*B* (Å²)
Pb(1)	0·2366 (3)	0·0862 (3)	0·4484 (2)	2·67 (5)
Pb(2)	0·2310 (2)	0·3741 (2)	0·3220 (2)	2·18 (4)
Pb(3)	0·0	0·8199 (4)	0·25	2·68 (7)
Sb(1)	0·0018 (4)	0·4816 (4)	0·1053 (3)	2·54 (8)
Sb(2)	0·2710 (4)	0·1904 (4)	0·0857 (2)	1·83 (6)
Sb(3)	0·0461 (3)	0·1250 (4)	0·1671 (2)	1·80 (6)
Sb(4)	0·5094 (3)	0·3047 (3)	0·0285 (2)	1·77 (6)
S(1)	0·1087 (12)	0·4160 (13)	0·0009 (9)	1·72 (21)
S(2)	0·1812 (14)	0·2662 (14)	0·1709 (9)	2·14 (25)
S(3)	0·3887 (10)	0·3613 (11)	0·1017 (7)	1·49 (21)
S(4)	0·3865 (11)	0·0766 (11)	0·1790 (8)	1·70 (21)
S(5)	0·3532 (14)	0·4954 (14)	0·2438 (10)	1·98 (24)
S(6)	0·1314 (12)	0·3104 (12)	0·4367 (8)	1·59 (21)
S(7)	0·3812 (12)	0·1926 (13)	0·3759 (8)	2·18 (25)
S(8)	0·3699 (14)	0·4742 (14)	0·4417 (9)	1·92 (23)
S(9)	0·0	0·2569 (23)	0·25	2·36 (38)

The asymmetric unit in plagionite (Table 2) contains sixteen atoms, all of which occupy a general position of the space group with the exception of Pb(3) and S(9), which occupy position 4 *e* 2. As in most Pb sulphosalts, the coordination of Pb is very irregular: Pb(1) has a distorted octahedral coordination with S neighbours at distances ranging from 2·74 to 3·16 Å (average: 2·97 Å); Pb(2) has six neighbours at distances between 2·83 and 3·17 Å (average: 2·97 Å) plus a seventh neighbour at 3·33 Å; Pb(3) has an irregular distribution of six neighbours at distances of 2·85 to 3·38 Å (average: 3·06 Å); two additional sulphurs at a much greater distance of 3·68 Å complete a square antiprismatic coordination for this atom. Three of the four Sb atoms have the [1+2+2] pyramidal coordination found for half of the Sb in stibnite, Sb_2S_3. Average distances for the three types of neighbours in the polyhedra are 2·47, 2·59 and 3·03 Å, respectively. The remaining Sb has a trigonal pyramidal coordination with an average bond length of 2·43 Å.

A projection along b of the polyhedra contained between $y=0$ and $y=1/2$ is presented in Fig. 1. The remainder of the cell content may be obtained when the depicted layer is shifted by the face-centring translation of the lattice, $\frac{1}{2}\frac{1}{2}0$. The most striking feature of the structure is the string of four Pb(1) and Pb(2) octahedra sharing edges along c. This cluster of octahedra is linked by the Pb(3) polyhedron to form a continuous chain parallel to $[10\bar{1}]$. The chain is flanked on either side by Sb pyramids. The strong short and intermediate bonds of each Sb square pyramid are directed toward the chain of Pb polyhedra. The long Pb(2)–S(9) bond and an Sb(3)–S(9)–Sb(3) bridge provide the only links between chains in (010). Two Pb(2) polyhedra in adjacent chains are further linked by corner sharing with a Pb(3) group immediately above the depicted layer, and also by a Pb(3) polyhedron below the layer.

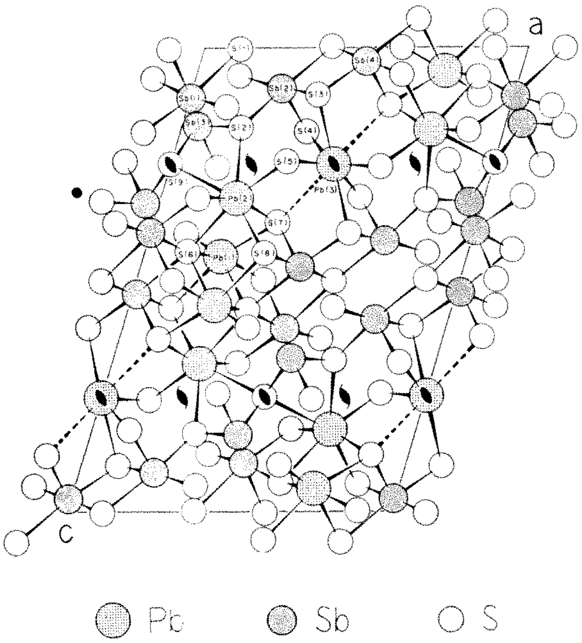


Fig. 1. Projection along *b* of the coordination polyhedra contained between $y = 0$ and $y = 1/2$ in plagionite. The *C* lattice provides an equivalent layer shifted ½½0 above the depicted polyhedra.

The arrangement of the Sb$^{[5]}$ polyhedra is another interesting feature. The bases of the square pyramids flanking each Pb chain are directed outwards from the chain and form a sheet parallel to (112). The Sb(1) and Sb(4) pyramids share an apical edge to form a limited chain similar to that in stibnite. In plagionite, however, this double chain shares an edge with the base of an Sb(2)$^{[5]}$ pyramid. A unit 6 polyhedra in width is thus formed rather than the quadruple chain found in stibnite. The opposed bases of Sb$^{[5]}$ pyramids on neighbouring Pb chains are parallel to (112). This feature explains the (112) cleavage observed in plagionite.

The characteristics of the structure of plagionite suggest a probable relation to other members of the group. Each successive member of the series contains an additional 8 Pb and 8 S per cell. This change in composition as well as the invariance of all lattice constants except *c* may be explained by adding one Pb and one S to a general position of the space group such that the number of Pb octahedra in the chain parallel to *c* increases by two. In accomplishing this, the array of Sb polyhedra may be left essentially unchanged. This would explain the common (112) cleavage in the family. Moreover, few additional bonds would be formed between Pb chains although the length of the chains is increased. This would account for the increasing ease of cleavage observed in the sequence plagionite–heteromorphite–semseyite. Inspection of Fig. 1, however, shows that the Sb polyhedra must be rearranged if the structure of fülöppite is to be derived by the removal of either Pb(1) or Pb(2) from the octahedral chain. This may explain the lack of cleavage in this mineral and the rather different value of β. Confirmation of this proposal must await completion of our structure determinations of the remaining minerals in the group.

This work was supported by a grant from the US National Science Foundation.

SEUNG-AM CHO
B. J. WUENSCH

Department of Metallurgy and Materials Science,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139.

Received October 20, 1969.

[1] Nowacki, W., *Schweiz. Min. Petrogr. Mitt.*, **49**, 109 (1969).

[2] Nuffield, E. W., *Univ. Toronto Studies, Geol. Ser.*, **50**, 49 (1946).

[3] Spencer, L. J., *Min. Mag.*, **12**, 55 (1899).

[4] Nuffield, E. W., and Peacock, M. A., *Univ. Toronto Studies, Geol. Ser.*, **49**, 17 (1945).

Karman Vortices in the Flow of Drag-reducing Polymer Solutions

THE effect on flow properties of adding small quantities of soluble polymers, which are able to change turbulence and form solutions of a different structure, has been reviewed[1]. Aqueous solutions of polyethylene oxide exhibit viscoelastic properties immediately after the solute has dissolved, but these disappear on ageing. The solution, however, retains its capacity for reducing turbulent resistance of friction in flow near a smooth surface for a long time, although shear flow of the solution accelerates considerably the disappearance of this capacity. Aqueous solutions of the polymer Guar gum do not display continuum viscoelastic properties, but additions of it do reduce turbulent frictional resistance.

The reduction of this resistance is explained by the viscoelastic associates of the polymer macromolecules of the solution, which behave as liquid drops in a steady motion or as solid particles in a regime of rapidly changing velocity.

Although the continuum viscoelastic properties of the solution are not very significant for turbulent drag-reducing near a smooth surface, they have considerable influence on the development of inertial turbulent vortices (which depend slightly on velocity). The effect is opposite in sign to that shown by the viscoelastic associates. Polymer additives have been shown[2] to affect Karman vortices in the flow behind a body. The Karman vortices for the first approximation can be classified as inertial vortices because they appear at a certain Reynolds number and later depend only slightly on its value. Variations of vortex regime as compared with the Newtonian case can be expected for flows both of elastic and inelastic solutions of polymers, but the variations will differ in sign.

In our experiments a device was used which represented a wide vessel at the bottom of which a short convergent nozzle with an outlet diameter of 1 cm was fixed. A calibrated wire was fixed in the outlet cross-section of the nozzle. A thermoanemometer transducer was mounted behind the wire at a distance of 10 diameters from the wire. A thermoanemometer with a hot wedge transducer coated with quartz, produced by DISA Electronic, was used. The transducer was fixed in such a way that the hot film was perpendicular to the wire, so that all vortices separated from the wire flowed past the film. The vortices covered different parts of the hot film length, depending on the diameter of the wire. In the experiments, flow velocity around the wire was measured both by the volume method and by making use of the thermoanemometer. The frequency of the succession of vortices was determined by comparing Lissajous figures from oscillations on the oscillograph screen, as registered by the thermoanemometer, and those with known frequency yielded by an audio-frequency oscillator. The method used made it possible also to carry out comparative measurements of amplitudes of velocity pulsations in the area behind the wire. (Absolute measurements of

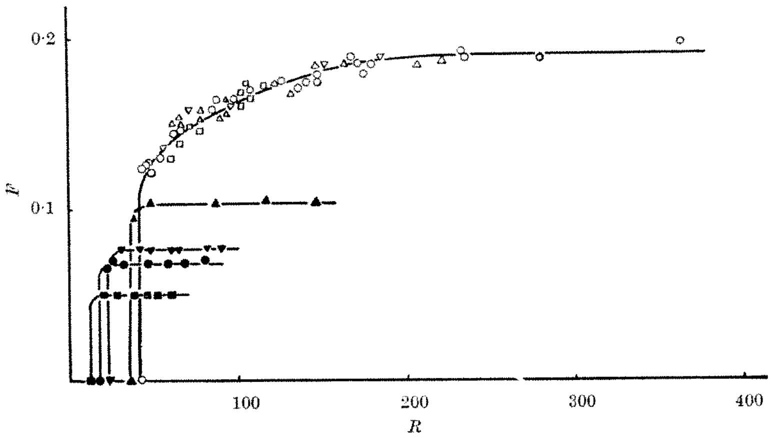


Fig. 1. Experimental results on the flow of water (open symbols) and polyethylene oxide solution (closed symbols) round wires of differing diameters: ●, 0·17 mm; ▲, 0·24 mm; ■, 0·31 mm; and ○, 0·57 mm. □■, 1; ○●, 2; ▽▼, 3; △▲, 4.

amplitudes were impossible owing to the considerable size of the transducer.)

The dimensionless frequency of the Karman vortex succession $F=fd/v$ is given in Fig. 1 as a function of the Reynolds number $R=vd/\nu$, where f is the frequency of the vortices, d is the wire diameter, v is the velocity of the liquid and ν is the kinematic viscosity coefficient. Experimental results shown by the open symbols were obtained from the flow of water around wires of various diameters. Data obtained for the flow of an aqueous solution of polyethylene oxide at a weight concentration of 10^{-4} are denoted by the closed symbols. The solution was kept for about four days after preparation; it preserved its continuum viscoelastic properties. It follows from the data given in Fig. 1 that the frequency of Karman vortices is lower for polyethylene oxide solutions than for the Newtonian liquid. The reduction in frequency is greater for smaller diameters of the wire. With decrease in wire diameter, the Reynolds number at which the first vortices appear also decreases. The vortex intensity in the flow of a viscoelastic solution of polyethylene oxide was found to be lower than that in the flow of water.

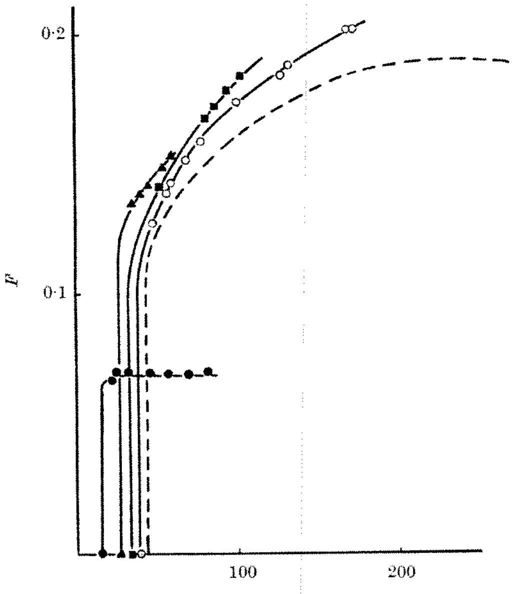


Fig. 2. The dimensionless frequency as a function of Reynolds number for the transition from elastic to inelastic polyethylene oxide solution (closed symbols) and for Guar gum (open symbols). ●, 1; ▲, 2; ■, 3; ○, 4.

In order to obtain an inelastic solution of polyethylene oxide containing associates, an elastic solution was passed through the centrifugal pump of a thermostat. Curves for the dimensionless frequency as a function of Reynolds number are given in Fig. 2, both for the transition of polyethylene oxide solution from elastic to inelastic associate-containing solution and for the subsequent breaking up of associates (closed symbols). The data were obtained for a solution of a concentration of 10^{-4} in the flow around a wire of 0·25 mm diameter. Curve 1 was obtained for an elastic solution, curves 2 and 3 for an inelastic one 30 and 60 min respectively after the associates were subject to the breaking up action of the pump vanes.

It follows from the data obtained that when a solution loses its viscoelastic properties, the frequency of the Karman vortex succession becomes at a certain moment somewhat higher than that in the Newtonian liquid (broken curve). A considerable increase in the amplitude of oscillations corresponding to the Karman vortices was observed, compared with the Newtonian case. With a further breaking up of associates the amplitude and frequency of oscillations decreased, tending to their values for water. An increase in the amplitude and frequency of oscillations was observed when the wire was in the flow of Guar gum solution. Data on the dimensionless frequency are given in Fig. 2 for Guar gum solution with a concentration of 5×10^{-4}, with a wire of 0·24 mm diameter (open symbols 4).

The data obtained are in good agreement with the theory that the intensity of inertial vortices increases in inelastic associate-containing solutions, while in elastic solutions it decreases. These data make the results obtained by Gadd[2] more precise.

We thank G. I. Barenblatt for helpful discussions and L. A. Rodionova for assistance in carrying out the experiments.

V. N. KALASHNIKOV
A. M. KUDIN

Institute of Mechanical Problems,
USSR Academy of Sciences,
Moscow.

Received April 24; revised September 5, 1969.

[1] Barenblatt, G. I., and Kalashnikov, V. N., *Izvestia Akad. Nauk SSSR, MJG*, **3** (1968).
[2] Gadd, G. E., *Nature*, **212**, 874 (1966).

Effect of Rotational Phase Transformations on Dry (Coulomb) Friction

ONE of the more peculiar of the solid-state crystalline phase transformations which occur in nature is that in which a highly ordered crystalline phase is exchanged for one exhibiting a large degree of rotational disorder. In such a transformation some weaker bonds are broken while stronger bonds remain. Parts of a molecule are then free to rotate about the fixed structure. For each of the two phases it is not uncommon to find considerably different values in interfacial tension at the crystal–melt interface. For carbon tetrachloride, one of the many substances exhibiting a rotational disordered phase, this interfacial tension decreases from 13·9 erg/cm² for the more ordered phase to 6·67 erg/cm² for the rotationally disordered phase[1].

In regard to Coulomb type (dry) friction, both the lowering of surface tension and/or the rotational freedom may be expected to produce dramatic changes. For example, rotational freedom on a molecular scale may be kinematically favourable to the sliding process. On the other hand, surface energy has long been thought to play an important part in friction and wear processes[2]. It is also notable that the experimental work to be described produces results consistent with these expectations.

The sliding system chosen for investigation was that of a hard steel hemisphere sliding on solid state CCl_4. CCl_4 was chosen from all those known to exhibit the previously described phase transformation for the following reasons. (i) The temperature (222·5 K) (ref. 3) at which it transforms is easily attainable in practice. (ii) The transformation temperature is sufficiently removed from its melting point (250·3 K) (ref. 3) to avoid any ambiguity in the experimental observations. (iii) Pure CCl_4 is easily obtainable.

Fig. 1 shows the simple experimental apparatus used. A dual walled (vacuum/liquid nitrogen) container houses the experimental elements. Initially an annular vessel (C) containing liquid CCl_4 is inverted on a glass plate and lowered into the container. The glass plate produces a smooth surface of CCl_4 on freezing. Introduction of liquid nitrogen in the container wall lowers the interior temperature to about 190 K. The glass plate is removed and the vessel of frozen CCl_4 is righted and fastened to the bottom end of a driveshaft as shown in the diagram. A steel hemisphere mounted on the end of a strain-gauged beam rests on the CCl_4 surface under a normal load of 110 g applied by a pulley-hung weight (W). The driveshaft is given several rotations clockwise and counterclockwise, to ensure a uniform grooved depression (nearly invisible to the naked eye). The apparatus is kept moisture free by a flow of dried argon. The experiment starts when the flow of liquid nitrogen is halted, which occurs when the interior temperature is about 180 K. As the interior temperature drifts slowly back towards room temperature a thermocouple, frozen into the CCl_4 surface, gives readings of the temperature. Friction forces generated by the constant to and fro motion of the driveshaft, which has an average sliding velocity of 4·3 cm s^{-1}, are recorded from the strain gauges. When the phase transformation temperature (222·5 K) is attained a timewise sequence of relatively constant temperatures is noted as the change of phase proceeds. The results of interest are shown in Fig. 2 as a plot of normalized friction force against CCl_4 surface temperature. The magnitude of the maximum friction force corresponding to an ordinate value of 1·0 on this graph is 9·4 g, giving a maximum coefficient of friction of 0·085. The main feature of interest is that exactly at the transition temperature the forces of friction fall to less than half their previous values. (No surface changes visible to the naked eye occur until melting.) Of course, a second drop in friction occurs as the melting temperature is attained.

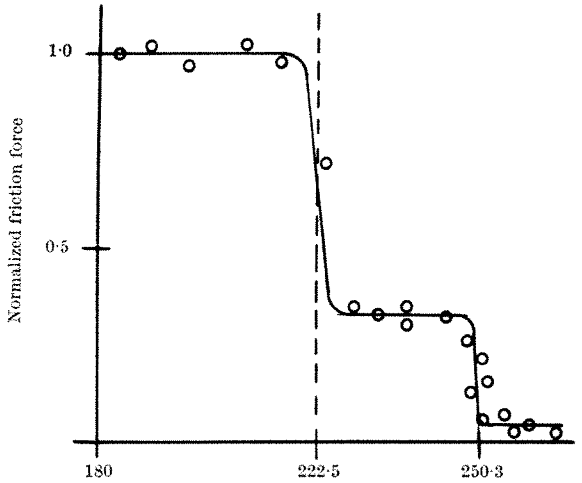


Fig. 2. Experimental results showing a decrease in friction at the transition temperature.

It may also be noted that these observations are reversible, that is, if at, say, 230 K the flow of liquid nitrogen is reinstated, then at 222·5 K, the phase transformation temperature, friction values will abruptly rise.

Further work is planned using a "plastic" crystal, carbon tetrabromide, in place of carbon tetrachloride. The interesting features of CBr_4 are (i) its surface tension values of 10·7 erg/cm^2 and 21·8 erg/cm^2 (ref. 1) for the disordered and ordered phases respectively, and (ii) the fact that its ability to rotate is hindered. This may make it possible to discern which property contributes to the lowering of friction, that is, the onset of rotational disorder or the lowering surface tension.

THOMAS E. SIMKINS

Experimental Mechanics and
Thermodynamics Laboratory,
US Army,
Watervliet, New York 12189.

Received October 29; revised December 8, 1969.

[1] Dunning, W. J., *Structure of Surfaces, Physics and Chemistry of the Organic Solid State*, *1* (edit. by Fox, D.), 432, 581 (Interscience Pub., 1963).

[2] Rabinowicz, E., *Compatibility Criteria for Sliding Metals, Friction and Lubrication on Metal Processing*, 90 (ASME, 1966).

[3] Ubbelohde, A. R., *Melting and Crystal Structure*, 73 (Clarendon Press, 1965).

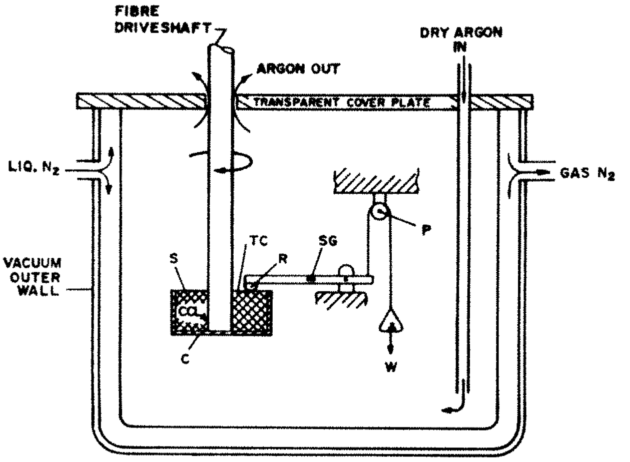


Fig. 1. Experimental apparatus. C, Metal container; P, pulley; R, hemispherical steel rider; S, smooth solid CCl_4 surface; SG, one of four strain gauges; TC, thermocouple; W, weight providing normal load.

Friction Reduction Characteristics of Fibrous and Colloidal Substances

THE phenomenon of friction reduction produced in smooth pipes by fibre suspensions in turbulent flow has been observed by several investigators over the past three decades, particularly by those examining the flow characteristics of wood-pulp fibre suspensions[1]. During a recent study of the friction-reducing properties of long-chain, water-soluble polymer molecules, an investigation was made to determine whether certain water-insoluble fibrous materials, or colloidal materials other than wood-pulp fibres, would also produce a friction-reduction effect. The measurements were made by means of a rheometer with a steel capillary tube 0·046 inches in diameter. Transducers mounted 5 inches apart along the capillary tube were used to measure the pressure drop. The sample was forced through the capillary by means of a constant-speed,

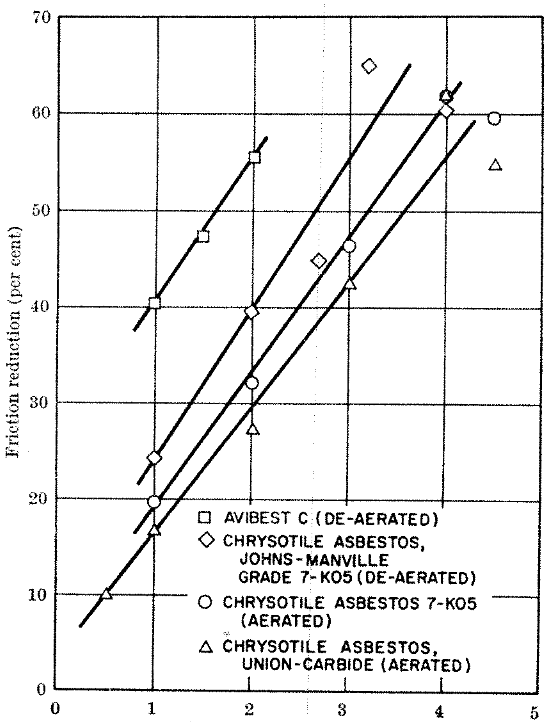


Fig. 1. Friction reduction of asbestos materials.

motor-driven plunger at a rate of approximately 13 cm³ per second. Friction reduction (FR) was computed as follows

$$\text{per cent FR} = 100\left(1 - \frac{\Delta P_2}{\Delta P_1}\right)$$

where ΔP_1 = pressure drop using water or other reference liquid alone, ΔP_2 = pressure drop using sample dispersion.

The measurements were made at room temperature (24° C). The dispersions were prepared by agitating the sample and the reference liquid for 5 min in a high-shear type blender at 21,000 r.p.m.

The first fibrous material selected for study, the natural inorganic polymer 'Chrysotile' asbestos (Johns-Manville grade 7*K*05), produced noteworthy results. A maximum friction reduction of 65 per cent was achieved at an asbestos concentration of 3·2 weight per cent. The *p*H of the dispersion was adjusted to between 4 and 4·5 with acetic acid before agitation in the blender. Deaerated dispersions proved to be more effective than aerated ones.

A recently developed microcrystalline product derived from 'Chrysotile' asbestos, 'Avibest C' (FMC Corporation), was also tested. This material has a rod-like particle shape, with a maximum particle dimension of 0·5 micron. It was surmised that 'Avibest C' would be more effective on a weight basis than 'Chrysotile' asbestos in reducing friction because of its smaller particle size. The results confirmed this belief (Fig. 1).

Other novel microcrystalline colloidal materials manufactured by the FMC Corporation were subsequently evaluated, and the results are shown in Fig. 2. 'Avitene H', an acid salt derived from a bovine hide collagen, produced a maximum friction reduction of 51·7 per cent at a concentration of 0·125 per cent. Its particle shape appears to be spheroidal[2]. 'Avicel CM' (later renamed 'Aviloid'), a cellulose product with a fibre-like particle shape, produced a maximum friction reduction of 47 per cent at a concentration of 1·5 per cent. The same concentration of 'Avamide-6', a microcrystalline nylon, did not reduce friction.

The materials that produced friction reduction varied in their properties according to production lot and period of ageing. For example, a new sample of 'Avicel CM' that produced a friction reduction of 47 per cent at a concentration of 1·5 per cent yielded only a 31 per cent reduction after 1·5 yr of ageing. 'Avibest C' gave more consistent results, even after ageing for the same period. The increasing stiffness of the gels formed by 'Avibest' and 'Avicel' dispersions at concentrations greater than 2 per cent made them too difficult to test in the narrow-capillary rheometer described here.

A variety of other water-insoluble materials that have either fibre-like or colloidal-size particles (or, in some cases, both) were evaluated for friction-reduction capability. The results are presented in Table 1.

Table 1. FRICTION CHARACTERISTICS OF FIBROUS AND COLLOIDAL DISPERSIONS IN TURBULENT FLOW (IN WATER, EXCEPT 'AVIBEST C')

Substance	Concentration (weight per cent)	Friction change Reduction (per cent)	Friction change Increase (per cent)	Maximum particle dimension (μm)
'Baymal' colloidal alumina (DuPont)	1·0		1·4	0·1
	3·0		4·6	
'Attagel 20'	2·0		4·0	0·14
Attapulgus clay	2·5		5·4	(average equivalent spherical diameter)
'Benaqua', clay product (National Lead Co.)	0·1	0·2		0·1
	1·0	10·2		
	1·5	13·4		
'Aquablak K' colloidal carbon	0·3		1·1	
	1·0		2·4	
Pigmentary potassium titanate	3·0	0·0		10·0
'Avibest C' in Dow-Corning 200 silicone fluid (1 cstk)	1·0	12·1		0·5
'Avibest C' in tetrahydrofurfuryl alcohol	0·5		5·7	0·5
	1·5		24·4	

Particles of colloidal alumina, although similar in size and shape to those of the microcrystalline cellulose developed by FMC[3], produced an increase of friction. The increase was greater at *p*H 8 than at *p*H 4. 'Attagel 20' and pigmentary potassium titanate, which have needle-like particles, failed to produce friction reduction. Benaqua, a magnesium silicate clay with a platelet particle form, produced a 13·4 per cent friction reduction at a concentration of 1·5 per cent. Above 1·5 per cent, the gel was too thick for testing in the rheometer. 'Avibest C', in Dow-Corning 200 silicone fluid, produced a 12 per cent friction reduction. A considerable increase in friction was obtained, however, with a dispersion of 'Avibest C' in tetrahydrofurfuryl alcohol.

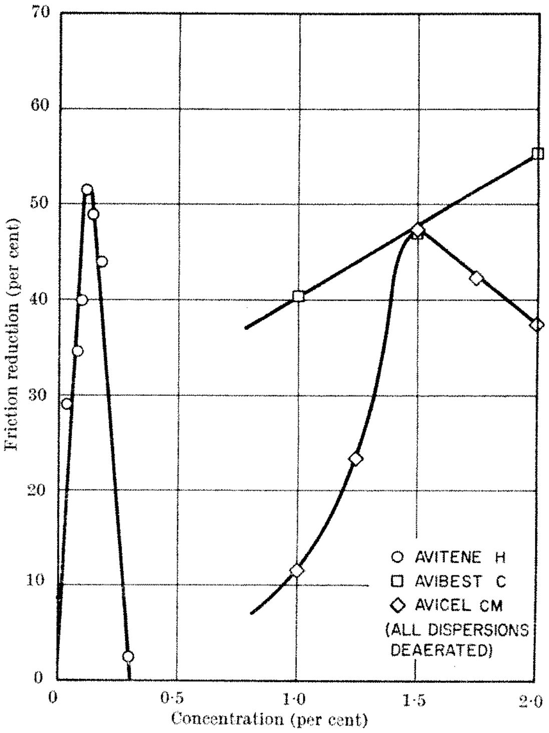


Fig. 2. Friction reduction of microcrystalline substances.

No data are available to explain the ability of some fibre-like substances to reduce friction, while others of a similar particle size and shape are unable to do so. It can only be hypothesized that the explanation lies in the types of chemical groups that exist on the surfaces of the particles, or in variations in particle flexibility. The vastly different results obtained with 'Avibest C' dispersions in different liquids are likewise not understood. A clearer, although not necessarily complete, understanding will be obtained when tests are performed with a number of other organic liquids.

A. B. ARRANAGA

Ocean Technology Department,
Naval Undersea Research and Development Center,
Pasadena, California.

Received October 14, 1969.

[1] Daily, J. W., and Bugliarello, G., *TAPPI*, **44**, 7 (1961).
[2] Battista, O. A., *Chemist*, **44**, 6 (1967).
[3] Battista, O. A., *J. Polymer Sci.*, Part C, 9 (1965).

BIOLOGICAL SCIENCES

ATPase Activity of the Sodium Pump needs Phosphatidylserine

IT has frequently been suggested that the chemical properties of phospholipids and their prevalence in cell membranes make them particularly suitable compounds to serve as carrier molecules for the transport of cations. Some support for this suggestion has been provided by studies of the cation binding properties of phospholipids[1,2] and of various model systems involving phospholipids[3-5]. Taken together, these investigations indicated that some phospholipids might be especially concerned in the active transport of Na^+ and K^+, but the evidence is not conclusive. The concept of such a carrier for ion transport antedates the work showing the involvement of ATP and its enzymatic hydrolysis in the Na^+ pump; and during the past decade there has been a shift of interest and emphasis towards the role of the membrane ATPase system in active cation transport[6]. During this period, however, evidence has accumulated that the Na^+, K^+-activated ATPase is eliminated when lipids are removed from membranes by solvent extraction, treatment with detergents, or exposure to phospholipases[7-9]. The question therefore arises whether phospholipid might provide a carrier system in which the protein of the ATPase is also involved, such that the ATPase activity depends on lipid as well as protein.

There have been several reports of the activation of ATPases by addition of lipid after the enzyme preparations had been treated with deoxycholate or phospholipase, but there is no agreement on the nature of the lipid and whether the ATPase activity is that of the Na^+ pump. For example, although activation by lecithin has been reported by some workers[10,11], others have failed to confirm this action[7,12], or have obtained equivocal results[13]. In one report, phosphatidylserine was shown to activate but not lecithin[12]. Again, activation by a number of different lipids has been claimed but not always confirmed[10,12,14-17]. In view of the previous chemical work implicating phospholipids as potential carrier substances, we have investigated the phospholipid requirement for the ATPase of the Na^+ pump.

Solubilization of a membrane-containing fraction (microsomal) from ox brain cortex with deoxycholate[11] removed most or all of the ouabain inhibited ATPase activity. *p*-Nitrophenylphosphatase activity, which is associated with the ATPase, was also reduced in the same way. Both these enzymatic activities were restored after adding sonicated dispersions of crude commercial samples of phosphatidic acid, phosphatidylinositol or phosphatidylserine but not phosphatidylethanolamine or phosphatidylcholine (lecithin). The commercial samples were impure, however, and, after they had been partially purified by thin-layer chromatography, definite activation was demonstrated only with that component of the crude samples which migrated like phosphatidylserine.

The action of this lipid has been investigated in detail. With a typical enzyme preparation, ouabain sensitive ATPase activity (in µmoles P_i liberated per g protein per min) was raised ten-fold, from 13 to 127. This difference was found only when the incubation medium contained both Na^+ and K^+, because in their absence phosphatidylserine did not stimulate. This shows that the activity dependent on phosphatidylserine was that part of the ATPase requiring Na^+ and K^+, and which is completely inhibited by ouabain. Another inhibitor which largely opposes the action of Na^+ and K^+ is oligomycin and this compound decreased the ATPase activity dependent on phosphatidylserine from 158 to 68. *p*-Nitrophenylphosphatase activity (in µmoles *p*-nitrophenol liberated per g protein per min) was also stimulated by phosphatidylserine. Sensitivity to ouabain was only 4 without phosphatidylserine but 21 after adding the lipid. By contrast with ATP hydrolysis, this activity was little affected by oligomycin. The enzymatic activity dependent on phosphatidylserine therefore exhibited properties characteristic of the ATPase of the Na^+ pump. It seems that this activity can be markedly reduced by treatment with a detergent.

A test was made to see if ouabain-sensitive ATPase activity was raised by phosphatidylserine in the ordinary enzyme preparation before it had been treated with deoxycholate. An increase from 77 to 170 was found with this lipid, thus raising the proportion of ouabain sensitive activity from 38 to 55 per cent. This result suggests that phospholipid may be lost during preparation of the membrane fraction.

The results have a bearing on two aspects of membrane phenomena. First, there is the requirement for phosphatidylserine for optimum activity of an enzyme which is membrane bound. The question of whether ouabain sensitive ATPase and *p*-nitrophenylphosphatase activity both reside in the same catalytic unit is not at present resolved[13,15,17], but it is nevertheless clear from our results that both activities depend on phosphatidylserine. Very few enzymes have been shown to require phospholipid for their activity and, indeed, characterization of enzymes usually involves purification to a greater or lesser extent, and ideally a crystalline protein is examined. The activation of ATPase and *p*-nitrophenylphosphatase by phosphatidylserine, and by none of the other four phospholipids which we have examined, suggests a requirement for a specific phospholipid. From the point of view of enzyme action, therefore, it seems that those properties which make the membrane bound ATPase of great interest physiologically are dependent not just on protein but on protein and lipid together. Characterization of the Na^+-activated ATPase as a chemical entity therefore presents a much more formidable problem than characterization in the classical sense, in which an enzyme consists solely of protein. The other aspect of our results relates to the functional consequence of ATPase activity which is the active transport of Na^+ and K^+ across the cell membrane. It seems likely that the carrier substance for the transport of these ions is a complex unit of phosphatidylserine and protein. The inhibition of transport by ouabain may arise from interference with the interaction between protein and phosphatidylserine. Other obvious implications of our results concern the micro-architecture of the cell membrane in which phosphatidylserine perhaps maintains the appropriate orientation of the protein molecule so that catalysis of ATP is associated with the transport of ions.

We thank the Medical and Science Research Councils for financial support.

K. P. Wheeler
R. Whittam

Department of Physiology,
University of Leicester.

Received September 25, 1969.

[1] Solomon, A. K., Lionetti, F., and Curran, P. F., *Nature*, **178**, 582 (1956).
[2] Kirschner, L. B., *J. Gen. Physiol.*, **42**, 231 (1958).
[3] Hoffman, J. F., Schulman, J. H., and Eden, M., *Fed. Proc.*, **18**, 70 (1959).
[4] Tobias, J. M., Agin, D. P., and Pawlowski, R., *J. Gen. Physiol.*, **45**, 989 (1962).
[5] Papahadjopoulos, D., and Bangham, A. D., *Biochim. Biophys. Acta*, **126**, 185 (1966).
[6] Glynn, I. M., *Brit. Med. Bull.*, **24**, 165 (1968).
[7] Askari, A., and Frantantoni, J. C., *Biochim. Biophys. Acta*, **92**, 132 (1964).
[8] Emmelot, P., and Bos, C. J., *Biochim. Biophys. Acta*, **120**, 369 (1966).
[9] Schatzmann, H. J., *Nature*, **196**, 677 (1962).
[10] Tanaka, R., and Abood, L. G., *Arch. Biochem. Biophys.*, **108**, 47 (1964).
[11] Tanaka, R., and Strickland, K. P., *Arch. Biochem. Biophys.*, **111**, 583 (1965).
[12] Fenster, L. J., and Copenhaver, J. H., *Biochim. Biophys. Acta*, **137**, 406 (1967).
[13] Emmelot, P., and Bos, C. J., *Biochim. Biophys. Acta*, **150**, 341 (1968).
[14] Ohnishi, T., and Kawamura, H., *J. Biochem. Tokyo*, **56**, 377 (1964).
[15] Formby, B., and Clausen, J., *Z. Physiol. Chem.*, **349**, 909 (1968).
[16] Tanaka, R., and Mitsumata, T., *J. Neurochem.*, **16**, 1163 (1969).
[17] Tanaka, R., *J. Neurochem.*, **16**, 1301 (1969).

Active Ionic Transport across Biological Membranes: Possible Role of Electrons and Protons

THE conversion of metabolic energy into useful work is a very efficient process in biological cells. The energy in ATP is transformed into mechanical, electrical and osmotic work at efficiencies which have been calculated to approach 100 per cent for acid secretion in the stomach[1], active Na^+ transport in frog skin[2] and sartorius muscle contraction[3]. These observations show that energy is converted in biological cells by a process which is not limited by a Carnot efficiency factor (about 2 per cent in warm blooded animals). The process could involve electrochemical "cold" combustion, in which chemical energy is transformed into electronic potential energy, in much the same way as the operation of a fuel cell[4]. Accordingly, Mitchell[5,6] likened the oxido/reduction and phosphorylation processes in his chemiosmotic hypothesis to a protonic fuel cell in which ATP synthesis was construed as a vectorial reaction driven by a "proton-motive" force across the mitochondrial membrane.

There is evidence[7] for such an electrochemical mechanism of energy conversion from metabolic energy to osmotic work (active transport) across certain biological membranes. (The mechanism can also be called electrodic: Bockris and Conway[8] define "electrodics" as "the study of processes which occur at the surface of an electronic conductor in contact with a liquid".) The evidence comes from the unique character of the non-linearity in the voltage-current characteristics displayed by live secretory tissues, in contrast to the linear relationship of dead tissue. When these characteristics are plotted as voltage against the logarithm of the current (Fig. 1), they display straight line relationships at displacements from resting potential larger than 40–50 mV. Using the theory of rate processes[9], the relationship at high voltage can be expressed as

$$i = i_0 \exp\left[\frac{(1-\beta)\ V^* F}{RT}\right]$$

where i_0 is the exchange current density (a constant), F is the Faraday, R is the gas constant, T is the absolute temperature, V^* is the displacement from resting potential and β is the symmetry factor. β has been calculated, from the magnitude of the slopes of a variety of tissues, to be close to 0·5, as shown in Table 1. The range of β values encountered experimentally is 0·35 to 0·70, representing the normal type of asymmetry accompanying uniquely determinable processes (from the β values) such as the present one, which is characterized by a single, almost symmetrical, rate-determining energy barrier[9–12]. The most likely process showing the voltage-current characteristics of Fig. 1 is an electrodic one in which the rate-determining step would be the passage of electrons between one of the solutions and an electron conductor in the membrane[13]. Electronic rate-determining steps of this type have been studied extensively at metal-solution[14] and semiconductor-solution[15] interfaces. The latter case is probably representative of the conditions in biological cells[16].

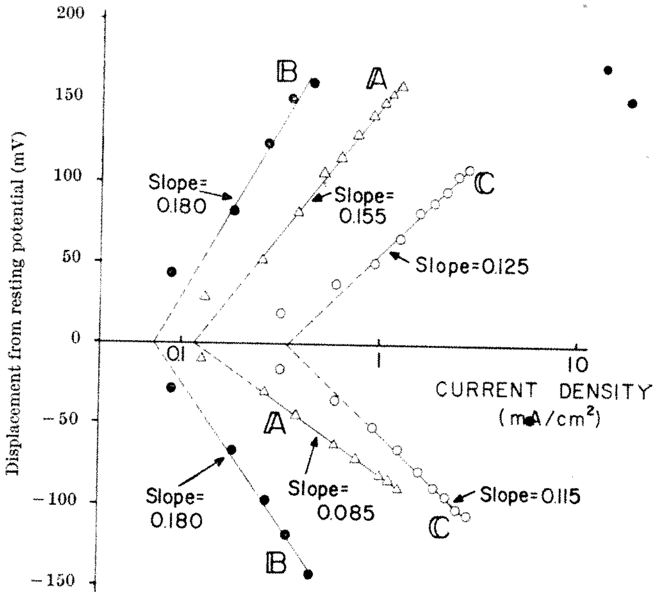


Fig. 1. Displacement from resting potential versus logarithm of current density for (*A*) frog gastric mucosa, (*B*) frog skin and (*C*) perch swimbladder.

Table 1. VALUES OF β CALCULATED FROM DATA ON SECRETORY TISSUES AND NERVE CELLS

Animal species and membrane type	Exponential slope in mV	Calculated value of β	Source of data
Frog gastric mucosa	125	0·52	(8)
Frog skin	180	0·67	(8)
Perch swimbladder	115	0·48	(8)
Rabbit oviduct	145	0·49	(25)
Loligo axon	110	0·45	(26)
Sepia axon	90	0·33	(27)
Lobster axon	155	0·61	(28)
Frog node	105	0·43	(29)

The electrons crossing one of the membrane-solution interfaces would take part in a redox reaction at the interface, in which a hydrogen atom is desorbed from the membrane to form a hydronium ion in the solution for each electron transferred to the electronic conductor in the membrane. The opposite would be the case for an electron which transferred from the electron conductor to one of the solutions. The complete model for electrodic current passage across the membrane is shown in Fig. 2, where it can be seen that for every electron which crosses the membrane in one direction, a proton traverses the full span from solution to solution.

This model has been derived strictly from the voltage-current characteristic of secretory tissue, yet it bears a very close resemblance to the protonic fuel cell described by Mitchell[5,6]. Because one of the properties of this electronic and protonic pathway is its dependence on intact metabolism[7], and because ATPases seem to be present in these tissues[17,18], specific models of secretory

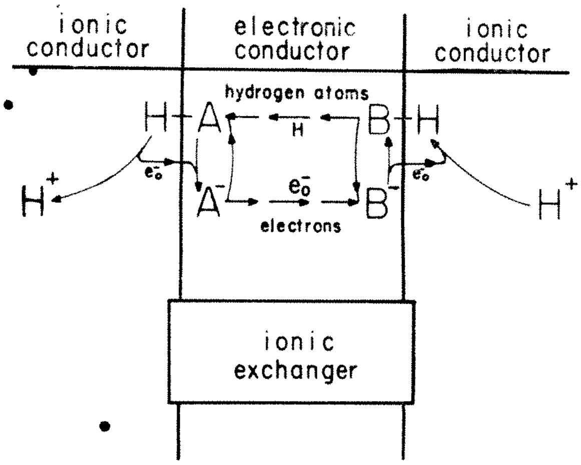


Fig. 2. Model for electrodic current passage through a membrane.

cells can be proposed on the assumption that the pathway actually is a reversible chemiosmotic-like ATPase, located at the secretory membrane and with the electrodic current flowing through it. ATP hydrolysis in such a vectorially oriented process could constitute a case of heterogeneous catalysis[14] involving redox reactions with electron transfers, which would result in the net transfer of H^+ ions across the membrane[5,6]. The local *p*H gradient created in this manner could cause active transfer of ions in a specialized ion-exchange structure with *p*H-dependent selectivity.

In the gastric mucosa, this ion-exchange structure could allow selectively for Cl^- ion movement by ion pair formation with the H^+ ions transported in the ATPase, resulting in the observed acid secretion[19].

In frog skin, this structure would favour Na^+ ions at the high *p*H side and K^+ and H^+ ions at the low *p*H side[20]. The Na^+ ions inside the cell would adsorb on the exchanger, traverse the membrane to the low *p*H side where the opposite selectivity exists, and exchange for K^+ or H^+ ions which would travel to the other side to maintain electroneutrality. The local *p*H gradient produced by the ATPase would thus provide the driving force for active Na^+ transport. Active transport of other ions and varying respiratory quotients could be modelled from the basic chemiosmotic ATPases, by assuming differentiated ion-exchange structures which result in the specialized active transport properties of various tissues. The resulting electrodic models of active ionic transport are found not only to be consistent with a variety of experimental observations, but also to provide novel interpretations for many observed phenomena such as the origin of the tissue potential difference, the nature of exchange diffusion, the actions of various drugs and many other properties[21].

Electrodic processes may be present in aspects of cellular function other than those related to the direct conversion of metabolic energy into useful work. One such example may be nerve excitation, for the steady state voltage-current characteristics of nerve (K^+ current as function of clamping voltage) are very similar in the depolarizing direction to those of secretory cells. After the series resistance inherent in the uncompensated feedback type of measurement[22] is subtracted from the original data, the voltage-current characteristics of nerves from a variety of species are described by equation 1 at V^* larger than 40–50 mV. The values of β obtained from these curves (Table 1) fall within the same range as those from secretory tissues, hinting at the possibility that electrodic processes may occur in nerve as well.

An electrodic model of nerve excitation can be proposed, based on a mechanism similar to that discussed for secretory tissue, but not metabolically dependent. The quantitative dependence of the K^+ current on applied potential[23] is modelled by a two step process which involves the primary transport of H^+ ions (electrodic current) and the secondary exchange of the H^+ ions for K^+ ions, resulting in the observed K^+ current. No specific assumptions need be made about the process of Na^+ activation in this model; the inactivation of Na^+, however, is assumed to occur through the lowering of the local external *p*H by protonic transport across the membrane. The excess H^+ ions could react with the negative membrane sites to prevent the passage of the Na^+ current as was suggested by Hille[24] for frog node of Ranvier. The reaction of H^+ ions with the membrane sites would prevent the H^+ ions from being immediately exchanged for K^+ ions from the nerve cytoplasm, and thus produce the observed delay in the rise of K^+ current to its steady-state value. This model qualitatively accounts for a variety of observations made on nerve, including the results of voltage-clamp experiments, effects of varying ionic concentrations on the bathing solutions and the action of various drugs on nerve excitation[21].

I thank Drs R. E. Davies and J. O'M. Bockris for their advice and encouragement. Support came from the US Public Health Service.

LAZARO J. MANDEL

Department of Biomedical Electronic Engineering,
Moore School,
University of Pennsylvania,
Philadelphia, Pennsylvania.

Received July 21; revised September 18, 1969.

[1] Crane, E. E., and Davies, R. E., *Biochem. J.*, **49**, 169 (1951).
[2] Ussing, H. H., *Protoplasma*, **63**, 292 (1967).
[3] Kushmerick, M. J., and Davies, R. E., *Proc. Roy. Soc.*, B, **174**, 315 (1969).
[4] Bockris, J. O'M., and Srinivasan, S., *Nature*, **215**, 197 (1967).
[5] Mitchell, P., *Nature*, **191**, 144 (1961).
[6] Mitchell, P., *Fed. Proc.*, **26**, 1370 (1967).
[7] Crane, E. E., and Davies, R. E., *Trans. Farad. Soc.*, **46**, 598 (1950).
[8] Bockris, J. O'M., and Conway, B. E., *Rec. Chem. Prog.*, **25**, 31 (1964).
[9] Glasstone, S., Laidler, K. J., and Eyring, H., *The Theory of Rate Processes* (McGraw-Hill, 1941).
[10] Zwolinsky, B. J., Eyring, H., and Reese, C. E., *J. Phys. Coll. Chem.*, **53**, 1426 (1949).
[11] Johnson, F. H., Eyring, H., and Polissar, M. J., *The Kinetic Basis of Molecular Biology* (Wiley, 1954).
[12] Parlin, R. B., and Eyring, H., in *Ion Transport Across Membranes* (edit. by Clarke, H. T.) (Academic Press, 1954).
[13] Bockris, J. O'M., and Reddy, A. K., *Modern Electrochemistry: An Interdisciplinary Study*, chap. 8 (Plenum Press, 1969).
[14] Srinivasan, S., Wroblowa, H., and Bockris, J. O'M., *Adv. Catal.*, **17**, 352 (1967).
[15] Myamlin, M., and Pleskov, Y., *Electrochemistry of Semiconductors* (Plenum Press, 1967).
[16] Gutmann, F., and Lyons, L. E., *Organic Semiconductors* (Wiley, 1967).
[17] Skou, J. C., *Physiol. Rev.*, **45**, 596 (1965).
[18] Kasbekar, D. K., and Durbin, R. P., *Biochim. Biophys. Acta*, **105**, 472 (1965).
[19] Davies, R. E., *Biol. Rev.*, **26**, 87 (1951).
[20] Moore, J. H., and Shechter, R. S., *Nature*, **222**, 476 (1969).
[21] Mandel, L. J., thesis Univ. of Pennsylvania (1969).
[22] Taylor, R. E., Moore, J. W., and Cole, K. S., *Biophys. J.*, **1**, 161 (1960).
[23] Cole, K. S., *Membranes, Ions, and Impulses* (University of California Press, 1968).
[24] Hille, B., *J. Gen. Physiol.*, **51**, 221 (1968).
[25] Brunton, W., thesis, Univ. Pennsylvania (1969).
[26] Hodgkin, A. L., and Huxley, A. F., *J. Physiol.*, **116**, 449 (1952).
[27] Cole, K. S., and Moore, J. W., *J. Gen. Physiol.*, **44**, 137 (1960).
[28] Julian, F. J., Moore, J. W., and Goldman, D. E., *J. Gen. Physiol.*, **45**, 1232 (1962).
[29] Dodge, F. A., and Frankenheuser, B., *J. Physiol.*, **143**, 83 (1958).

Discreteness of Conductance Change in Bimolecular Lipid Membranes in the Presence of Certain Antibiotics

NET ion movements across biological or synthetic lipid membranes may take place by various mechanisms, underlying all of which there is a rather ill-defined and small ion leakage or background conductance. Most ions

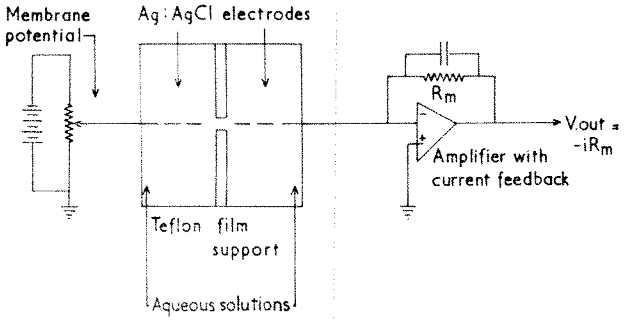


Fig. 1. Schematic diagram of the apparatus used to measure conductance.

permeate by means of pathways involving either permanent or transient modifications of the basic structure of the membrane. If permanent pathways are involved, a given membrane conductance may be accounted for by routes which are either numerous and of low conductance or few and of high conductance. For transient pathways, duration must also be considered. Thus, if a carrier is invoked, the duration will be the time the carrier, complexed with an ion, spends shuttling across the membrane. For a pore, the duration is the time for which it remains open to ions. At present little is known concerning the number, conductance and duration of the ionic pathways in any membrane of the types mentioned. Limited information is available for the nerve membrane, although this is rather imprecise and indirect[1].

The artificial bimolecular lipid film is an almost ideal structure in which to study single conductance pathways. There are many substances which render these model membranes highly permeable to ions although we know of only one publication giving evidence of discrete conductance pathways[2]. This was for a poorly characterized proteinaceous material[3]; the magnitude of the unit conductance change was about $10^{-10}\ \Omega^{-1}$. The conductance of a carefully prepared bimolecular lipid film is so low that in certain circumstances it should be possible to detect the conduction corresponding to a single molecule of a permeability-inducing substance even if this is considerably less than $10^{-10}\ \Omega^{-1}$. In this study the antibiotics nonactin, nystatin and gramicidin A were examined from this standpoint.

Films were formed from solutions of lecithin or glycerol mono-oleate in *n*-decane, in a hole on a Fluon support, by the injection manometer method[4]. For systems containing nystatin, however, cholesterol was also added. Conductance was measured by applying a known potential (normally 100 mV) to one side of the film and recording the current necessary to maintain the other side at zero (Fig. 1). Much care was required to isolate the apparatus adequately from mechanical and electrical noise. The time resolution of conductance fluctuations depended on the magnitude of the fluctuation. Transient increases in conductance of $5 \times 10^{-13}\ \Omega^{-1}$ could be observed, provided they lasted for about 100 ms. The corresponding resolution at higher conductances was 10 ms at $2 \times 10^{-11}\ \Omega^{-1}$ and 1 ms at $10^{-10}\ \Omega^{-1}$.

In the absence of antibiotic, the conductance of the black films was constant within the resolution of the apparatus. When antibiotic molecules were present the conductance increased, as has previously been noted[5-7,10]. At very low levels of conductance the manner of the increase depended markedly on the nature of the antibiotic. With nonactin the conductance increased smoothly on the formation of a black film. No step changes were observed down to a level of $5 \times 10^{-13}\ \Omega^{-1}$, but because of the resolution of the apparatus any step this small which was reversed in less than about 100 ms would not have been seen. For nystatin (reproducible) fluctuations in the conductance were also absent, the largest possible step being $2 \times 10^{-13}\ \Omega^{-1}$. Again no transient decreases in conductance were observed.

By contrast, when the films contained gramicidin A in low concentrations the conductance fluctuated with time in a well defined step-like fashion (Fig. 2). At high concentrations the steps overlap to give a high conductance in which the fluctuations can no longer be resolved. The step heights at first glance appear uniform, but in fact have a distribution of sizes which, in 1·0 M NaCl, has a peak at approximately $2{\cdot}4 \times 10^{-11}\ \Omega^{-1}$. The effects of electrolyte concentration and applied potential on the step height which corresponds to the distribution peak are shown in Figs. 3 and 4, respectively. The frequency of occurrence of steps increases with applied potential.

Nonactin forms 1 : 1 complexes with univalent ions[8], and it has been suggested that the macrocyclic molecule carries ions across the membrane[9]. The present findings neither contradict nor support this mechanism. The absence of steps for nystatin is interesting, for it has been inferred that the nystatin aggregates in the film to form pores of approximately 8 Å in diameter[11]. If such pores are considered to be right cylinders filled with electrolyte solution, it is very unlikely that they would have conductances of less than $2 \times 10^{-13}\ \Omega^{-1}$. The fact that conductance changes greater than this were not detected indicates that if pores exist they remain open for less than 100 ms.

Gramicidin A is nearly twice the size of either nonactin or nystatin, a fact which may help to explain the pronounced unit conductance changes it produces. In saturated NaCl and for 100 mV applied potential the net transfer of charge per conducting channel corresponds to 2×10^7 ions/s. This suggests that the effective ionic mobility across the membrane is little less than for the inorganic ion in water, and consequently a pore, rather than a carrier mechanism, is indicated. The commencement and termination of a step might correspond to the arrival in, and departure from, the film of a gramicidin molecule. There is, however, evidence against this mechanism. Another possible explanation arises from the suggestion that dimers of gramicidin may be the conducting unit in the black film[6]: commencement and termination of a step could correspond to the formation and dis-

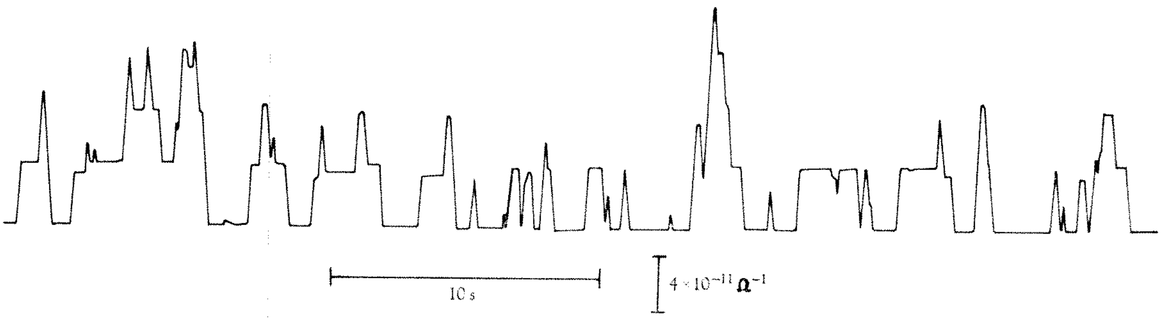


Fig. 2. A sequence of step changes in conductance for membranes formed from glycerol mono-oleate in *n*-decane in presence of gramicidin A. The aqueous phase was 1 M KCl.

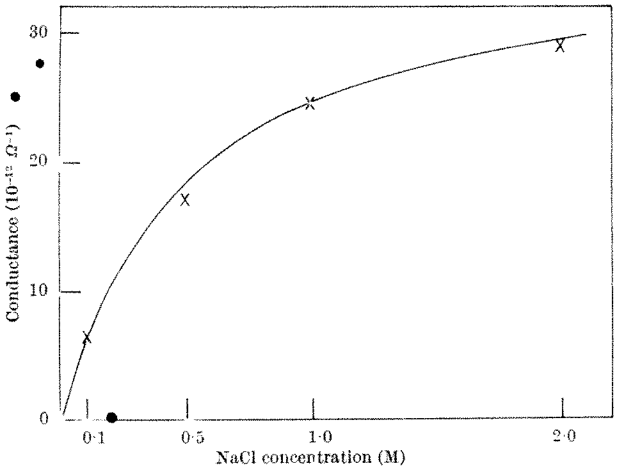


Fig. 3. The unit step of conductance as a function of NaCl concentration. The membranes were as for Fig. 2.

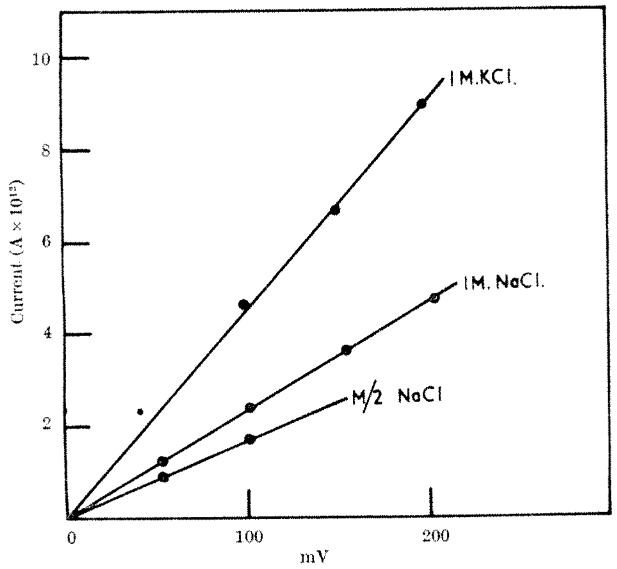


Fig. 4. Current–voltage characteristics for the unit step change in conductance. The membranes were as for Figs. 2 and 3.

sociation of a dimer. These and related questions will be discussed in a more detailed report.

The nystatin and nonactin were kindly given to us by Dr H. van Zutphen and Dr K. Heckmann, respectively, and we thank Dr E. Gross for a sample of pure valine gramicidin A. S. B. H. is supported by a US National Science Foundation graduate fellowship.

S. B. HLADKY
D. A. HAYDON

Laboratory of Biophysical Chemistry and Colloid Science,
University of Cambridge,
Free School Lane, Cambridge.

Received August 27; revised October 29, 1969.

[1] Hille, B., *J. Gen. Physiol.*, **51**, 199 (1968).
[2] Bean, R. C., Shepherd, W. C., Chan, H., and Eichner, J., *J. Gen. Physiol.*, **53**, 741 (1969).
[3] Kushnir, L. D., *Biochim. Biophys. Acta*, **150**, 285 (1968).
[4] Duyvis, E. M., dissertation Univ. Utrecht, (1962).
[5] van Zutphen, H., van Deenen, L. L. M., and Kinsky, S. C., *Biochem. Biophys. Res. Commun.*, **22**, 393 (1966).
[6] Tosteson, D. C., Andreoli, T. E., Tieffenberg, M., and Cook, P., *J. Gen. Physiol.*, **51**, 373s (1968).
[7] Mueller, P., and Rudin, D. O., *Biochem. Biophys. Res. Commun.*, **26**, 398 (1967).
[8] Kilbourn, B. T., Dunitz, J. D., Pioda, L. A. R., and Simon, W., *J. Mol. Biol.*, **30**, 559 (1967).
[9] Eisenman, G., Ciani, S. M., and Szabo, G., *Fed. Proc.*, **27**, 1289 (1968).
[10] Andreoli, T. E., and Monahan, M., *J. Gen. Physiol.*, **52**, 300 (1968).
[11] Finkelstein, A., and Cass, A., *J. Gen. Physiol.*, **52**, 145s (1968).

Genetic Curing of Blindness in *Phycomyces blakesleeanus*: a Quantitative Assessment of Dominance

Delbrück and Reichardt[1] have pointed out the possible potential of *Phycomyces* as an organism for investigating mechanisms of sensory perception. This fungus produces single-celled sporangiophores which are sensitive to light, gravity and stretch. It also shows an avoidance response which is a proclivity to grow away from solid objects. Either tropic responses or changes in rates of growth are produced.

By way of an attempt to identify the receptor pigment involved in light-responses, I have thought of the following possibility. Using a suitable non-phototropic mutant, and assuming that the "visual" defect is due to a lack of photopigment, suitable solutions which might contain the pigment or its messenger RNA (for example, purified fractions from extracts of the wild type fungus) might be used to try to restore the response to light. Such non-nuclear or biochemical "curing" of the visual defect would constitute a biological assay for the receptor or a precursor. At present there is no suitable assay for the photopigment. However, before trying biochemical restoration of vision it is essential to test whether a defect in response to light can be cured genetically. The likelihood of success with biochemical curing would tend to be greater if, when wild type nuclei were added to the photomutant, the resulting improvement in vision were large. Experiments to determine quantitatively the extent of genetic curing are reported here.

Strain C2, genotype *car-5*(−), a mutant which forms white sporangia and differs from the wild type in the synthesis of β-carotene[2] but not in its light-sensitivity, was mutagenized again using N-methyl-N′-nitro-N-nitrosoguanidine[3] at a concentration of 0·1 mg/ml. in 6·5 mM citrate/sodium phosphate buffer, *p*H 7·0. Agar plates were seeded with mutagenized spores. Photomutants were selected by Heisenberg's[4] technique, that is, I took those that grew upwards in a glass-bottomed box illuminated from below. These mutants are not entirely unresponsive to light but have a threshold of intensity higher than the wild type, that is, they tend to be "night-blind". They show some responses characteristic of the wild type—geotropism and avoidance. Although the poorer sensitivity to light might result from a dearth of receptor pigment, other possibilities (for example, a modified receptor pigment, a change in the process of transduction or a modified mechanism of dark adaptation) cannot be excluded.

From measurements of threshold intensity for light-perception using the technique of Varjù *et al.*[5], a photomutant (strain C146, genotype *car-5 mad-117*(−)) having a threshold corresponding to an intensity several hundred times greater than that for the wild type was chosen for attempts to improve its sight. For experiments of this kind, a technique had to be devised to ensure that sufficient donor material entered the cell and was transported into a newly sprouted sporangiophore. A simple, efficient micro-injection technique was devised for transferring cell-sap from one sporangiophore to another. A red mutant (strain C9, genotype *car R21*(−)) which accumulates lycopene due to a block in the cyclization step of β-carotene biosynthesis[2], was used as a donor. This mutant is fully photosensitive. Sporangiophores of the recipient mutant were used at a stage before the sporangium had formed. About 12 h after injection a new sporangiophore sprouted from the apical end and formed a yellow sporangium. Plated spores from such sporangia produced colonies of white, red and various shades of yellow. The latter are heterokaryons and were used for determining a quantitative relationship between threshold intensity and nuclear proportion. Mycelia of several shades of yellow were used. Samples of mycelia about 10 mm² were transferred to plastic plates containing glucose, asparagine and yeast, all solidified by agar[3], and

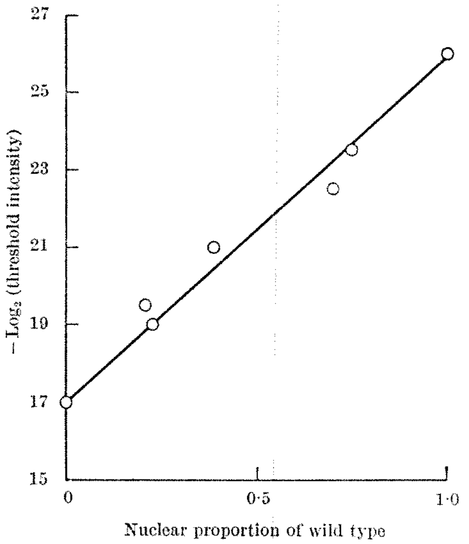


Fig. 1. $-\mathrm{Log}_2$ (threshold intensity) plotted against nuclear proportion. Curve shows restoration of vision using a heterokaryon made from a fully photosensitive red mutant and a white, night-blind mutant.

allowed to produce sporangia. Spores corresponding to each shade of yellow mycelium were collected and kept for the determination of nuclear proportion. Heisenberg and Cerdá-Olmedo[3] have shown that the nuclear ratio is constant in all parts of the mycelium, sporangiophores and sporangia and that random samples of nuclei are packaged into spores. Their technique was used here for determining the proportions of red (phototropic) and white (relatively non-phototropic) nuclei in each selected yellow mycelium.

Thresholds for light-perception corresponding to each nuclear proportion were then determined. Pieces (10 mm^2 approximately) of young mycelia from each of the chosen yellows were placed in vials each containing 1·5 ml. of growth medium (potato–dextrose–agar and 1 ml./l. of 0·05 per cent (w/v) thiamine). After 24 h many frail sporangiophores appeared, followed usually after 5 days by several sturdy sporangiophores. To prevent the production of frail sporangiophores the sample of mycelium was plated on growth medium in plastic dishes and young, robust sporangiophores were transferred individually to vials of medium for the determination of threshold after further growth.

Fig. 1 shows the effect of nuclear proportion on $-\log_2$ (threshold intensity). The latter quantity, based on a scale defined by Delbrück and Reichardt[1], may be expected to increase with the concentration or "effectiveness" of the photoreceptor pigment. The experimental variability is $\log_2$ intensity $= \pm 1$ approximately. From these results it seems that, although there is a considerable dominance of the photomutant, there is indeed curing of night-blindness by the admixture of wild type nuclei. If the data in Fig. 1 follow an exponential relationship, it might be conjectured that a missense mutation of a structural gene is involved, giving rise to an altered polypeptide subunit (for example, ref. 6) which associates with other subunits in an oligomeric form. Only the homo-oligomer of the wild type subunit would constitute the active photoreceptor. But this is one of many possibilities.

Cerdá-Olmedo has suggested (personal communication) that from a plot of threshold against nuclear proportion, as determined in this work, photomutants be classified by the value of the ordinate at $P = 0·5$. For strain C146 the value of the ordinate at a nuclear ratio of 0·5 is 21·5. High values for this parameter would obviously denote dominance of the wild type. This remains to be investigated.

Further evidence for the genetic curing of night-blindness was found by making the heterokaryon, C5 * C146, using the microsurgical technique mentioned here. C5 (genotype *car-10*(−)) is similar to C2 in that it forms white sporangia and differs from the wild type in its synthesis of β-carotene[2]. Sporangiophores from the resulting yellow heterokaryotic mycellia again showed an improved response to light. Because of the absence of the red colour-marker, a detailed quantitative examination was not made.

That blindness can be cured genetically is encouraging for work on possible biochemical restoration of vision. The simplicity and reliability of the micro-injection technique itself lead to further opportunities for studying the genetics and development of *Phycomyces*. For example, a quantitative analysis of the type shown here is necessary for any extensive investigation of complementation between photomutants.

I thank Professor Max Delbrück for his encouragement and Dr E. Cerdá-Olmedo for his help. This work was supported by the Gosney Fund of the California Institute of Technology and by the Council for Scientific and Industrial Research, Pretoria.

R. H. Villet

Division of Biology,
California Institute of Technology,
Pasadena, California 91109.

Received October 14, 1969.

[1] Delbrück, M., and Reichardt, W., in *Cellular Mechanisms in Differentiation and Growth* (edit. by Rudnick, D.), 3 (Princeton University Press, 1956).

[2] Meissner, G., and Delbrück, M., *Plant Physiol.*, **43**, 1279 (1968).

[3] Heisenberg, M., and Cerdá-Olmedo, E., *Mol. Gen. Genet.*, **102**, 187 (1968).

[4] Bergman, K., Burke, P. V., Cerdá-Olmedo, E., David, C. N., Delbrück, M., Foster, K. W., Goodell, E. W., Heisenberg, M., Meissner, G., Zalokar, M., Dennison, D. S., and Shropshire, W., *Bact. Rev.*, **33**, 143 (1969).

[5] Varjù, D., Edgar, L., and Delbrück, M., *J. Gen. Physiol.*, **45**, 47 (1961).

[6] Hayes, W., *The Genetics of Bacteria and Their Viruses*, second ed., 712 (Blackwells, Oxford and Edinburgh, 1968).

Anti-trachoma Activity of Rifampicin and Rifamycin SV Derivatives

RIFAMPICIN effectively inhibits the replication of trachoma agents both in infected cell cultures *in vitro*[1] and in embryonated eggs *in vivo*[2]. It acts during the initial developmental stages, characterized by active RNA synthesis[3]. The anti-trachoma inhibitory effect of rifampicin exceeded that of rifamycin SV[1,2], indicating that the presence of a hydrazone sidechain in carbon 3 of the chromophoric part of rifamycin SV might be concerned in the activity. To determine the function of the side chain, we have compared the effect of four compounds synthesized by adding to the formyl-rifamycin derivative of rifamycin SV different side chains at carbon 3. We have found that the presence of a hydrazone side chain in the rifamycin SV molecule enhanced the anti-trachoma activity of the drug *in vitro* and was essential for the inhibition of trachoma agent *in vivo*.

We used the T'ang strain of trachoma agent (TRIC// RC–PK/PK–2/OT) for the *in vitro* studies. For the *in vivo* studies we have also used the Bour strain (TRIC//USA–Cal, CAL–1/OT) of trachoma agent and the 6BC strain of psittacosis agent. The agents were propagated and titrated in cell cultures and embryonated eggs as previously reported[1,2].

The compounds (Lepetit) were added to infected FL cell cultures and embryonated eggs at concentrations from 0·001 to 100 μg/ml. All the derivatives inhibited the development of trachoma agent in cell cultures *in vitro* (Table 1).

The drugs could, however, be divided into two groups: (*a*) rifampicin and compound 33 which completely inhibited the replication of the T'ang strain of trachoma agent *in*

Table 1. INHIBITION OF T'ANG TRACHOMA AGENT BY RIFAMYCIN SV DERIVATIVES

Compound*	Drug concentration (μg/ml.)				
	0·001	0·01	0·1	1·0	10·0
	Per cent inhibition				
31 (Rifampicin)	0	100	100	100	100
33	0	100	100	100	100
18	0	55	100	100	100
11	0	0	0	100	100
26	0	0	0	100	100

* As reported by Maggi *et al.*[4].

Table 2. EFFECT OF RIFAMPICIN AND OTHER RIFAMYCIN SV DERIVATIVES ON THE REPLICATION OF TRACHOMA AND PSITTACOSIS AGENTS IN EMBRYONATED EGGS *in vivo*

Agent	Strain	Drug concentration (μg/egg)														
		Compound 31 (rifampicin)			Compound 33			Compound 18			Compound 11			Compound 26		
		1	10	100	1	10	100	1	10	100	1	10	100	1	10	100
		Per cent inhibition														
Trachoma	Bour	100	100	100	100	100	100	0	0	58	0	0	70	0	0	34
Trachoma	T'ang	100	100	100	72	84	100	0	10	58	0	0	0	0	0	50
Psittacosis	6BC	40	50	100	0	67	100	Not done			Not done			Not done		

vitro at a concentration of 0·01 μg/ml., and (*b*) compounds 18, 11 and 26 which inhibited trachoma agent at a concentration 10 and 100-fold higher than that of rifampicin. When the effect was studied in embryonated eggs *in vivo*, it was found (Table 2) that 1 μg/egg of rifampicin (compound 31) or compound 33 inhibited the replication of the Bour strain of trachoma agent. Compound 33 was slightly less effective than rifampicin in inhibiting the T'ang strain. By contrast, 100 μg/egg of compounds 18, 11 and 26 only partially inhibited the replication of the two trachoma strains in embryonated eggs. The findings *in vitro* and *in vivo* demonstrate that rifamycin SV derivatives which lack the hydrazone side chain in position 3 have a low anti-trachoma activity. The reason for the lower sensitivity of psittacosis agent to rifampicin (Table 2) is being studied.

Inhibition of replication of trachoma agent by rifamycin derivatives arises from their ability to prevent the synthesis of trachoma RNA in the developing inclusion bodies (unpublished results of B. Gutter and Y. B.). The inhibitory effect of rifampicin on trachoma RNA synthesis, like the effect on bacterial RNA polymerase[5], is to be attributed to inhibition of the agent's DNA-dependent RNA polymerase (unpublished results of I. Sarov and Y. B.). Recent studies[6] demonstrated that the macrocyclic ring of rifamycin SV is responsible for the binding of the drug to bacterial RNA polymerase; other parts of the molecule affect the permeation into intact microbial cells. Compound 31 (rifampicin) and compound 33 (Table 3), both having a hydrazone side chain in carbon 3 of the rifamycin SV chromophoric ring, are the most effective anti-trachoma compounds among the derivatives that we have studied (Table 3). The three other compounds, 18, 11 and 26, were less effective *in vitro* and very high concentrations were needed to inhibit trachoma agent *in vivo*. Compound 18, which has a short hydrazone side chain, resembled rifamycin SV in its low anti-trachoma activity (Table 3). We conclude that at least two parts of the rifampicin molecule are needed for inhibition of trachoma agent: the macrocyclic ring of the rifamycin SV molecule and the hydrazone side chain.

Table 3. RELATIONSHIP BETWEEN MOLECULAR STRUCTURE OF SIDE CHAIN IN RIFAMYCIN SV DERIVATIVES AND THEIR ANTI-TRACHOMA ACTIVITY *in vitro* AND *in vivo*

Compound[4]	Structure of side chain in position 3	Minimal inhibitory dose *In vitro* (μg/ml.)	Minimal inhibitory dose *In vivo* (μg/egg)
31 (Rifampicin)	$-CH=N-N(CH_2)_2N(CH_3)CH_2CH_2$	0·01	1·0
33	$-CH=N-N(CH_3)CH_2CH_2N(CH_3)_2$	0·01	1·0
Rifamycin SV	$-H$	0·10	> 100·0
18	$-CH=N-N(CH_3)_2$	0·10	> 100·0
11	$-CH=N-C_3H_2NS$	1·00	> 100·0
26	$-CH=N-N=CH-C_6H_5$	1·00	> 100·0

We found recently that compound 18, which lacked anti-trachoma activity, was as effective as rifampicin in inhibiting the replication of poxvirus, so demonstrating that although the hydrazone side chain of rifampicin is responsible for the anti-trachoma and anti-poxvirus activity of the drug, different parts of the hydrazone side chain were responsible for the inhibitory activities: the first part of the side chain can inhibit poxvirus replication while the terminal part is essential for the enhancement of the anti-trachoma activity of the drug. The efficiency of these derivatives of rifamycin SV in the cure of human trachoma still has to be studied.

We thank Dr L. Zeller, Professors P. Sensi and L. Silvestri from Lepetit, Milan, Italy, for the rifamycin SV derivatives. This work was aided by a grant from the World Health Organization, Geneva, and by a contribution to our Department of Virology by Ted and Frances Chanock, Los Angeles.

YECHIEL BECKER
YAEL ASHER
NEHAMA HIMMEL
ZICHRIYA ZAKAY-RONES

Department of Virology,
Hebrew University–Hadassah Medical School,
Jerusalem.

Received October 30, 1969.

[1] Becker, Y., and Zakay-Rones, Z., *Nature*, **222**, 851 (1969).
[2] Becker, Y., Asher, Y., Himmel, N., Zakay-Rones, Z., and Maythar, B., *Nature*, **224**, 33 (1969).
[3] Bernkopf, H., Mashiah, P., and Becker, Y., *Ann. NY Acad. Sci.*, **98**, 62 (1962).
[4] Maggi, N., Pallanza, R., and Sensi, P., *Anti-microbial Agents and Chemotherapia*, 765 (American Society for Microbiology, 1965).
[5] diMauro, E., Snyder, L., Marino, P., Lamberti, A., Coppo, A., and Tocchini-Valentini, G. P., *Nature*, **222**, 533 (1969).
[6] Wehrli, W., and Staehelin, M., *Biochim. Biophys. Acta*, **182**, 24 (1969).

Effect of a DNA Tumour Virus on Antibody Forming Cells in Neonatally Infected Hamsters

LEUKAEMOGENIC viruses, such as Friend, Rauscher or Moloney virus, may markedly suppress the immune responses of infected mice, both at the cellular and humoral levels[1-10]. AKR mice developing spontaneous leukaemia also have a marked suppression of serum antibody formation preceding clinical symptoms[11]. Several current theories of carcinogenesis suggest that such deficiencies of specific immunological competence may be a necessary but not exclusive prerequisite for tumour development[12-16]. In this regard, the induction of tumours by viruses other than those associated with murine leukaemia may also depend on an inadequate immune system and/or induction of specific immunological tolerance. Because most tumour viruses induce neoantigens in infected cells, it seems probable that an immunocompetent individual would respond to the virus induced antigens[15]. Loss of immunological competence could be related to development of the tumours. This study was undertaken to determine if a non-leukaemia DNA tumour virus could affect the immunological responsiveness of experimental animals. For this purpose, SV40 tumour virus was inoculated into newborn hamsters and their subsequent immune response to sheep erythrocytes was determined at the serum and cell level, using the haemolytic plaque assay in agar gel and serological procedures. The responses of non-infected hamsters were determined simultaneously.

Newborn hamsters were injected intraperitoneally with SV40 virus prepared in monkey kidney cell cultures[17], using at least six litters for each experiment. An equal number of animals were untreated controls. Tumour development was determined by close periodic physical examination during the subsequent six months[17]. More than 95 per cent of the animals injected with virus at birth developed multiple tumours at ages of 90 to 150 days. None of the control animals developed tumours.

To determine the immunological competence of these animals, groups of four or five infected or control hamsters were challenged by intraperitoneal inoculation of 0·1 ml. of a 10 per cent suspension of washed sheep red blood cells. Antibody plaque forming cells (PFC) were determined in spleens of individual hamsters 4 days later, using the plaque assay[18]. Haemolysins and haemagglutinins were determined in serum specimens by microtitre serological tests.

Table 1. EFFECT OF AGE ON APPEARANCE OF HAEMOLYSIN FORMING CELLS IN SPLEENS OF HAMSTERS IMMUNIZED WITH SHEEP ERYTHROCYTES

Age at time of challenge immunization (days)	Average number of PFCs per spleen on day: 2	4	7	10	15	20
1– 2	< 5	< 5	< 5	39	—	135
6– 8	665	4,830	246	193	185	169
11–14	2,431	16,653	5,950	2,050	1,670	1,130
18–21	28,341	80,369	42,395	4,350	3,160	—
26–28	45,421	139,531	32,340	9,321	2,159	1,330
> 35	39,753	151,350	38,461	11,215	2,350	1,653

Groups of hamsters were injected intraperitoneally, at age indicated, with 0·1 ml. 10 per cent sheep erythrocytes; five or more animals were killed on day indicated and the average number of PFCs per spleen was determined for each group.

Normal, non-infected hamsters injected with erythrocytes at 4 to 6 weeks of age exhibited a vigorous immune response when tested 4 days after immunization (Table 1). There was a direct relationship between the age of the animal at the time of challenge and the number of PFCs. For example, normal hamsters immunized when 1 week old rarely had more than 5,000 PFCs per spleen 4 days later (at 11 days of age). Immunization of 2 week old hamsters resulted in a peak of nearly 20,000 PFCs per spleen. Hamsters which were 3 or 4 weeks old had three to five times as many PFCs, the number approaching that for the mature animals.

Hamsters injected at birth with the tumour virus also responded with significant numbers of PFCs to the red blood cells. There was little or no detectable difference between the number of PFCs in spleens of infected animals at 3 to 6 weeks of age and the corresponding controls (Table 2). Infected animals tested at 1 or 2 weeks of age had about 50 per cent fewer PFCs than control animals of the same age. The response of non-infected animals at this time was, however, generally less than 10 to 15 per cent of that which occurred in the adult control groups.

The serum antibody response to sheep erythrocytes was generally low in 1 or 2 week old hamsters, regardless of treatment. Titres were moderately higher in 3 and 4 week old hamsters, reaching a level of 1 : 200 to 1 : 600 in the 4 to 6 week old groups. There was essentially no difference in the mean titre, or range of titre, between infected and control hamsters at any age.

These results revealed little, if any, effect on the immune responsiveness of young adult hamsters to sheep erythrocytes during the latent period of SV40 virus induced oncogenesis. There was, however, a suggestion of a slight to moderate depression of development of immune competence to this antigen during the early period of immunological maturation (1 to 2 weeks of age). The significance of such a moderate and temporary deficiency at the cellular level in such young animals is not clear, but it is obvious that at 3 or 4 weeks of age, or later, SV40 infected animals responded as well as non-infected controls. Further, the serological titres, which were generally quite low in the younger animals, were similar for all infected and control groups. Thus immune suppression—at least to sheep erythrocytes—was not a significant factor in tumour induction by a DNA tumour virus, such as SV40, as it is in mice infected with an RNA leukaemogenic virus.

Table 2. EFFECT OF SV40 INFECTION AT BIRTH ON THE IMMUNE RESPONSE OF HAMSTERS TO SHEEP ERYTHROCYTES AT VARIOUS AGES

Age at time of challenge immunization (days)	Mean peak immune response to sheep erythrocytes: Control mice PFC/spleen	Control mice PFC/10^6 spleen cells	Control mice Serum titre	SV40 infected mice PFC/spleen	SV40 infected mice PFC/10^6 spleen cells	SV40 infected mice Serum titre
1	< 100	< 1	< 1 : 4	< 100	< 1	< 1 : 4
7	1,738	163	1 : 8	889	59	1 : 4
14	19.965	229	1 : 27	17,861	183	1 : 17
21	62,695	289	1 : 78	49,215	275	1 : 67
28	139,581	314	1 : 219	132,850	319	1 : 211
42	153,580	386	1 : 135	156,385	335	1 : 202

Groups of hamsters were untreated or injected intraperitoneally with SV40 virus on the day of birth; five or more animals were immunized intraperitoneally with 0·1 ml. 10 per cent sheep erythrocytes at age indicated and killed 4 days later.

This study was supported in part by grants from the US National Science Foundation, the American Cancer Society and a Damon Runyon research grant. H. G. is a scholar of the Leukemia Society of America.

HERMAN FRIEDMAN
HERMAN GOLDNER

Department of Microbiology and
Cancer Laboratory,
Albert Einstein Medical Center, and
Department of Microbiology,
Temple University School of Medicine,
Philadelphia.

Received July 29; revised September 23, 1969.

[1] Peterson, R. D. A., Hendrickson, R., and Good, R. A., *Proc. Soc. Exp. Biol. and Med.*, **114**, 517 (1963).

[2] Dent, P. B., Peterson, R. D. A., and Good, R. A., *Proc. Soc. Exp. Biol. and Med.*, **119**, 969 (1965).

[3] Siegel, B. V., and Morton, J. I., *Immunology*, **10**, 559 (1966).

[4] Siegel, B. V., and Morton, J. I., *Proc. Soc. Exp. Biol. and Med.*, **123**, 467 (1966).

[5] Cremer, N. E., Taylor, D. O. N., and Hagens, S. J., *J. Immunol.*, **96**, 495 (1966).

[6] Salaman, M. H., and Wedderburn, N., *Immunology*, **10**, 445 (1966).

[7] Odaka, T., Ishii, H., Yamaura, K., and Yamamoto, T., *Jap. J. Exp. Med.*, **36**, 277 (1966).

[8] Chan, G., Rancourt, M. W., Ceglowski, W., and Friedman, H., *Science*, **159**, 437 (1968).

[9] Ceglowski, W. S., and Friedman, H., *Proc. Soc. Exp. Biol. and Med.*, **126**, 662 (1967).

[10] Ceglowski, W. S., and Friedman, H., *J. Immunol.*, **101**, 594 (1968).

[11] Metcalf, D., and Moulds, R., *Int. J. Cancer*, **2**, 53 (1967).

[12] Old, L. J., and Boyse, E. A., *Ann. Rev. Med.*, **15**, 167 (1964).

[13] Sjoren, H. O., *Prog. Exp. Tumor Res.*, **6**, 289 (1965).

[14] Hellstrom, K. E., and Moller, G., *Prog. Allergy*, **9**, 158 (1965).

[15] Klein, G., *Ann. Rev. Microbiol.*, **20**, 232 (1966).

[16] Moloney, J. B., *Ann. Rev. Med.*, **15**, 393 (1964).

[17] Goldner, H., Girardi, A. J., Larson, J. M., and Hilleman, M. R., *Proc. Soc. Exp. Biol. and Med.*, **117**, 851 (1964).

[18] Jerne, N. K., and Nordin, A. A., *Science*, **140**, 405 (1963).

Effects of Adenoviruses on the Chromosomes of Normal Human Cells and Cells Trisomic for an *E* Chromosome

NON-RANDOM damage to the chromosomes of human embryo kidney (HEK) cells by adenovirus type 12 (*in vitro*) has been reported by Zur Hausen[1]. We have obtained similar results with adenovirus type 12 in fibroblasts grown from human embryo lung (HEL) and from skin biopsy samples, as well as in the epithelial HEK cells.

HEK and HEL cells were obtained from embryos removed by therapeutic abortion. Fibroblast cultures were also obtained from outgrowths of skin biopsy samples taken from normal and genetically abnormal children and adults. The cells were grown in 5 cm plastic Petri dishes. The medium used for cell cultivation was Ham's F10 (ref. 2) + 10 per cent tryptose phosphate broth and 20 per cent foetal calf serum. All cultures were incubated at 37° C in an atmosphere of 5 per cent CO_2 in air. Adenovirus strains were supplied by Dr H. G. Pereira (National Institute for Medical Research, Mill Hill, London), grown in HEK cells and purified by the method of Russell, Valentine and Pereira[3].

For cytogenetic studies, cells washed with phosphate buffered saline were infected by adsorbing the virus inoculum (input multiplicity = 1) for 3 h at 37° C. After the adsorption period the cells were washed with phosphate buffered saline and reincubated with growth medium. Colcemid was added to the cultures 24 h after virus adsorption. Three hours later, the cells were removed from the dishes with 0·25 per cent trypsin, centrifuged at 800 r.p.m. and resuspended in 0·075 M KCl. After incubation at 37° C for 5 min, the cells were again centrifuged and then fixed in a mixture of three parts absolute methyl alcohol and one part glacial acetic acid. Slide preparations were made by drying drops of fixed cell suspension on slides at room temperature, followed by staining with acetic orcein; they were then mounted in euparal.

In Fig. 1, the No. 1 and No. 17 chromosomes show the typical damage found after *in vitro* infection with adenovirus type 12. Experiments with other adenovirus serotypes (types 2, 5, 7 and 18) have resulted in random damage to the chromosomes, and, where a No. 17 was involved, the damage has occurred at positions on the chromatids different from that usually affected by adenovirus type 12. In Fig. 2, the distribution of chromosomal damage induced by type 12 is compared with that found after *in vitro* infection with type 18. The results with type 18 are more compatible with a random distribution of damage: a histogram based on the relative lengths[4] of the individual chromosomes has a similar appearance (Fig. 3). There may be some degree of specificity in the response of the No. 17 chromosomes to adenovirus type 18 infection.

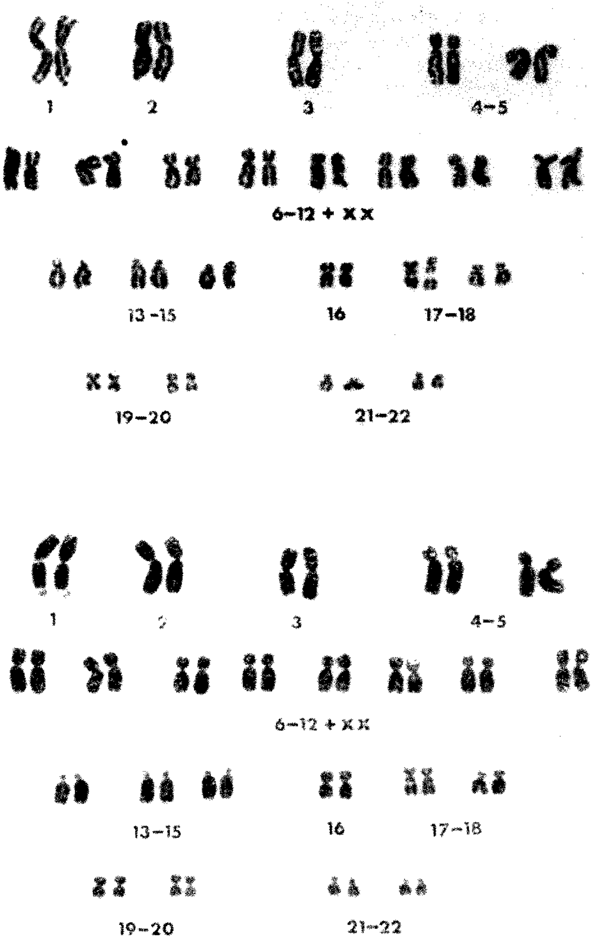


Fig. 1. Karyotypes of two cells showing non-random damage to chromosomes No. 1 and No. 17 induced by adenovirus type 12 *in vitro*.

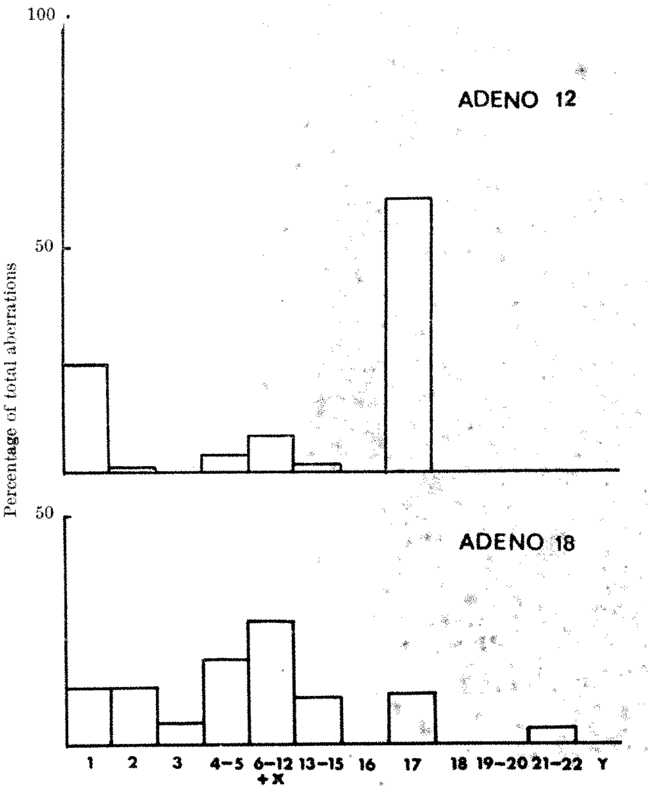


Fig. 2. Distribution of damage to chromosomes induced by adenovirus types 12 and 18.

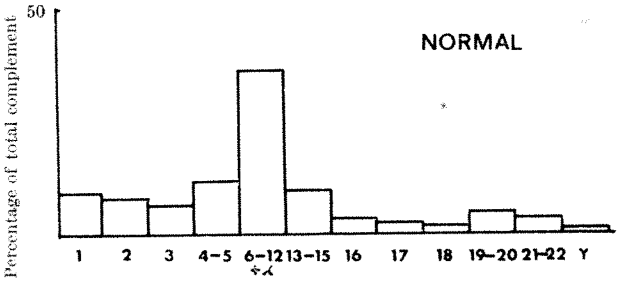


Fig. 3. Distribution of chromosomal material based on chromosome lengths (Denver classification).

Trisomy *E* (Edwards' syndrome), in which severe malformations—usually fatal in the first year of life—occur, was originally described as being associated with an extra chromosome, No. 17 (ref. 5). Patau *et al.*[6] stated that the extra chromosome was a No. 18 and autoradiographic studies carried out by Yunis *et al.*[7] supported this contention.

Using the effect of adenovirus type 12 on chromosome No. 17 as a marker, the response of the five *E* group autosomes to infection by this virus was examined. Experiments were carried out as described for normal cells, 100 metaphases being examined for each specimen. Damage to the No. 17 chromosomes was similar to that found in normal human cells, but in no cell has the morphology of more than two *E* group autosomes been affected by the virus.

Table 1. NON-RANDOM DAMAGE BY ADENOVIRUS TYPE 12 TO *E* GROUP CHROMOSOMES FROM NORMAL AND GENETICALLY ABNORMAL (TRISOMY *E*) CELLS

	No damage to *E* group	Damage to one *E*	Damage to two *E*	Damage to three *E*	Total cells
Normal	76	78	46	0	200
Trisomy *E*	158	26	16	0	200

In Table 1 the numbers of cells with damage to *E* group chromosomes are shown, based on 100 cells from each of two normal subjects and 100 cells from each of two subjects with trisomy *E*. The decreased number of cells

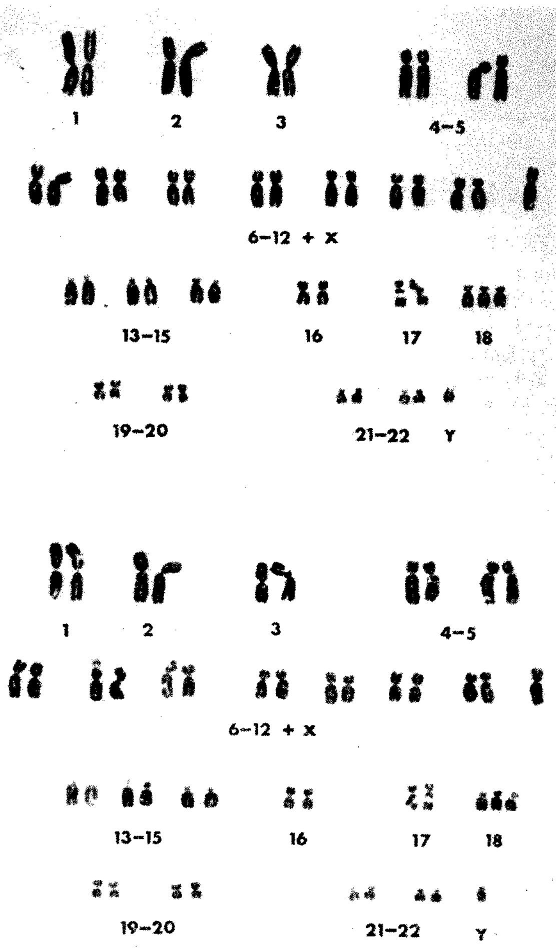


Fig. 4. Karyotypes of two cells, from an infant with Edwards' syndrome, infected *in vitro* with adenovirus type 12.

with damage to *E* group chromosomes in the trisomy *E* samples reflects the difference in virus susceptibility of these cells in comparison with the normal cells.

Fig. 4 shows the chromosomes of two virus-infected cells from a male infant with Edwards' syndrome, in which the typical non-random damage induced by adenovirus type 12 to the No. 17 chromosomes can be seen.

These results offer further evidence to support identification of the extra chromosome in Edwards' syndrome as a No. 18.

I thank Dr H. G. Pereira for virus samples, Dr Angela Taylor for a skin specimen from a case of Edwards' syndrome, Professor D. G. Harnden and Dr Patricia Jacobs for encouragement and advice and Mrs Katie Vause for technical assistance.

J. K. McDOUGALL*

MRC Clinical and Population Cytogenetics Unit,
Western General Hospital,
Crewe Road,
Edinburgh, 4.

Received October 3, 1969.

* Present address: Department of Cancer Studies, University of Birmingham, Birmingham 15.

[1] Zur Hausen, H., *J. Virol.*, **1**, 1174 (1967).
[2] Hamm, R. G., *Exp. Cell Res.*, **29**, 515 (1963).
[3] Russell, W. C., Valentine, R. C., and Pereira, H. G., *J. Gen. Virol.*, **1**, 509 (1967).
[4] Levan, A., and Nicholls, W. W., *Hereditas*, **51**, 378 (1964).
[5] Edwards, J. H., Harnden, D. G., Cameron, A. H., Crosse, V. M., and Wolff, O. H., *Lancet*, i, 787 (1960).
[6] Patau, K., Therman, E., Smith, D. W., and DeMars, R. I., *Chromosoma*, **12**, 280 (1961).
[7] Yunis, J. J., Hook, E. B., and Mayer, M., *Lancet*, ii, 286 (1964).

Transformation and Virus Growth by Murine Sarcoma Viruses in Human Cells

In the study of neoplastic transformation of human cells in culture, the quantitative systems so far described have used only the DNA-containing tumour viruses SV40 (ref. 1) and adenovirus type 12 (ref. 2). Various factors, both genetic[3] and environmental[4], influence the efficiency of transformation with these viruses. We describe here a quantitative transformation system using an RNA-containing tumour virus, murine sarcoma virus (MSV)[5]. The transformation of human cells by MSV is inefficient, but in optimal conditions (logarithmically growing cells and certain highly susceptible cell strains) both cell transformation and virus growth can be demonstrated.

Human fibroblast strains derived from a variety of sources, including embryonic lung and kidney, newborn foreskin, and adult skin, were grown in Dulbecco's medium supplemented with 10 per cent calf serum. MSV stocks were obtained from two sources: (1) a cloned line of Molony sarcoma virus (MSV–M)-transformed Balb/3T3 mouse cells[6]; (2) a Kirsten sarcoma virus (MSV–Ki)-transformed rat cell line[7] supplied by Dr V. Klement (Children's Hospital, Los Angeles). Both *in vitro* transformed lines produce high titres of MSV. The virus pools, obtained from supernatants two days after medium change, were first clarified by low speed centrifugation and then passed through 0·45 µm 'Millipore' filters. Human strains were infected within 24 hours after inoculation of 2–3 × 10^5 cells into 15 cm^2 Falcon flasks. After a one hour adsorption period medium was added and then changed twice weekly.

As early as 5–6 days after infection, focal areas of more refractile cells were seen. These areas increased in size during the next week. Fig. 1*a* shows an example of a focus 12 days after virus exposure. Most of the cells were fusiform in shape, and many contained large cytoplasmic vacuoles. The number of foci did not increase after two weeks, but after that time in certain strains a pronounced proliferative effect with multilayered cell growth was noted (Fig. 1*b*). The number of transformed foci was counted at 14 days; the same virus preparations produced foci somewhat more rapidly on mouse and rat cells, and here foci were scored at 7 days. Balb/3T3 was used to titre MSV–M and the rat NRK line was used to assay MSV–Ki. While each virus will grow on a variety of rodent cell cultures, the above cells were found to be the most susceptible.

Table 1. FOCUS-FORMING ABILITY OF MURINE SARCOMA VIRUSES ON RODENT AND HUMAN CELLS

	Rodent cells (FFU/ml.)*	Human cells (FFU/ml.)†	Relative focus forming ability ‡
MSV–Ki	3·4 × 10^5	1·1 × 10^3	3 × 10^2
MSV–M	5·0 × 10^4	5	1 × 10^4

* Assayed at 7 days after infection: MSV-Ki titred on NRK and MSV-M titred on Balb/3T3.

† Assayed at 14 days on newborn foreskin strain 8727 and adult skin fibroblast strains R.M. and B.F.

‡ Titre (foci × dilution) on mouse cells/titre (foci × dilution) on human cells.

Human cell cultures were stained for murine sarcoma–leukaemia antigen by an indirect fluorescent antibody method using rat anti-MSV serum and fluorescein-conjugated goat anti-rat serum (unpublished data of W. P. Rowe, J. W. Hartley and R. E. Wilsnak). Seven days after infection focal areas showing characteristic cytoplasmic staining were seen; they contained 5–10 cells per focus. Uninfected human cultures showed no staining, nor did MSV-infected cultures that were stained with control rat serum.

Eighteen human fibroblast strains, each derived from a different person, were tested. Eleven have shown clear evidence of focus formation by MSV. The number of foci

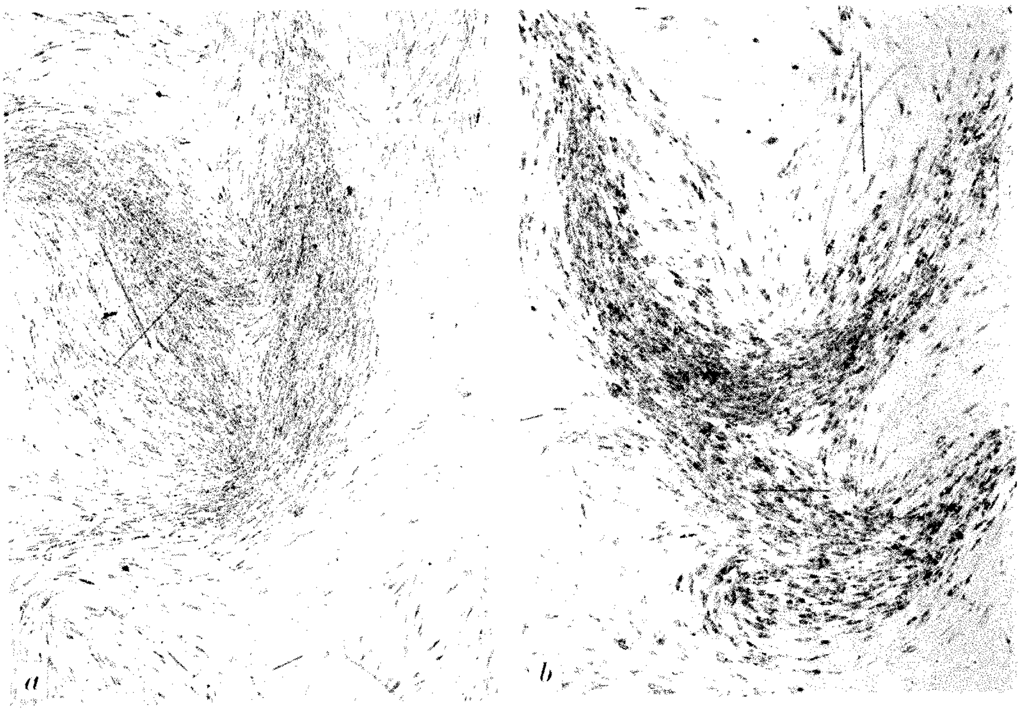


Fig. 1. *a*, A transformed focus appearing on human strain B.F. at 12 days after infection; *b*, a more advanced focus seen at 16 days on strain R.M. (× 20.)

ranged from one or two per plate up to several hundred with the most susceptible strains. For MSV–Ki, roughly 3×10^2 focus forming units (FFU) were needed to produce one focus on a susceptible human cell; with the virus from MSV–Balb/3T3 approximately 30 times as many FFU were required (Table 1). Dilution of each virus stock resulted in a decrease in the number of foci most consistent with a "one hit" pattern. That the foci were due to MSV and not to the associated helper leukaemia virus was shown by using sarcoma virus-free leukaemia stocks at comparable or higher titres and finding no foci on any of the human cells tested.

The cultures at two weeks after infection showed evidence of virus replication by several methods. Electron microscopic sections of foci showed typical budding C-type particles[8]. Sucrose gradient banding of the supernatant fluid showed a peak of radioactivity at a buoyant density of 1·16 g/cm^3, the density characteristic of MSV[9]. Focal areas of transformed cells were isolated by cloning. After six transfers and after over three months, morphologically transformed cells could still be seen. These cultures still contained fluorescent antibody staining cells and continued to produce C-type particles. Using filtered supernatant preparations from MSV-infected human cells, biological activity was demonstrated by focus formation both on mouse and human cells. The virus obtained still produced foci considerably more efficiently on mouse cells than on human cells, suggesting little, if any, host cell modification. DEAE–dextran, which increases the efficiency of transformation with several RNA-containing tumour viruses, also increased the efficiency in this system, but did not alter the relative focus forming ability.

This communication shows that murine tumour viruses can reproducibly infect and morphologically transform many strains of human cells. Certain avian sarcoma viruses have also been shown to infect and morphologically alter human cells[10]. With the murine viruses Bernard *et al.*[11] have reported morphological alteration and virus growth using one human embryonic strain, WI38. The ability of the sarcoma viruses of avian and murine origin both to grow in and also to transform human cells of diverse origin increases the possibility that these or similar agents may, on occasion, cross species barriers to cause tumours in man.

STUART A. AARONSON
GEORGE J. TODARO

Viral Carcinogenesis Branch,
National Cancer Institute,
Bethesda,
Maryland 20014.

Received October 10; revised December 12, 1969.

[1] Aaronson, S. A., and Todaro, G. J., *Virology*, **36**, 254 (1968).
[2] Todaro, G. J., and Aaronson, S. A., *Proc. US Nat. Acad. Sci.*, **61**, 1272 (1968).
[3] Todaro, G. J., Green, H., and Swift, M. R., *Science*, **153**, 1252 (1966).
[4] Pollock, E. J., and Todaro, G. J., *Nature*, **219**, 520 (1968).
[5] Huebner, R. J., *Proc. US Nat. Acad. Sci.*, **58**, 835 (1967).
[6] Todaro, G. J., and Aaronson, S. A., *Virology*, **38**, 174 (1969).
[7] Klement, V., Hartley, J. W., Rowe, W. P., and Huebner, R. J., *J. Nat. Cancer Inst.* (in the press).
[8] Bernhard, W., *Cancer Res.*, **20**, 712 (1960).
[9] Biswal, N., Grizzard, M. B., McCombs, R. M., and Benyesh-Melnick, M., *J. Virol.*, **2**, 1346 (1968).
[10] Stenkvist, B., and Ponten, J., *Acta Path. Microb. Scand.*, **62**, 315 (1964).
[11] Bernard, C., Lasneret, J., Boucher, M., and Boiron, M., *CR Acad. Sci.*, D **268**, 624 (1969).

Effect of ALS Globulins on Amino-acid Incorporation by Human Peripheral Lymphocytes

THE mechanism by which antilymphocyte globulins act on immunocompetent cells remains unknown. Recent studies have demonstrated that antilymphocyte sera (ALS) and phytohaemagglutinin (PHA) display a similarity in their action on the lymphocyte[1–6]. We have studied the action of these substances on the incorporation of amino-acids by human peripheral lymphocytes.

Human blood (400 ml.) drawn by venous puncture was collected on heparin (2·5 mg for 15 ml. of blood). Lymphocytes were isolated by filtration on synthetic fibre according to a technique described previously[7]. After filtration

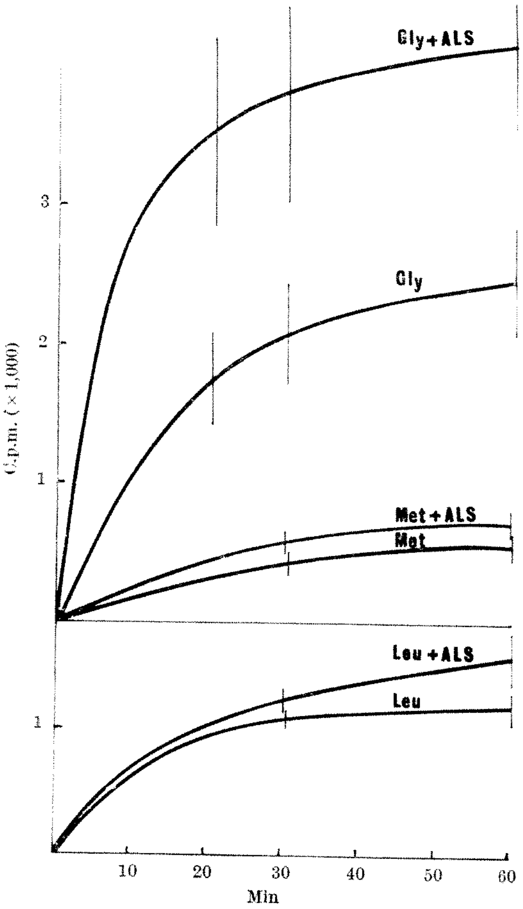


Fig. 1. Action of horse ALS on the rate of incorporation *in vitro* of glycine (Gly), methionine (Met) and leucine (Leu). 15×10^6 lymphocytes were incubated in 1·5 ml. of Krebs–Ringer phosphate buffer (*p*H 7·4), containing ^{3}H or ^{14}C-labelled amino-acid (3·33 mmole; 0·33 μCi/mmole) with or without ALS (0·1 ml. of a solution rich in IgG, 15 mg/ml.). The titre of the preparation was 1/1,024. The rate of incorporation (c.p.m.) is expressed as a function of time. The confidence limit (95 per cent) is indicated by a vertical line.

the blood was precipitated by dextran (molecular weight 500,000), and the supernatant was centrifuged in the cold at 250*g* for 20 min. The red cells in the pellet were haemolysed by adding a solution of NH_4Cl (0·87 per cent). The mixture was centrifuged in the cold at 250*g* for 20 min and the final pellet washed in saline and resuspended in saline.

Lymphocytes were obtained in a purity of 95 per cent, the remainder being polymorphonuclear leucocytes. The yield of lymphocytes was about 20 ml. of a suspension containing 20×10^6–25×10^6 cells/ml. They were incubated in the presence of amino-acids labelled with ^{14}C or ^{3}H for relatively short periods (60 min). When phytohaemagglutinin was used (PHA-M, Difco, in vials of 5 ml.), it was added to the incubation medium at the same time as the labelled amino-acid. The same procedure applies to the use of fractions of ALS.

Horse antilymphocytic sera were prepared by Dr Carraz of the Pasteur Institute of Lyon, by immunization with human thoracic duct lymphocytes[8]. The antiserum obtained was decomplemented, then absorbed on human red cells. A fraction enriched in antilymphocyte globulins was prepared by precipitation of the serum at 4° C with ammonium sulphate (40 per cent saturation), which is finally eliminated by dialysis. The precipitate obtained was taken up in saline to the same volume as the initial serum. Rabbit antilymphocytic sera were obtained by immunizing rabbits with human peripheral lymphocytes. The precipitate obtained with ammonium sulphate (40 per cent saturation) was dissolved and fractionated in the cold by electrophoresis on Pevikon media with 0·18 M borate buffer (*p*H 8·2), according to a previously reported technique[9]. In this way the γG-globulin (IgG) and albumin fractions are isolated. The same technique was used to prepare IgG and albumin fractions from normal decomplemented and absorbed horse and rabbit sera.

The potency of the ALS was measured by its cytotoxic action on normal human lymphocytes: the lymphocytotoxic test of Engelfriet[10] with trypan blue was used. The titres given are the highest dilutions which give more than 15 per cent dead cells. The titres for the different horse antilymphocyte globulins were 1/1,024; for rabbit IgG fractions the range was 1/256–1/512.

The influence of horse ALS on the rate of incorporation of methionine, leucine and glycine by human lymphocytes *in vitro* is illustrated in Fig. 1. A significant increase in the rate of incorporation is obtained immediately in the case of glycine; in the presence of an identical amount of antilymphocyte globulin it takes about 30 min of incubation for leucine and 60 min for methionine to have a significant effect. Subsequent studies (unpublished) have demonstrated that the incorporation of leucine and glycine by human lymphocytes, like that by other cells, requires different carriers at the level of the cellular membrane. We postulate that the stimulation by ALS is related to its direct action on certain of these transport systems. (The interpretation of the increased rate of incorporation in the case of leucine must take into account the fact that certain lots of ALS have sufficient cytotoxic power to damage the cellular membrane in about 30 min.)

The action of ALS is also dependent on its origin (Fig. 2). Equal amounts of various IgG fractions from horse ALS, all having the same cytotoxic titre, increase the rate of incorporation of glycine to different extents. On the other hand, IgG fractions purified from the different rabbit ALS preparations studied so far had an inhibitory action. This constitutes additional proof for the heterogeneity of antilymphocyte globulins. In every case, however, their action is immediate and manifest in the first 30 min. Within the limits of the concentrations used, the rate of incorporation of glycine increases with the dose of ALS (Fig. 3). The inhibitory action of the corresponding proteins of normal horse serum is also dependent on the dose.

Fig. 4 indicates that PHA exerts an inhibitory action on the rate of incorporation of glycine, this effect being directly related to concentration. There therefore seems to be a difference between the mode of action of horse

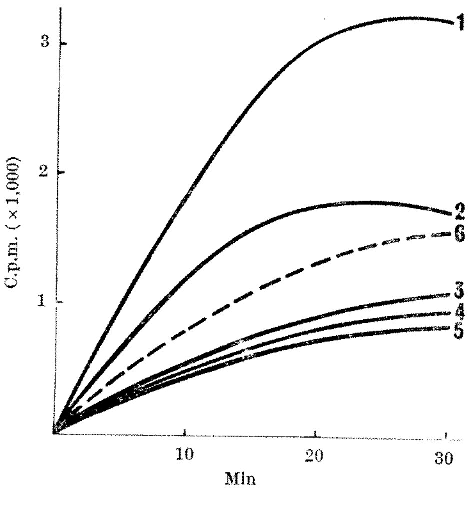


Fig. 2. Action of ALS of different origins on the rate of incorporation of glycine by peripheral human lymphocytes. 1 and 2: preparations of horse ALS rich in IgG (15 mg/ml.); the titre of both lots was 1/1,024. 3, 4 and 5: rabbit IgG fractions (13 mg/ml.) purified from the corresponding ALS. The titres of the different fractions were respectively 1/256, 1/512 and 1/256. 6: incorporation of glycine without ALS.

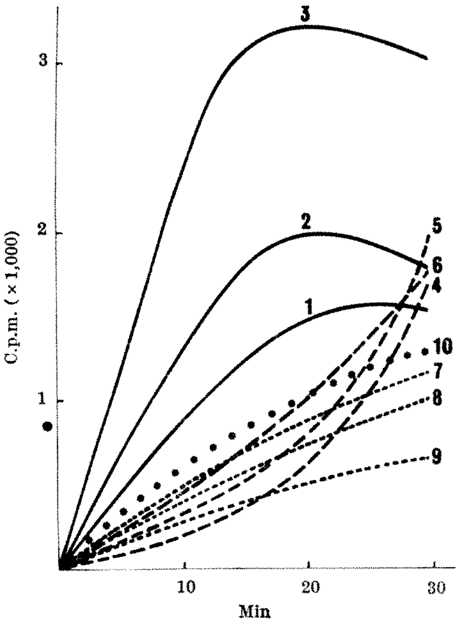


Fig. 3. Influence of the amount of ALS on the rate of incorporation of glycine by peripheral human lymphocytes. 1, 2 and 3: preparations of horse ALS rich in IgG (15 mg/ml.) at increasing doses (0·3 mg, 2·25 mg and 3·75 mg, respectively). 4, 5 and 6: IgG fractions (15 mg/ml.) purified from normal horse serum in increasing doses (0·3 mg, 2·25 mg and 3·75 mg, respectively). 7, 8 and 9: albumin fractions of normal horse serum, brought to the same volume as the initial serum (0·02 ml., 0·15 ml. and 0·25 ml., respectively). 10: incorporation of glycine without heterologous proteins.

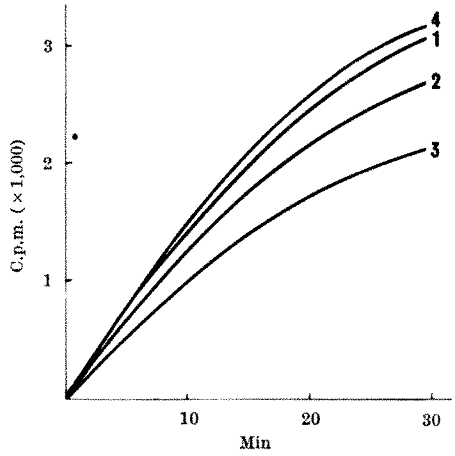


Fig. 4. Influence of different concentration of PHA on the rate of incorporation of glycine by human peripheral lymphocytes. 'Bacto-PHA-M' (Difco Laboratory) was dissolved in 5 ml. of saline. 1, 2 and 3: final dilution of the stock solution of PHA (1/100, 1/50 and 1/10, respectively). 4: incorporation of glycine in the absence of PHA.

ALS and PHA, the rate of incorporation of glycine in peripheral lymphocytes being increased by ALS but decreased by PHA. We are investigating whether this activation is the result of a direct action on the transport system for glycine by the lymphocyte membrane.

We thank Dr M. Carraz, Pasteur Institute, Lyon, for providing us with horse ALS; and M. Terrier for technical assistance.

H. Bétuel
G. B. Richard
L. Colobert
L. Revol

Blood Transfusion Centre and
Laboratory of Medical Biochemistry,
Faculty of Medicine,
Lyon.

Received May 1; revised September 24, 1969.

[1] Vassar, P. S., and Culling, C. F. A., *Nature*, **202**, 610 (1964).
[2] Borberg, H., Woodruff, J., Hirschhorn, R., Gesner, B., Miescher, P., and Silber, R., *Science*, **154**, 1019 (1966).
[3] Chessin, L. N., Borjeson, J., and Welsh, P., *Fed. Proc.*, **25**, 306 (1967).
[4] Lindhall-Kiessling, K., and Peterson, R. D. A., *Fed. Proc.*, **26**, 527 (1967).
[5] Oppenheim, T. J., Hersh, E. M., and Block, J. B., *J. Reticuloendothel. Soc.*, **3**, 373 (1966).
[6] Mosedale, G., Felstead, K. J., and Parke, T. A. C., *Nature*, **218**, 983 (1968).
[7] Bétuel, H., Gautheron, D., and Revol, L., *Rev. Franc. Etudes Clin. Biol.* (in the press).
[8] Carraz, M., Traeger, J., Fries, D., Perrin, J., *et al.*, *Rev. Inst. Pasteur Lyon*, **1**, 17 (1967).
[9] Richard, G. B., thèse, Lyon (1966).
[10] Engelfriet, C. P., and Britten, A., *Vox Sang.*, **11**, 334 (1966).

Acute Childhood Leukaemia in Relation to the HL-A Human Transplantation Genes

The expectation that certain human diseases might show a high statistical correlation with certain transplantation genes is based on analogy with mouse systems[1]. The H-2b gene in mice is associated with a low incidence of tumours and with great longevity in three different inbred strains[2]. Another transplantation gene, the H-2k gene, confers in homozygous form an almost 100 per cent susceptibility to the Gross leukaemia virus[3] and a non-susceptibility to the Tennant leukaemia virus[4].

Attempting to find out whether in man certain HL-A genes might correlate with human leukaemia, we adopted two rules. First, we would deal with a very well defined type of leukaemia. We therefore investigated acute childhood leukaemia because of its rather consistent lymphatic origin, its clinical course and its restricted age distribution. The second rule was that we would look for an HL-A correlation on the basis of genotypic as well as phenotypic data. The H-2k gene in mice is manifested by the serological specificities 1, 3, 5, 8, 11 and 25. Many of these, in combination with further specificities, are found on other mouse transplantation genes, for example, on H-2a; nevertheless, only one particular collection of specificities denotes the H-2k gene. Failure to appreciate these rules may have prevented Kourilsky *et al.*[5] from finding any correlation between the HL-A system and "acute leukaemia" in man.

We investigated ten families in each of which one child was afflicted with acute lymphatic leukaemia and in which at least one other sibling was available for HL-A typing. Including leukaemics and their siblings, a total of twenty-three children plus all twenty parents were studied. The HL-A type of each person was determined with the microlymphocytotoxicity test[6]. We used anti-sera determining specificities HL-A 1, 2, 3, 9, "Thompson", Da-17, and Lc-26 at the first sublocus of the HL-A system. At the second sublocus, the following were determined: HL-A 5, 7, 8, Lc-15, "Gallegos", and "Merritt"[1]. A collection of known HL-A specificities which migrate as a unit in families is known as a "haplotype"; from a practical serological standpoint the terms "gene" and "haplotype" are similar. Because each HL-A haplotype is reflected by none or by one (but almost never more than one) specificity from each sublocus, a total of fifty-six possible haplotypes can be envisaged. This figure assumes a "null" haplotype at each sublocus. By immunogenetic analysis of our typing data[1], the actual haplotype of each member of our ten families was deduced, including the haplotypes of the ten leukaemic children. Separate but similar studies of twenty-five random normal families provided us with sufficient random, parental haplotypic data (100 parental HL-A chromosomes) to estimate normal frequencies in our population. Whether any particular haplotype appeared more often than expected among the leukaemics might then be determined.

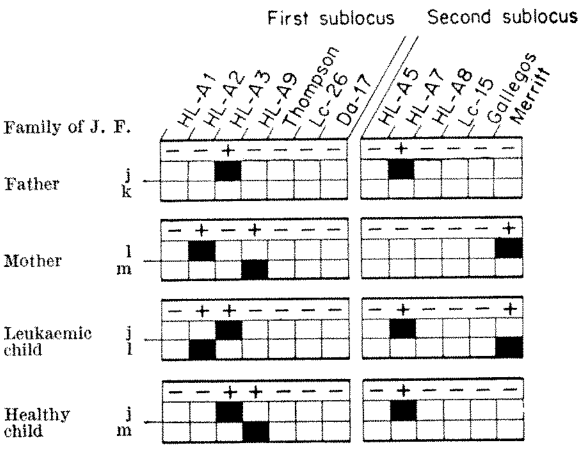


Fig. 1. Immunogenetic analysis of a leukaemic family. Phenotypic data are given as + or − under each specificity and for each family member. Genotypes can now be derived by simple inspection assuming the two subloci and the rule of complete mutual exclusivity at each of them.

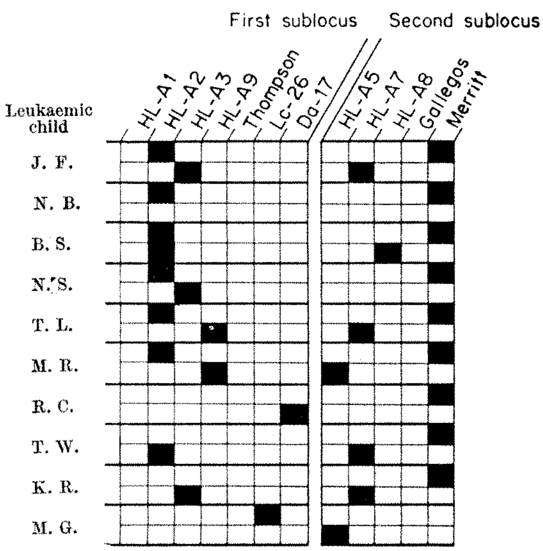


Fig. 2. HL-A genotypes of ten unrelated children with acute lymphatic leukaemia.

Results obtained with one of the ten families are shown in Fig. 1. The HL-A haplotype of the leukaemic child was HL-A 3, 7/2, Merritt. We shall concentrate on this second, HL-A 2, Merritt haplotype, designated the l-haplotype in Fig. 1. In our normal population the frequency of the l-haplotype was only five per cent, but six of our ten leukaemic children had this haplotype on one of their two HL-A chromosomes. The probability of this or a greater frequency happening by chance is less than 0·0025.

Fig. 2 gives the haplotypes of all ten unrelated leukaemic children. The frequency of the l-haplotype is evident. We note also that none of the twenty haplotypes carried the HL-A 1 specificity. The frequency of HL-A 1 in any combination was 0·25 in our normal population. The probability therefore of all ten leukaemics being HL-A 1-negative is about 0·016. We note furthermore (Fig. 2) that nine children were phenotypically Merritt positive. The frequency of the Merritt specificity in any combination was 0·20, for it also occurred in association with specificities other than HL-A 2. In these circumstances the probability of nine of ten persons with one or more haplotypes carrying the Merritt specificity is less than 0·035.

There can be little doubt as to the statistical significance of at least the first of these probabilities in view of its magnitude. Assuming for the sake of discussion that acute childhood leukaemia has a viral origin, the possession of the HL-A 2, Merritt haplotype may indicate an increased susceptibility to a particular leukaemia virus present near Los Angeles. Different associations might be found in other localities. Healthy persons also possess the HL-A 2, Merritt haplotype, including parents and siblings of the leukaemic children studied here. We are thus dealing not with an absolute but with an enhanced susceptibility, and additional factors must come into play in the pathogenesis of the leukaemia. Whether the presence of the Merritt specificity irrespective of HL-A 2 is meaningful in showing enhanced susceptibility to leukaemia, and whether the presence of the HL-A 1 specificity indicates resistance to the form of leukaemia studied here, must await the accumulation of a larger series. The ability to predict that persons with such and such an HL-A genotype are "at risk" with respect to acute leukaemia, persons with another genotype "at risk" for some other disease, would be of obvious value. The present study suggests that exploration of the HL-A system in the manner outlined might yield these kinds of data.

We thank Edith Zeller for technical assistance. This work was supported in part by a research grant from the National Institutes of Health, US Public Health Service, and the Gynne Hazen Cherry Memorial Laboratory.

Roy L. Walford
Simon Finkelstein
Robert Neerhout
Pat Konrad
Edward Shanbrom

Departments of Pathology and Pediatrics,
UCLA Medical Center, Los Angeles,
and the Research Division,
Hyland Laboratories.

Received October 13; revised December 5, 1969.

[1] Walford, R. L., *The Isoantigenic Systems of Human Leukocytes: Medical and Biological Significance* (Munksgaard, Copenhagen, 1969).
[2] Walford, R. L., *The Immunologic Theory of Aging* (Munksgaard, Copenhagen, 1969).
[3] Lilly, F., Boyse, E. A., and Olds, L. J., *Lancet*, ii, 1207 (1964).
[4] Tennant, J. R., *J. Nat. Canc. Inst.*, **34**, 633 (1965).
[5] Kourilsky, F. M., Dausset, J., Feingold, J., Dupuy, J. M., and Bernard, J., *Advances in Transplantation*, 515 (Munksgaard, Copenhagen, 1968).
[6] Troup, G. M., and Walford, R. L., *Amer. J. Clin. Pathol.*, **51**, 529 (1969).

Enzyme Patterns in Tetraparental Mouse Liver

Tetraparental mice are produced by fusing two eight-cell stage embryos. If the two embryos originate from different inbred strains, allelic differences between the strains produce mosaic patterns in the adult tetraparental which make possible inferences about development[1-3]. Mintz and Baker have elegantly used this concept to confirm that somatic muscle cells fuse during development[4]. They used an isozyme variant of isocitrate dehydrogenase, and found hybrid enzyme patterns in somatic muscle tissue. Only parental patterns were demonstrable in other tissues, such as the liver.

Mouse liver contains other enzymes which have known genetic variants. An interesting example is β-glucuronidase, which has high activity in some strains, low activity in others and intermediate activity in high–low hybrids. The high and low traits segregate as simple mendelian alleles (ref. 5, and personal communication from L. A. Herzenberg). The intermediate activity of β-glucuronidase in hybrid livers makes the enzyme a potentially useful marker to determine the mix of a tetraparental liver. This use was contemplated for an experiment different from that described here, but when the assay was carried out, some surprising variation in the data led to the experiment described here. The results suggest that the liver may be clonal in structure.

The livers of six C57Bl/10SnJ–C3H/HeJ tetraparental mice were removed after death, washed immediately in phosphate-buffered saline to remove excess blood and frozen between two blocks of dry ice. They were kept at or below −78·5° C until use. Samples weighing roughly 45 mg were chipped from the frozen organ, weighed, diluted 1 : 100 with distilled water, and homogenized in the cold with a Potter–Elvehjem glass homogenizer. Because the β-glucuronidase mutation affects intracellular location as well as certain physical properties of the enzyme[6], this stage was carried out in a rigidly standardized manner, in conditions known not to disrupt mitochondria (a setting of 4·5 on a Tri-R Stir-R homogenizer, using six passes of the pestle (personal communication from T. P. Bennett). The β-glucuronidase activity was measured by the method of Talalay *et al.*[7], as modified by Paigen[6], and all assays were carried out on the uncentrifuged crude homogenate. Each assay was performed in duplicate. The enzyme exhibits first order kinetics in the conditions used. Artificial mixtures of C57Bl/10SnJ and C3H/HeJ liver homogenates had activities that were proportionally intermediate. Assaying the six tetraparental livers on the first trial gave values ranging between the two parental values. The same picture emerged on the second trial, but the glucuronidase value for a given piece was in some cases quite different from that for the previous piece from the same tetraparental liver. The parental and hybrid values were much more consistent. To study this variation systematically, three of the tetraparental livers that had varied the most (T_3, T_4 and T_7) and one that had not varied more than the non-chimaeric pieces (T_{11}) were selected and analysed in seven experiments. In each experiment six approximately equal pieces of a tetraparental liver were analysed at the same time as six identically treated pieces of hybrid liver; once, twice or three times on a given liver. The mean, range and standard error of the mean for each set of six tetraparental pieces and paired hybrid pieces are given in Table 1. The standard errors of the two types of liver are significantly different, even when T_{11} is included (*T* test, $P < 0{\cdot}001$).

Table 1. β-GLUCURONIDASE ACTIVITY, IN μg PHENOLPHTHALEIN/G LIVER RELEASED FROM PHENOLPHTHALEIN GLUCURONIDE IN ONE HOUR AT 38° C

	Tetraparental livers				F₁ livers			
Exp. No.	Liver No.	Mean, 6 samples	Range	Standard error	Liver No.	Mean, 6 samples	Range	Standard error
1	T_3	1,370	1,100–1,900	132	F_7	2,090	2,015–2,150	27·4
2	T_4	2,200	1,015–3,210	302	F_6	1,805	1,575–1,965	66·7
3	T_4	2,120	1,645–2,615	160	F_7	1,955	1,855–2,050	26·2
4	T_4	2,205	1,930–2,565	117	F_4	1,910	1,785–1,995	33·8
5	T_7	2,610	1,595–3,065	219	F_5	2,200	2,065–2,360	47·9
6	T_7	1,440	1,060–2,150	152	F_4	1,980	1,900–2,040	27·8
7	T_{11}	3,605	3,390–3,960	85	F_7	2,050	1,925–2,235	37·3

The means and standard errors of six livers each from C57Bl/10SnJ, C3H/HeJ, and C57Bl/10SnJ–C3H/HeJ hybrids are 3,620 ± 223, 417 ± 9·3, and 2,050 ± 82·3 γ/g respectively.

The most obvious interpretation of the differential variation is that the cells producing the different values for β-glucuronidase are clonally distributed in the tetraparental liver. This conclusion is strengthened by rough internal consistency within the data. Tetraparental livers with an overall β-glucuronidase value close to that of the hybrid control in general have a higher variance than those whose activities approach parental values, as would be expected on a binomial sampling model. For example, liver T_{11}, the overall estimate of which is about the same as the C57Bl/10SnJ parent, has a sample variance which is just slightly above the highest hybrid variance. The high value for β-glucuronidase in this liver, coupled with its expected low variance, serves as an internal control, making it unlikely that differential treatment of tetraparental and hybrid livers produced the differential variance.

The binomial nature of the experimental situation allows an estimate of the number of clones found in the liver. Intuitively, if the clone size is very small relative to sample size (which was approximately 1/20 of the liver) there will be very little variance and, as this ratio increases, so will the variance, until one is dealing with a bimodal distribution. The theoretical variance of a given clone size and constant sample size may be obtained from the binomial distribution, if the assumption is made that all clones are equal in size and the average clone size is smaller than the average sample size. The variance V, expressed on a zero to one scale, then equals pq/N, and $N = pq/V$. In this equation p and q are the overall estimates of parental mix in the liver, expressed as fractions of the parental strains (again on a zero to one scale) and N is the number of clones in a sample. The experimental variance is assumed to be that of the hybrid liver, and is subtracted. The estimate, derived from all six experiments (excluding T_{11}), is $N = 17{\cdot}2 \pm 4{\cdot}12$ standard error of the mean, range $= 8{\cdot}06$ to $32{\cdot}8$. The conclusion that the liver is indeed clonal awaits confirmation by enzyme histochemistry.

I thank my colleagues for lively discussions and the use of their facilities. I especially thank Mr Jerry Bona for mathematical consultation. This work was performed under an institutional grant from Harvard University.

THOMAS G. WEGMANN

Biological Laboratories,
Harvard University,
Cambridge, Massachusetts 02138.

Received September 25, 1969.

[1] Tarkowski, A. K., *Nature*, **190**, 857 (1961).

[2] Mintz, B., *Amer. Zool.*, **2**, 432 (1962).

[3] Wegmann, T. G., and Gilman, J. G., *Devel. Biol.* (in the press).

[4] Mintz, B., and Baker, W. B., *Proc. US Nat. Acad. Sci.*, **58**, 592 (1967).

[5] Law, L. W., Morrow, A. G., and Greenspan, E. M., *J. Nat. Cancer Inst.*, **12**, 909 (1952).

[6] Paigen, K., *Exp. Cell. Res.*, **25**, 286 (1961).

[7] Talalay, P., Fishman, W. H., and Huggins, C., *J. Biol. Chem.*, **166**, 757 (1946).

Effect of Serum from Irradiated Rats on Gastrointestinal Function

A STRONG gustatory aversion results when an animal consumes a distinctively flavoured fluid during exposure to sublethal doses (10–30 r.) of ionizing radiation[1]. Similar aversive reactions are observed when wild rodents consume poisoned food baits[2]; or when laboratory rats drink flavoured water and are subsequently injected with an emetic drug[3]; or when these animals are injected with a small amount of serum (1·2 ml.) from a donor exposed to massive irradiation (30 krad) from cobalt 60 (ref. 4).

The gastrointestinal tract exhibits transient functional changes when exposed to ionizing radiation[5,6]. Thus it seems that the gustatory aversion which follows radiation exposure is a conditioned response resulting from the association of a particular flavour with "radiation sickness". A circulation by-product of energy absorption which causes gastrointestinal disturbance could account for the malaise as well as the aversive reactions observed. This hypothesis is not only of significance for the behavioural investigations, but is also of potential importance for clinical understanding and management.

To test this hypothesis we investigated the effect of both crude serum and serum fractions from irradiated donor rats on *in vitro* preparations of rat ileum and uterus. The *in vitro* preparation was in a standard 50 ml. chamber in which 2–3 cm pieces of tissue were bathed in oxygenated (5 per cent CO_2, 95 per cent O_2) Tyrode solution at 38·5° C. Recordings were made using a Harvard Apparatus Co. mechano-transducer and paper chart reader. After each test, the preparation was washed with 200 ml. of

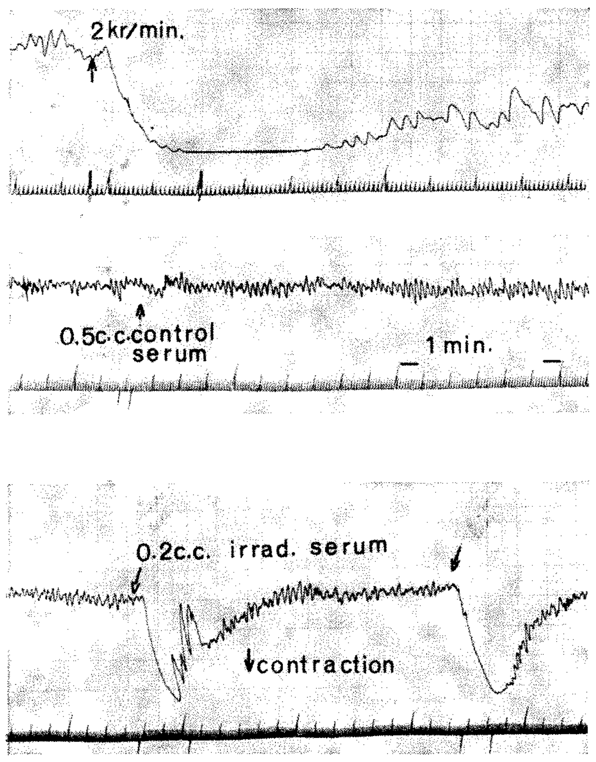


Fig. 1.

fresh Tyrode solution. Initially we found that serum from both irradiated and control rats produced an immediate increase in tone of several minutes' duration when topically administered *in vitro*. In many cases, however, the effect of the serum from irradiated donors was more pronounced than that from control donors.

Testing the assumption that both samples contained bradykinin which caused the increased tone and masked the irradiation effect, all *in vitro* solutions were perfused with sodium salicylate (10–20 mg/50 ml.), a potent blocker of bradykinin. In these conditions the addition of 0·2 ml. of the irradiated serum (10, 20 or 30 krad) to the bath caused a marked and prolonged contractile effect in thirty ileum preparations, while the control serum had little effect. Fig. 1 shows the response of an *in vitro* gut preparation to irradiated serum (bottom trace) along with a response to direct X-ray exposure (top trace). Control serum (middle trace) produced no changes. These data support the notion that radiation conditioned aversion is due to a humoral agent which produces noxious stimuli by affecting gastrointestinal smooth muscle function.

In an attempt to ascertain the nature of this putative humoral agent we used a 'Sephadex' gel fractionation procedure and tested the fractions on muscle bath preparations of rat or guinea-pig ileum. The initial fractionation, using 'Sephadex *G*–25', yielded two groups of active fractions in the irradiated serum, and one group of active fractions in the control serum. Both irradiated and control sera contained a fraction which elutes early (a protein or polypeptide). Assuming that this early fraction contained bradykinin, we pretreated ileum strips with sodium salicylate (20 mg/50 ml. Tyrode) which had effectively blocked the contraction of the gut evoked by control serum, but not that in response to serum from irradiated rats.

The other active fraction, found only in the serum from irradiated donors, elutes from the 'Sephadex' column at a position corresponding to some of the common biogenic amines. A number of pharmacological blocking agents were used to identify this second active fraction. Ten separate bioassays were performed with 1/1,000 and with 1/10,000 'Methysergide' (MS491 Sandoz), a serotonin blocking agent. Pretreatment of ileum strips with salicylate and atropine did not block the effect of irradiated serum, but salicylate and MS491 either blocked the response completely or markedly inhibited it. Because MS491 blocks not only serotonin but also histamine, a stronger antihistamine was tested.

In fifteen separate preparations pretreated with salicylate and chlortrimeton (1/1,000) the usual effect of irradiated serum was either totally or markedly blocked. Chlortrimeton also blocked the usual contractile response evoked by exposure of *in vitro* ileum preparations to X-ray (10 s at 1,000 r./min).

These findings strongly suggest that the aversion conditioning evoked by injection of serum from irradiated donors is caused by the effect of histamine on the gut. It also seems that the immediate transient contractile response of the gut to direct radiation exposure may involve the release of bound histamine, as indeed may the early phase of "radiation sickness".

This work was done at the Department of Biology, Boston University, and the Neurosurgical Service, Massachusetts General Hospital, and supported by the US Atomic Energy Commission and the US National Institutes of Health. F. R. E. is a career awardee of the National Institutes of Health.

CHARLES K. LEVY*

Department of Biology,
Boston University.

FRANK R. ERVIN

Department of Psychiatry,
Harvard Medical School.

JOHN GARCIA

Department of Psychology,
State University of New York
at Stony Brook.

Received March 17, 1969.

* Present address: Department of Zoology, PO Box 30197, University College, Nairobi, Kenya.

[1] Garcia, J., Kimeldorf, D. J., and Hunt, E. L., *Psychol. Rev.*, **68**, 383 (1961).
[2] Barnett, S. A., *The Rat—A Study in Behavior* (Aldine Press, Chicago, 1963).
[3] Garcia, J., and Koelling, R. A., *Radiat. Res.*, suppl., **7**, 439 (1967).
[4] Garcia, J., Ervin, F. R., and Koelling, R. A., *Nature*, **213**, 682 (1967).
[5] Conard, R. A., *Radiat. Res.*, **5**, 167 (1956).
[6] Levy, C. K., *Current Topics in Radiation Research*, **3**, 97 (1967).

Pituitary Growth Hormone Essential for Regulation of Serum Cholesterol

PITUITARY growth hormone was found to be essential in the normal regulation of the rat's serum cholesterol during a study of the cause(s) of the hypercholesterolaemia usually observed in the hypophysectomized rat.

To determine whether the hypercholesterolaemia occurring in the hypophysectomized rat was solely the result of impaired thyroid function, we first measured the serum cholesterol changes taking place in five groups of ten mature male rats (Long Evans strain) after (*a*) simple thyroidectomy (T), (*b*) thyroidectomy, castration and adrenalectomy (T–C–A); (*c*) removal of only the anterior lobe of the pituitary; (*d*) removal of only the posterior lobe of the pituitary; and (*e*) removal of both the anterior and posterior pituitary lobes. Following surgery, all rats (and also ten unoperated controls) were fed a liquid low-fat, low-cholesterol (40 mg/100 ml.) diet (55 ml. per day: an amount which corresponds to the average daily diet of normal rats). The rats were anaesthetized with ether and bled from the tail weekly.

Average serum cholesterol values (measured by the method of Martinek[1]) for the rats which were totally hypophysectomized (Fig. 1) rose progressively during the postoperative period. The value of 185 mg/100 ml. for

Whatman Biochemicals
Elastase
Lactate
Glyceraldehyde
Asparaginase
E coli strain K 12 CA 244 (Catalogue No
Frozen cell sludge Approximately 100 gm wet wt
Store deep frozen (-20°C) on arrival
Whatman
Springfield Mill

Enzymes in the Whatman tradition - purity and reliability

Whatman – already famous for the quality of their filtration and chromatographic materials – are now extending their field of operations to include enzymes and related biochemicals for all applications where purity and reliability are of high importance. The new Whatman range includes Lactate dehydrogenase, L-asparaginase, Elastase and Glyceraldehyde-3-phosphate dehydrogenase. They are all made to an extremely high standard of purity, and detailed specifications are given in the new Whatman Biochemicals' catalogue. The free loose leaf catalogue is now available and supplementary sheets will be sent to all catalogue holders as new products are introduced. Prices are extremely competitive.

Whatman – enzymes you can trust

Whatman Biochemicals Limited,
Springfield Mill, Maidstone, Kent, England.
Telephone: Maidstone 61688/9 Telex: 96113

I would like to receive a free copy of the new Whatman Biochemicals Catalogue.

NAME ____________________

ORGANISATION ____________________

ADDRESS ____________________

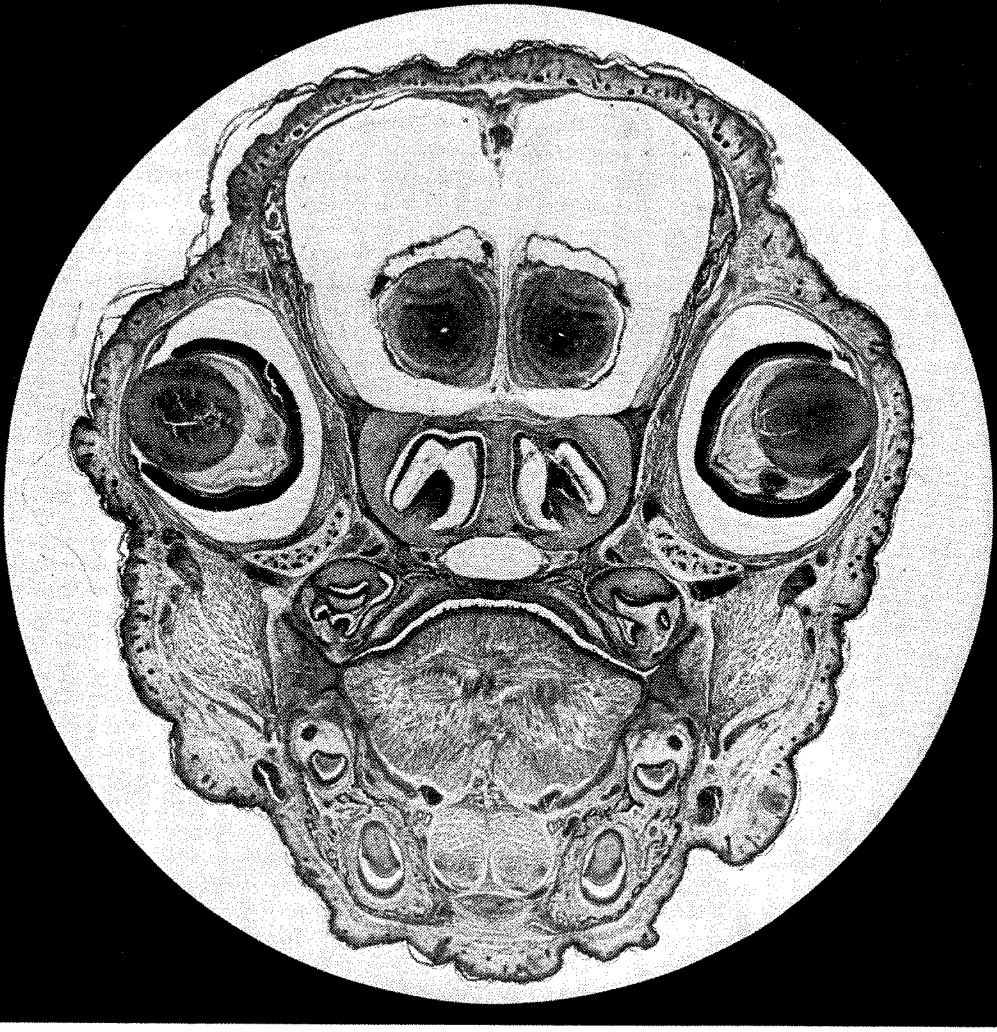
____

Whatman® Whatman Biochemicals Limited

® Registered trademark of W. & R. Balston Limited.

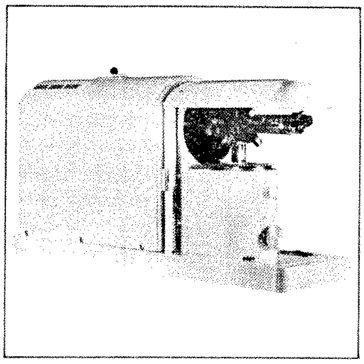
WB/PA/701/N

Take your classes down to microscopic size.



If you want to show a room-full of students what's going on under a microscope—instantly, clearly, and at several magnifications—the Zeiss Revolver Microprojector will do it for you. The mouse embryo illustrated above, showing the rudiments of the eyes, is an example of what can be shown. To change magnifications and go from an over-all view to successively smaller and more selective areas of your specimen, you simply revolve the nosepiece, bringing any of six objectives into play. Moreover, you can move the slide at any time to change the area under observation.
Two of the six objectives are the famous Zeiss LUMINARS, for low-power projection; the other four are PLANACHROMATS for magnifications up to 100X. All the objectives assure you a flat field, sharp from edge to edge. And a choice of four projection lenses lets you vary image diameters from about 3 ft. to 10½ ft. over projection distances of 10½ ft. to 65½ ft. The built-in multi-purpose illuminator takes either a CSI lamp or an XBO xenon lamp. The instrument is compact (35″ x 10½″ base; 19½″ high) and simple to operate.

For complete details write to the Sole UK Agents for Carl Zeiss of West Germany
Degenhardt & Co Ltd,
31/36 Foley Street,
London W1P 8AP.
Tel: 01-636 8050 (15 lines)



ZEISS
The great name in optics
CARL ZEISS **Degenhardt**

rats subjected to removal of just the anterior lobe was, moreover, approximately the same at the end of 3 weeks as that (190 mg/100 ml.) for the total hypophysectomized rats. For rats subjected to removal of just their posterior lobe the value (68 mg/100 ml.) was approximately the same at the end of 3 weeks as that (72 mg/100 ml.) for controls. It seems that the hypercholesterolaemia in the totally hypophysectomized rats arose solely from the absence of the anterior lobe.

As Fig. 1 demonstrates, hypophysectomized rats differed from thyroidectomized rats in that serum cholesterol rose not only more quickly but also to a much higher value during the 3 week postoperative period. On the other hand, those rats which were not only thyroidectomized but also castrated and adrenalectomized failed to exhibit a higher average serum cholesterol than those which were only thyroidectomized. These results suggested that the higher serum cholesterol of the hypophysectomized rats was not the result of hypofunction of either the sex or adrenal glands which is known to occur after removal of the pituitary. It is evident, moreover, that neither the absence of ACTH nor the sex stimulating hormones in the hypophysectomized rat were concerned in the hypercholesterolaemia.

A second study further indicated that the hypercholesterolaemia of the hypophysectomized rat could not be attributed solely to thyroid hypofunction following the absence of thyroid stimulating hormone (TSH). Thus when ten thyroidectomized and ten hypophysectomized rats were administered relatively physiological amounts of TSH (in the form of 2 mg of thyroid substance daily) after surgery, whereas the average serum cholesterol of the thyroidectomized rats did not increase, that of the hypophysectomized rats rose to an average level of 120 mg/100 ml., a value significantly higher than that of the controls and thyroidectomized rats treated with thyroid substance (Fig. 1).

A third study confirmed that the deranged cholesterol metabolism of the hypophysectomized rats arose from something more than thyroid hypofunction. Thus when ten hypophysectomized, nine thyroidectomized and five normal control rats were injected intravenously with sodium ^{3}H-acetate and killed 1 h later, analyses of their liver and serum cholesterol revealed (Table 1) that the percentage of incorporation of tritium in newly synthesized hepatic or plasma cholesterol was far less in the hypophysectomized than in the thyroidectomized rats.

In a fourth experiment, we demonstrated that not only the cholesterol but also the triglyceride metabolism of the hypophysectomized rat differed from that of the thyroidectomized rat. Thus 12 h after the intravenous injection of 250 mg Triton[2] into ten hypophysectomized rats, ten thyroidectomized rats and ten controls, the rise in serum cholesterol and triglyceride (Table 2) was much less in the hypophysectomized rats than in the thyroidectomized rats. Clearly the hypophysectomized rats were discharging less cholesterol and far less triglyceride into their blood than the thyroidectomized rats[2].

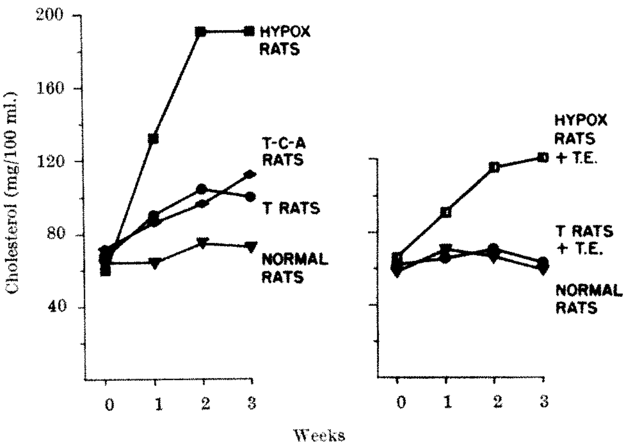


Fig. 1. Left: increase of serum cholesterol over 3 weeks in hypophysectomized (Hypox), thyroidectomized (T) and thyroidectomized-castrated-adrenalectomized (T-C-A) rats. Right: hypophysectomized (Hypox) rats given thyroid extract (T.E.) and thyroidectomized (T) rats given thyroid extract.

We concluded that hypercholesterolaemia taking place in the hypophysectomized rat was not the result solely of thyroid hypofunction and was probably totally independent of the sex and adrenal glands. The results also suggested that some other hormone of the pituitary anterior lobe besides TSH, ACTH, FSH and ICSH must be important in the regulation of serum cholesterol and that its absence leads to an increase in this sterol.

To identify this hormone, separate groups of male rats, immediately following extirpation of their pituitary glands, were given (*a*) ACTH (1 unit twice daily), (*b*) testosterone (0·5 mg three times a week), (*c*) thyroid extract (2 mg daily) and (*d*) purified bovine pituitary growth hormone (supplied by Dr C. **H.** Li, 100 µg three times daily). Normal and untreated hypophysectomized rats were also used. All rats were fed the low-fat, low-cholesterol liquid diet and were bled weekly to determine serum cholesterol concentration.

As Table 3 illustrates, neither the daily injection of ACTH nor the thrice weekly injection of testosterone altered the ensuing hypercholesterolaemia of the hypophysectomized rats. Thyroid extract—as was observed in the initial experiments—reduced but did not completely prevent the rise in plasma cholesterol of the hypophysectomized rats. Surprisingly, the administration of growth hormone was as effective as the thyroid extract in reducing the postoperative hypercholesterolaemia of the hypophysectomized rats.

In a second study, various combinations of the above hormones were administered to hypophysectomized rats. Groups of hypophysectomized rats were given (*a*) thyroid + ACTH + testosterone + growth hormone, (*b*) thyroid + ACTH + testosterone, (*c*) thyroid + growth hormone and (*d*) growth hormone + ACTH + testosterone, in the amounts already given. For control purposes, normal intact and untreated hypophysectomized rats and normal rats given both thyroid extract and growth hormone were observed. The animals were fed and bled as described.

Table 1. SPECIFIC ACTIVITY OF TRITIUM IN LIVER AND SERUM CHOLESTEROL OF THYROIDECTOMIZED AND HYPOPHYSECTOMIZED RATS

No. rats	Av. wt (g)		Liver Av. wt (g)	Liver Av. cholesterol Total (mg)	Liver Av. cholesterol (mg/g)	Cholesterol (disint./min/mg)	Av. serum cholesterol mg/100 ml.	Av. serum cholesterol disint./min/mg
		Control						
5	222		9·3	33·8	3·6	715	61	1,065
		Range:	(8·9–12·1)	(27–48)	(3·2–4·8)	(335–1,550)	(58–67)	(350–1,720)
		S.E.	±0·85	±4·8	±0·34	±239	±1·9	±263
		Thyroidectomized						
9	217		8·1	27·9	3·4	460	101*	303*
		Range:	(7·1–9·7)	(13·5–49·7)	(1·9–6·0)	(170–848)	(60–135)	(182–640)
		S.E.	±0·31	±3·8	±0·43	±94	±7·9	±56
		Hypophysectomized						
10	187		6·0†	36·2	6·0†	149†	202†	82†
		Range:	(5·4–6·9)	(28–51)	(4·5–8·3)	(71–217)	(130–280)	(36–127)
		S.E.	±0·17	±2·1	±0·33	±13·4	±15·0	±9·0

*** Mean is significantly ($P < 0·01$) different from corresponding value for normal control rats.**
† Mean is significantly ($P < 0·01$) different from corresponding value for thyroidectomized and normal control rats.

Table 2. SERUM CHOLESTEROL AND TRIGLYCERIDE RISE IN THYROIDECTOMIZED AND HYPOPHYSECTOMIZED RATS AFTER TRITON INJECTION

No.	Rats		Av. wt (g)	Av. serum cholesterol (mg/100 ml.) Before Triton	12 h after Triton	Av. net increase	Av. serum triglyceride (mg/100 ml.) Before Triton	12 h after Triton	Av. net increase
	Normal								
10			240	63	352	289	39	2,196	2,157
	Range:		(145–270)	(51–78)	(256–422)	(200–371)	(13–74)	(1,340–3,290)	(1,313–3,277)
		S.E.		±3·4	±12·2	±18·7	±5·9	±150	±148
	Thyroidectomized*								
10			232	100†	305†	214†	35	1,660†	1,625†
	Range:		(188–258)	(84–114)	(268–359)	(145–293)	(13–66)	(1,350–2,100)	(1,330–2,087)
		S.E.		±3·1	±10·8	±12·1	±4·8	±80	±71
	Hypophysectomized*								
10			223	137‡	283	145‡	20	661‡	641‡
	Range:		(197–263)	(99–192)	(234–400)	(115–295)	(7–44)	(323–1340)	(310–1310)
		S.E.		±8·2	±10·1	±17	±3·8	±89	±11

* Rats were subjected to thyroidectomy or hypophysectomy 1 week before study.
† Mean is significantly ($P < 0{\cdot}01$) different from corresponding mean for control normal rats.
‡ Mean is significantly ($P < 0{\cdot}01$) different from corresponding mean for thyroidectomized and control normal rats.

Table 3. EFFECT OF VARIOUS HORMONES ON SERUM CHOLESTEROL OF HYPOPHYSECTOMIZED RATS

	No.	Average weight (g) Initial	3 weeks	Gain or loss	Average serum cholesterol (mg/100 ml.) Initial	Week 1	Week 2	Week 3	Av. per 3 weeks
I. Normal rats									
A. Untreated	14	211	249	+38	68 (±3·1)*	65 (±4·0)	64 (±3·8)	69 (±4·1)	66 (±3·2)
B. Treated with thyroid extract plus growth hormone	10	220	253	+33	61 (±3·4)	71 (±4·1)	68 (±4·3)	60 (±5·1)	66 (±4·4)
II. Untreated hypophysectomized rats	25	208	182	−26 (±3·1)	58 (±3·5)	148 (±6·8)	168 (±8·8)	197 (±9·1)	171 (±5·9)
III. Hypophysectomized rats treated with:									
A. ACTH	7	197	174	−23 (±2·8)	58 (±3·1)	153 (±6·9)	153 (±19·0)	180 (±17·3)	162 (±12·5)
B. Testosterone	6	198	190	−8 (±2·7)	64 (±2·8)	149 (±5·9)	167 (±20·2)	188 (±16·8)	168 (±14·5)
C. Thyroid extract	12	236	217	−19 (±2·2)	63 (±2·9)	94 (±3·9)†	110 (±9·1)†	115 (±14·0)†	113 (±5·7)†
D. Growth hormone	15	202	209	+7	59 (±3·1)	95 (±7·9)	118 (±6·8)†	104 (±6·6)†	108 (±3·9)
IV. Hypophysectomized rats treated with:									
A. Thyroid extract									
(1) Plus ACTH + testosterone + growth hormone	10	228	227	−1	64 (±3·4)	57 (±4·4)‡	68 (±4·1)‡	73 (±2·8)‡	66 (±2·9)‡
(2) Plus ACTH + testosterone	8	182	176	−6	61 (±2·8)	115 (±5·6)†	104 (±4·5)†	109 (±8·2)†	109 (±3·9)†
(3) Plus growth hormone	15	201	193	−8 (±1·6)	62 (±3·8)	77 (±5·1)‡	74 (±3·3)	62 (±4·2)‡	71 (±2·4)‡
B. Growth hormone plus ACTH + testosterone	10	175	182	+7	58 (±2·9)	111 (±5·2)†	125 (±6·2)†	130 (±10·1)†	122 (±6·6)†

* *S.E.* of mean.
† Mean is significantly different from corresponding value for control untreated hypophysectomized rats ($P < 0{\cdot}001$).
‡ Mean is significantly different from corresponding value for control untreated hypophysectomized rats ($P < 0{\cdot}001$) but essentially the same as that for control normal untreated rats.

The results again indicated that either thyroid or growth hormone could inhibit significantly the development of hypercholesterolaemia in the hypophysectomized rat. When thyroid was administered with ACTH, testosterone and growth hormone, hypercholesterolaemia was completely prevented in the hypophysectomized rat (Table 3). When thyroid was given with just ACTH and testosterone, however, the partial inhibition of the hypercholesterolaemia achieved was no greater than that from administration of thyroid alone (compare III-C with IV-A-1 in Table 3). But when growth hormone was given with thyroid, the onset of hypercholesterolaemia was completely prevented. When ACTH and testosterone were given with growth hormone, the degree of inhibition of hypercholesterolaemia was no greater than that with growth hormone alone (compare III-D and IV-B). In the normal intact rat, the combined administration of both thyroid and growth hormone (I-B) did not influence the normal cholesterolaemic state present in these rats.

The requirement not only of physiological doses of thyroid but also similar doses of growth hormone for the normal cholesterolaemic status adds to the evidence for the essential part of growth hormone in cholesterol regulation. The administration of either hormone singly or together to the normal intact rat did not, however, reveal this. Apparently the normal animal possesses sufficient of these hormones so that moderate additions of either of them led to virtually no change in plasma cholesterol.

Although the administration of growth hormone has long been known to alter the plasma fatty acid level in the experimental animal[3-5] and humans[6,7], its action in the regulation of serum cholesterol was obscured partially by the fact that it usually has been administered to normal animals and in such large doses that a diabetic state ensued[5]—a complication which might counteract any possible hypocholesterolaemic effects of growth hormone itself. Further, this complication is perhaps present in most acromegalic patients for they are usually studied only after the severity of their pituitary hypersecretion of growth hormone has induced a diabetic state, which again might obscure any hypocholesterolaemic effects of excess growth hormone. It is interesting in this connexion that Camitta and Gray[8] recently described a patient suffering from acromegaly with diabetic lipaemia and hypercholesterolaemia who promptly exhibited a relative hypocholesterolaemia after his diabetes was alleviated by insulin. Finally, although growth hormone reputedly has a biological life of more than 9 h (ref. 9), this value was based on the persistence of its growth-affecting rather than on its lipid-affecting properties. Consequently, to demonstrate its part in the regulation of plasma cholesterol, one may need to administer growth hormone frequently, as we have done.

This work was supported by grants from the National Heart Institute, the Burroughs–Wellcome Fund, and the Tarrant County Texas Heart Association. We thank Dr C. H. Li of the University of California for a generous supply of growth hormone and suggestions concerning this study.

MEYER FRIEDMAN
SANFORD O. BYERS

Harold Brunn Institute,
Mount Zion Hospital and Medical Center,
1600 Civisadero Street,
San Francisco.

STEPHEN R. ELEK

University of Southern California,
School of Medicine,
Los Angeles,
California.

Received September 8, 1969.

[1] Martinek, R. G., *Clin. Chem.*, **11**, 495 (1965).
[2] Byers, S. O., and Friedman, M., *Amer. J. Physiol.*, **198**, 629 (1960).
[3] Campbell, J., in *The Hypophyseal Growth Hormone, Nature and Actions* (edit. by Smith, R. W., Gaebler, O. H., and Long, C. N. H.) (Blakiston Division, McGraw-Hill, New York, 1955).
[4] De Bodo, R. C., and Altszuler, V., *Vitamins and Hormones*, **15**, 205 (1957).
[5] Wilgram, G. F., Campbell, J., Lewis, L., and Patterson, J., *Diabetes*, **8**, 205 (1959).
[6] Raben, M. S., and Hollenberg, C. H., *J. Clin. Invest.*, **38**, 484 (1959).
[7] Raben, M. S., *New England J. Med.*, **266**, 31 (1962).
[8] Camitta, F. D., and Gray, T. K., *Diabetes*, **18**, 44 (1969).
[9] Van Dyke, D. C., Simpson, M. E., Li, C. H., and Evans, H. M., *Amer. J. Physiol.*, **163**, 297 (1950).

Dietary Sucrose and Platelet Behaviour

WE have reported[1] that six out of a group of nineteen healthy young men responded to a diet rich in sucrose by an increase in body weight, in plasma insulin level and in platelet adhesiveness. From this experiment we have suggested that only some people are susceptible to "sucrose-induced hyperinsulinism" and that this may be associated with their susceptibility to occlusive atherosclerotic disease. A further study[2] of two groups of middle-aged men, one suffering from peripheral vascular disease and the other apparently free from arterial disease, supported this suggestion.

Platelet adhesiveness may well play an important part in atherogenesis, so we thought it worth while to study in more detail the effect of dietary sucrose on platelet behaviour. We have therefore examined the electrophoretic mobility of platelets, according to the technique of Hampton and Mitchell[3]. This technique is more precise than that for platelet adhesiveness; moreover, mobility and adhesiveness are likely to be related phenomena[4].

Hampton and Mitchell[3] showed that the mobility of platelets of apparently healthy persons is not much affected by low concentrations (0·005 μg/ml.) of adenosine diphosphate (ADP) or noradrenaline, but is considerably increased by concentrations of 0·05 μg/ml. On the other hand, the mobility of platelets of persons with atherosclerosis is increased by the lower concentration of ADP but very little affected at the higher concentration of ADP, while the mobility in the presence of noradrenaline is the same as that of platelets from healthy persons.

We have studied three subjects previously shown to develop sucrose-induced hyperinsulinism, and three subjects shown not to do so. There were two men and one woman in each group. All seemed to be healthy by clinical examination, and showed no abnormalities in a twelve-lead electrocardiogram. After 5 days of preliminary study, including measurement of diet, they were told to substitute sucrose for starch in their diet as much as possible for the next 2 weeks, and then to revert to their normal diet. Before the beginning of the high sucrose diet, at its completion, and 2 weeks after its completion, the subjects were weighed and venous blood taken for determination of blood glucose[5], plasma insulin by immuno-assay[6] and platelet mobility[3]. The latter technique involves making a concentrated suspension of platelets by mixing one part of "platelet-rich plasma" (PRP) with nine parts of "platelet-poor plasma" (PPP). The PRP fraction is obtained by slow centrifugation of citrated blood, and careful collection of the layer just above the red and white cells; the PPP fraction is obtained by subsequent high speed centrifugation and collection of the supernatant plasma.

All six subjects behaved as they had in previous investigations. The three who had shown sucrose-induced hyperinsulinism did so again; the insulin concentrations and the body weights had increased significantly by the end of 14 days on high sucrose diets, and had fallen again at the end of the next 14 days on normal diets (Table 1). The remaining three subjects, who had not shown these changes previously, also failed to show them in this study.

At the beginning of the study, the platelet mobility of both groups of subjects was the same, and similar to that of normal people[3] (Fig. 1). At the end of the period of the high sucrose diets, however, the mobility was changed in the subjects with hyperinsulinism, but not in the other subjects. In the former, the mobility was similar to that found in atherosclerosis; maximal mobility with ADP occurred when this substance was present in the lower concentration of 0·005 μg/ml. (Fig. 2). After 14 days on normal diets, the behaviour of the platelets of these subjects had partly, but not completely, reverted to the "normal" behaviour seen at the beginning of the experiment (Fig. 3).

Bolton *et al.*[7] have shown that the abnormal platelet behaviour can be transferred to "normal" platelets by

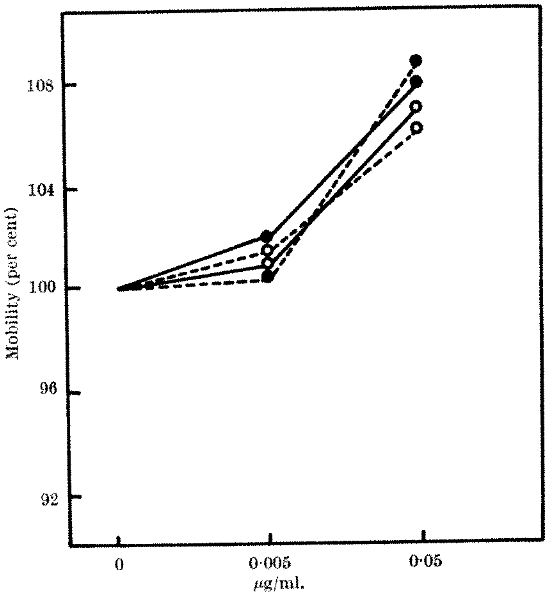


Fig. 1. Mean platelet electrophoretic mobility in presence of ADP (●) or noradrenaline (○), before sucrose diet. ———, Three subjects who later developed hyperinsulinism after sucrose; - - -, three subjects who did not develop hyperinsulinism.

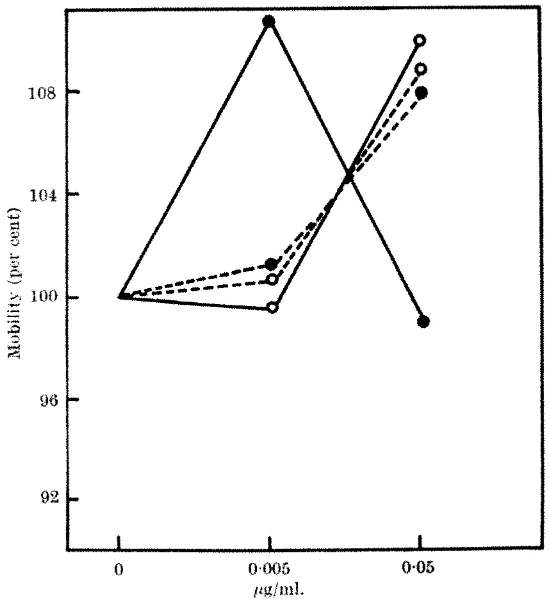


Fig. 2. Mean platelet electrophoretic mobility in presence of ADP (●) or noradrenaline (○), after 14 days on high sucrose diet. ———, Three subjects who developed hyperinsulinism; - - -, three subjects who did not develop hyperinsulinism.

Table 1. EFFECT OF HIGH-SUCROSE DIET IN SIX SUBJECTS (MEAN VALUES)

Diet	Group 1						Group 2					
	kcalories per day	Weight (kg)	Sucrose (g/day)	Insulin (μU/ml.)			kcalories per day	Weight (kg)	Sucrose (g/day)	Insulin (μU/ml.)		
				0	30 min	120 min				0	30 min	120 min
Normal	3,050	69·7	142	23	67	36	3,230	73·1	150	22	68	34
High sucrose	3,130	71·9	427	28	135	80	3,350	73·6	418	21	69	33
Normal	—	69·6	—	24	71	39	—	73·3	—	23	70	34

Group 1, three subjects previously shown to develop "sucrose-induced hyperinsulinism".
Group 2, three subjects previously shown not to develop "sucrose-induced hyperinsulinism".
Insulin measured before and at intervals of 30 min and 120 min after 50 g of glucose orally.

the plasma from subjects with atherosclerosis. This was also true of the behaviour of the platelets in our subjects with sucrose-induced hyperinsulinism. When the plasma (PPP) from these subjects, at the end of 14 days of high sucrose intake, was added to the platelets (PRP) from the other subjects, the mobility with ADP was changed to that characteristic of "abnormal" platelets (Fig. 4).

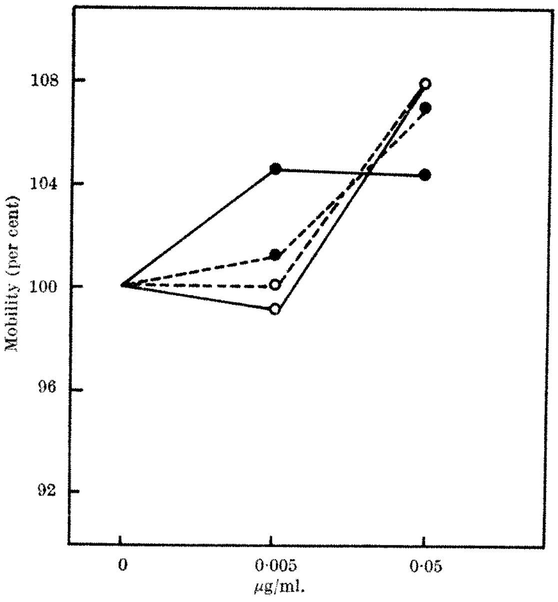


Fig. 3. Mean platelet electrophoretic mobility in presence of ADP (●) or noradrenaline (○), 14 days after completion of high sucrose diet. ———, Three subjects who had developed hyperinsulinism; - - -, three subjects who had not developed hyperinsulinism.

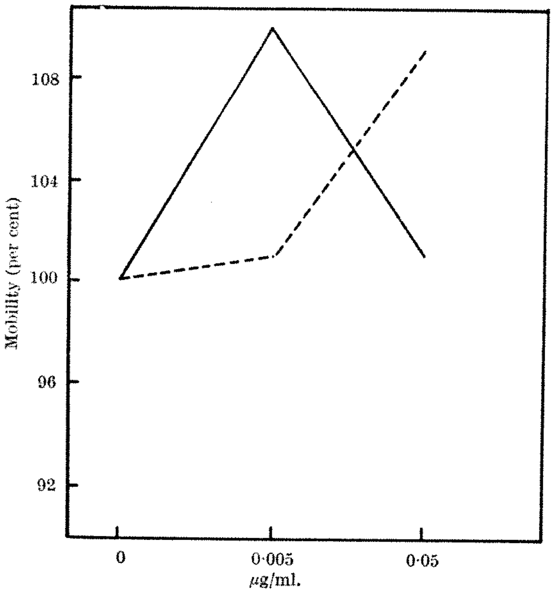


Fig. 4. Effect of ADP on "normal" platelets (PRP) suspended in "normal" or in "abnormal" plasma (PPP). Mean of three subjects. ———, Normal platelets in abnormal plasma; - - -, normal platelets in normal plasma.

We considered the possibility that the abnormality produced by dietary sugar was a direct result of the higher level of insulin in the plasma, but this was unlikely, for after 2 weeks on normal diet the hyperinsulinism induced by sucrose had virtually disappeared, while a fair degree of platelet abnormality was still seen (Table 1, Fig. 3). Nevertheless, we measured platelet mobility in the presence of 200 μU/ml. of porcine insulin, and found that it was quite unaffected.

We thank the British Heart Foundation for financial support.

STEPHEN SZANTO
JOHN YUDKIN

Department of Nutrition,
Queen Elizabeth College,
London W8.

Received December 9, 1969.

[1] Szanto, S., and Yudkin, J., *Postgrad. Med. J.*, **45**, 602 (1969).
[2] Yudkin, J., Szanto, S., and Kakkar, V. V., *Postgrad. Med. J.*, **45**, 608 (1969).
[3] Hampton, J. R., and Mitchell, J. R. A., *Lancet*, ii, 764 (1966).
[4] Elkeles, R. S., Hampton, J. R., and Mitchell, J. R. A., *Lancet*, ii, 315 (1968).
[5] Huggett, A. St G., and Nixon, D. A., *Lancet*, ii, 368 (1957).
[6] Hales, C. J., and Randle, P. J., *Biochem. J.*, **88**, 137 (1963).
[7] Bolton, C. H., Hampton, J. R., and Mitchell, J. R. A., *Lancet*, ii, 1101 (1967).

Effect of Early Malnutrition on the Reaction of Adult Rats to Aversive Stimuli

A GREAT deal of interest has been shown in the relationship between early malnutrition, especially protein–calorie malnutrition, and intellectual capacity or learning ability. But early malnutrition may cause other changes in behaviour. An animal's motivation for food or water may be affected if it is deprived of them in early life[1–4]. In a non-appetitive situation Barnes *et al.*[5] noted a "motivational and emotional" difference in previously malnourished rats in learning a water maze. Moreover, adult pigs which had been fed protein-poor diets early in life and then given a full diet took longer to extinguish a conditioned avoidance response than controls, although there was little difference in the rate of acquisition of the response[6]. This has also been reported in rats when there was more intertrial responding in previously malnourished animals[7]. We have investigated whether a brief period of malnutrition very early in life increases the emotional reactivity of the adult to aversive stimulation.

Holtzman rats were distributed in litters consisting of eight pups to a dam. During suckling, half the dams were given a purified diet containing 21 per cent protein (as 25 per cent casein) and the rest were given diets containing 10 per cent protein (as 12 per cent casein) to restrict the availability of milk during the pre-weaning period. All pups were allowed to nurse for 3 weeks, and then the males were taken for further study. These animals were fed a purified diet containing either 21 or 2·5 per cent protein (as 25 and 3 per cent casein respectively) for the next 4 weeks, after which all animals received the 21 per cent protein diet. The behavioural tests started when rats were about 16 weeks old. Three tests were performed, each with different animals.

First we used a modified open field apparatus, a large wooden box (27·0 × 34·5 × 45·5 cm), open on top with a 'Plexiglas' front. The bottom was marked with six squares. In three 10 min sessions the animals became adapted to the apparatus. For the fourth session they were placed in the apparatus as usual, but half-way through the session a 28 V solenoid attached to the rear of the chamber was activated to make a loud, sharp noise. The amount of locomotor activity (number of squares crossed) before and after the noise was recorded.

In the second test we examined the effect of early malnutrition on passive avoidance behaviour. The animal was placed on a small (12·7 × 7·6 cm) platform which was elevated 7·6 cm from an electrified grid floor. Immediately after the animal stepped off the platform it received a 0·8 mA electric shock for 0·5 s. The animal was returned to its home cage for 60 s and then replaced on the platform. The time taken to leave the platform was then recorded (although after 5 min the animal was removed).

In the last behavioural test of this series we investigated whether previously malnourished rats would avoid an electric shock more actively than others. The animals were placed in a small compartment and required to press a bar to postpone a brief electric shock (1 mA lasting 0·5) for 20 s (S—S = R—S = 20). The animals were given five sessions lasting 165 min.

Fig. 1 shows growth patterns during the experiment. The restriction of protein both before and after weaning had a significant effect ($P < 0{\cdot}001$) on the rate of growth and the final weight—the less protein the smaller the animal.

Table 1. RESULTS OF BEHAVIOURAL TESTS AND EXPERIMENTAL CONDITIONS

Pre-weaning diet: Post-weaning diet:	Control Control	Restricted Control	Control Restricted	Restricted Restricted
Test 1 (per cent locomotor reduction)	$56{\cdot}0^a$ (7)	$51{\cdot}8^a$ (7)	$65{\cdot}5^b$ (7)	$74{\cdot}3^c$ (7)
Test 2 (reciprocal latency)	$0{\cdot}095^a$ (7)	$0{\cdot}020^b$ (7)	$0{\cdot}052^b$ (7)	$0{\cdot}020^c$ (7)
Test 3 (bar press per min)	$3{\cdot}97^a$ (8)			$5{\cdot}59^b$ (8)

Values within a row not designated with the same letter are statistically significant ($P < 0{\cdot}01$).
The numbers of animals in each group are shown in parentheses.

The results of the behavioural tests are given in Table 1. The typical response to the noise in the first experiment was first to run to a corner, crouch and show considerable signs of emotional stress—defaecation and urination. Later this response waned, but the animals usually remained fairly close to the corner and further exploration of the box was inhibited. The scores represent the percentage reduction of motility (number of squares crossed in the last 5 min compared with the 5 min before the noise) after the activation of the solenoid. Clearly, rats that had been malnourished by receiving the 2·5 per cent protein diet made a greater response to the noise than the control rats. Moreover, pre-weaning restriction of protein was not itself sufficient to alter the response in this measure.

Rats tested for passive avoidance responded according to the same pattern, and the animals malnourished in early life made a significantly greater passive avoidance response than controls, as their longer time on the platform shows. This test revealed a significant effect of pre-weaning restriction of protein. There were no differences in the initial delay when the animals left the platform.

Not only did the animals malnourished early in life react more intensely to an aversive stimulus, but they responded faster in the Sidman avoidance situation, as the results of the third test show (Table 1). Another measure recorded in the Sidman avoidance situation, but not shown in Table 1, was the time for which the bar was depressed. This measure also reflected the greater response of these animals to the aversive conditions.

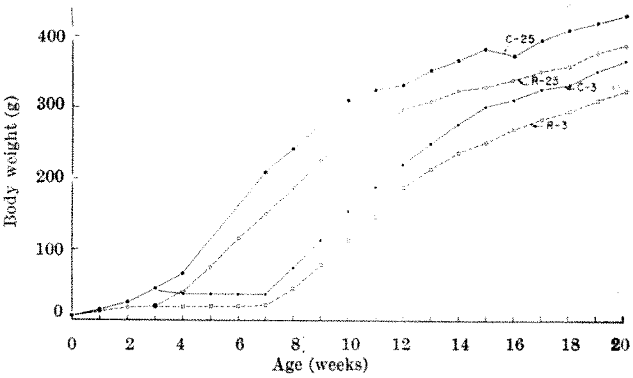


Fig. 1. Growth curves of rats malnourished early in life. C, Rats suckled by dams given 25 per cent casein (control pre-weaning); R, rats suckled by dams given 12 per cent casein; 25, rats on a diet containing 25 per cent casein during the first 4 weeks after weaning; 3, rats on a diet containing 3 per cent casein during the first 4 weeks after weaning.

When casually observed in their home cage, rats that have been malnourished are not distinguishable from the controls apart from being smaller. The behavioural difference becomes apparent only when they are placed in a moderately stressful situation. This suggests that one effect of malnutrition during early development is to produce a rather long lasting, if not permanent, lowering of the threshold of the stress–response system. There may be some support for this idea in the finding that the adrenals of early malnourished animals are consistently larger than in controls. Several workers have suggested that adrenal–pituitary secretions play a part in the behavioural response to stress[8-10].

It is not yet clear whether early malnutrition results in deficient learning ability[11,12]. The data presented here, however, suggest that severe malnutrition experienced very early in life results in other long lasting, if not permanent, behavioural abnormalities.

This work was supported in part by the National Institute of Child Health and Human Development, the US National Institutes of Health, and the Nutrition Foundation.

DAVID A. LEVITSKY
RICHARD H. BARNES

Graduate School of Nutrition,
Cornell University,
Ithaca, New York 14850.

Received September 15; revised December 29, 1969.

[1] McV. Hunt, J., *J. Abnorm. Soc. Psychol.*, **36**, 338 (1941).
[2] McV. Hunt, J., Schlosberg, H., Solomon, R. L., and Stellar, E., *J. Comp. Physiol. Psychol.*, **40**, 291 (1947).
[3] Marx, M. H., *J. Comp. Physiol. Psychol.*, **45**, 43 (1952).
[4] Mandler, J. M., *J. Comp. Physiol. Psychol.*, **51**, 513 (1958).
[5] Barnes, R. H., Cunnold, S. R., Zimmermann, R. R., Simmons, H., MacLeod, R. B., and Krook, L., *J. Nutrit.*, **89**, 399 (1966).
[6] Barnes, R. H., Moore, A. U., Reid, I. M., and Pond, W. G., in *Malnutrition, Learning, and Behavior* (MIT Press, 1968).
[7] Frankova, S., and Barnes, R. H., *J. Nutrit.*, **96**, 485 (1968).
[8] Latane, B., and Schachter, S., *J. Comp. Physiol. Psychol.*, **55**, 369 (1962).
[9] Levine, S., and Soliday, S., *J. Comp. Physiol. Psychol.*, **55**, 214 (1962).
[10] Weiss, J. M., McEwen, B. S., Silva, M. T. A., and Kalkut, M. F., *Science*, **163**, 197 (1969).
[11] Latham, M. C., in *Nutrition and Intellectual Growth in Children*, Bulletin 25-A, 33 (Association for Childhood Education International, 1969).
[12] Winick, M., in *Nutrition and Intellectual Growth in Children*, Bulletin 25-A, 25 (Association for Childhood Education International, 1969).

Spread of Responses in the Cerebral Cortex to Meaningful Stimuli

THE primary visual receiving area of the mammalian cerebral cortex is readily excited by sudden alterations in the intensity of light falling on either retina. Techniques developed by Hubel and Wiesel[4] have made it possible to record the responses of individual cortical neurones to appropriately placed retinal stimuli of the right shape. The response of single units to the proper form of physiological stimulus is sometimes so clear that a single flash or movement of a pattern of light in the visual field will invariably produce a burst of discharges from the recorded neurone. Most neurones in the lightly anaesthetized or unanaesthetized visual cortex, however, are less predict-

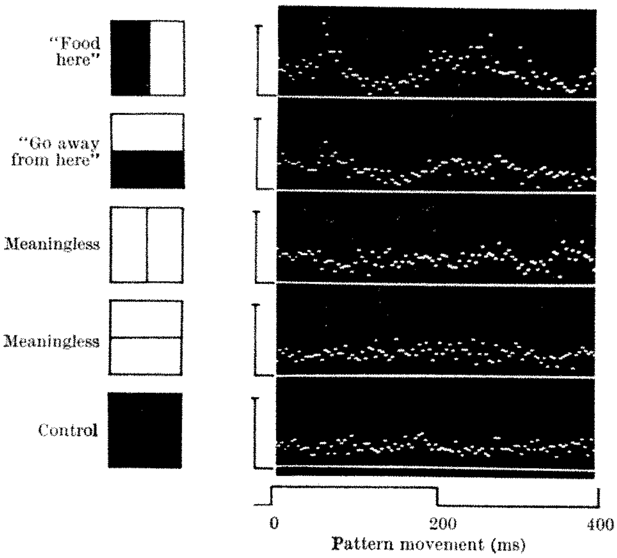


Fig. 1. Post-stimulus histograms obtained from a single cell (cat *A*, cell 1) in the anterior contralateral supra-sylvian gyrus in response to the various patterns shown. Ordinate, count/4 ms × 600. Recording time, 4 min. Pattern movements were at right angles to the pattern border.

able in their behaviour, and their response to retinal excitation is best demonstrated by averaging procedures[2]. A post-stimulus histogram (PSH) is obtained, which displays the number of neuronal discharges occurring at various times after each of a series of identical retinal stimuli. Thus the PSH indicates the distribution of probability of unit discharge, following visual excitation.

We have found that, in the cat, the responses of cortical neurones lying outside the primary receiving area are extremely weak, so that it is often impossible to produce any detectable response from neurones in unanaesthetized parietal cortex. This fact is not altogether surprising, for an intact animal would not be expected to pay the least attention, or to respond in any way, to the sort of patterns projected on the retina during physiological experiments. To the intact animal such patterns would be "meaningless". On the other hand, a more widespread cortical response to retinal excitation with "meaningful" patterns might be expected; this would have produced some predictable motor response from the intact animal. We have therefore begun a series of experiments designed to compare the spread, outside the visual area, of responses to "meaningful" and "meaningless" patterns presented in the visual field.

Cats were trained to respond to simple geometrical patterns by teaching them to choose between two possible sources of food on the basis of the pattern displayed. In this way, they learned that one pattern meant "food here", and the other meant "go away from here or you will get a mild shock". When an animal was trained to 80–90 per cent efficiency, it was anaesthetized with volatile anaesthetic and the forebrain was neurologically isolated from the rest of the nervous system without damage to its blood supply[1]. Artificial respiration was supplied and, after the operation, the preparation was taken off anaesthetic. This procedure produces an unanaesthetized brain with no motor outputs and only one functional input—the visual pathway. Despite surgical interference, the cortical neurones still display spontaneous activity and the units in the primary visual area respond to retinal excitation in a manner very similar to that seen in the lightly anaesthetized animal. To excite the visual system, we projected patterns on a pearl-glass screen about 30 cm in front of the cat. These patterns were focused on one retina by a 10 dioptre lens and were provided with a rectangular oscillation of approximately 30 s arc and 3 c.p.s. frequency[3]. An averaging computer was used to record the probability of unit discharge at various times after the two movements of the stimulating pattern. We excited the retina with two classes of pattern: (*A*) "meaningful" patterns which produced predictable motor responses in the trained animal: (*B*) similar patterns which the animal had not seen before ("meaningless"). All these were patterns which, in our experience, produce approximately equal excitation of cells in primary visual area.

Table 1. RELATIVE MAGNITUDE OF RESPONSES TO MEANINGFUL AND MEANINGLESS PATTERNS

			Patterns listed in order of magnitude of response			
Cortical area	Cat	Cell	Greatest	....	....	Least
Parietal	*A*	1 (Figure)	+	+	○	○
		2	+	+	○	○
		3	+	+	○	○
	B	1	+	+	○	
		2	+	○	+	
		3	+	○	+	
	C	1	+	+	○	
		2	+	+	○	
		3	+	○	+	
Auditory	*B*	4	+	○	+	
		5	+	○	+	
Sensori-motor	*A*	4	+	+	○	○
		5	+	○	○	+
	C	4	+	+	○	
Percentage of meaningful patterns in column			100	54	39	10

+, Meaningful patterns: "food here" or "go away from here". ○, Meaningless patterns. Brackets indicate equal response.

Fig. 1 shows five post-stimulus histograms obtained from the same cell in the "parietal" area (anterior part of the contralateral supra-sylvian gyrus). The first four histograms show responses to the various patterns indicated. The fifth represents a control, recorded with the projector lamp switched off, but the rest of the apparatus active; this indicates the sampling error to be expected. It is clear that the two meaningful patterns produced a greater disturbance of the neurone's behaviour than did the two meaningless patterns. We have obtained similar results from fourteen cells in three cats. These results are summarized in Table 1. Response magnitude was assessed visually in terms of the amount of deviation from a horizontal line, taking into account the sampling error to be expected. The first column of the table contains only meaningful patterns, with the meaningless patterns in the second and third columns (only cat *A* was tested with two forms of meaningless stimulus). It would clearly be dangerous to base generalizations on such a small number of experiments, but we feel that these preliminary results were of sufficient interest to be reported.

B. DELISLE BURNS
A. C. WEBB

National Institute for Medical Research,
Mill Hill, London NW7.

Received October 8, 1969.

---

[1] Bliss, T. V. P., Burns, B. D., and Uttley, A. M., *J. Physiol.*, **195**, 339 (1967).
[2] Burns, B. D., *The Uncertain Nervous System*, 194 (Edward Arnold, London, 1968).
[3] Burns, B. D., and Pritchard, R., *J. Physiol.*, **197**, 149 (1968).
[4] Hubel, D. H., and Wiesel, T. N., *J. Physiol.*, **148**, 574 (1959).

Subcellular Localization of Serotonin in an Identified Serotonin-containing Neurone

KNOWLEDGE of the subcellular localization of serotonin (5-hydroxytryptamine) in neurones is important for an understanding of the precise physiological role of this amine in nervous tissue. Some of the most pertinent pharmacological and electrophysiological data on the neuronal role of serotonin have been obtained from work with molluscs[1,2]. In particular there is strong evidence that the amine acts as a transmitter on one particular type of neurone in the gastropod brain[2]. Fractionation studies with homogenates of bivalve ganglia, which are extremely rich in serotonin, have shown that the amine is bound to particles, but associated with particles of different density from those binding acetylcholine[3]. The precise type of particle or particles associated with serotonin, however, is not definitely known[4].

In the ganglia of gastropod molluscs are very large neurone somata, some of which contain amines[5]. We have examined in detail one particularly large serotonin-containing neurone, which is readily located in different preparations, in each cerebral ganglion of the slug *Limax maximus*. Results of the experiments show that serotonin is bound within small granulated vesicles of 40 to 100 nm in diameter and also, in certain conditions, in particles which resemble lysosomes.

Using standard methods of amine-fluorescence histochemistry[6], a giant yellow-orange fluorescing neurone was observed in each metacerebral ganglion of *Limax maximus*. The fluorescence was judged specific for serotonin using the usual criteria of colour, reducibility, fading and absence without paraformaldehyde sublimation. After the yellow fluorescence had faded, no green fluorescence indicative of catecholamines was observed.[7] A group of small brilliantly green fluorescing cells close to each giant cell provided convenient material for comparison. Reserpine, injected in amounts of 0·1 to 1 mg during 24 to 48 h, greatly reduced or abolished the fluorescence. Further evidence for the presence of serotonin and absence of catecholamines was provided by the effects of different drugs on the fluorescence and by bioassay of individual cells.

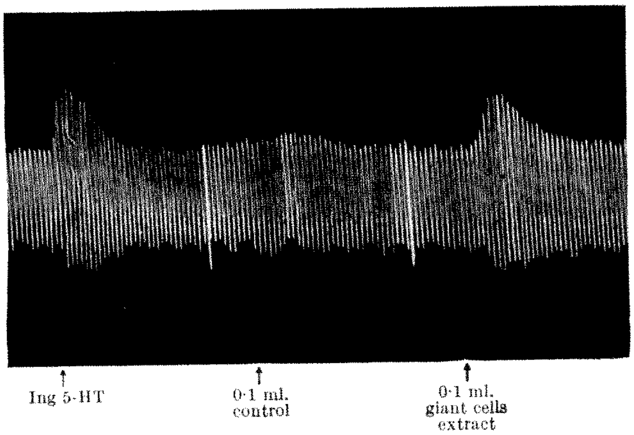


Fig. 1. Responses of an isolated heart of *Helix pomatia* to serotonin (5-HT), to an extract prepared from individually isolated giant serotonin cells and to a similarly prepared extract of an equivalent number of non-fluorescing cells (control).

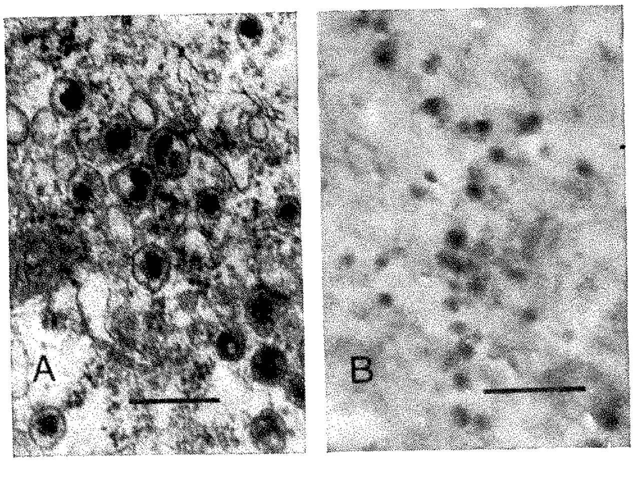


Fig. 2. *A*, Small group of granular vesicles in a giant serotonin cell fixed in glutaraldehyde and osmium and stained with lead citrate and uranyl acetate. (The bar represents 0·2 μm.) *B*, Electron dense deposits, representing sites of amine localization, in a giant serotonin cell. Tissue was processed by Wood's method[12]. The deposits are the same size as the granule centres in *A*. (The bar represents 0·5 μm.)

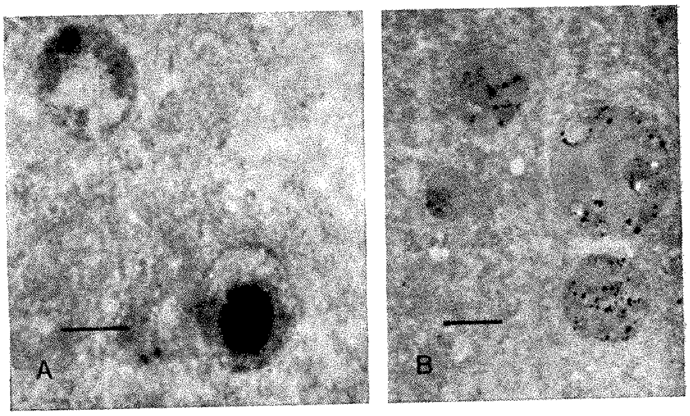


Fig. 3. Staining, indicative of an amine, in particles which resemble lysosomes in the giant serotonin cell. *A*, Tissue fixed and stained by Wood's method[12]. (The bar represents 0·5 μm.) *B*, Tissue processed by the method of Cannata *et al.*[15]. (The bar represents 1 μm.)

The intensity of yellow fluorescence was reduced by injecting *p*-chlorophenylalanine, which blocks serotonin formation[7], though not altered with α-methyl-*m*-tyrosine, an inhibitor of tyrosine hydroxylase[8]. Injections of the precursor of serotonin, 5-hydroxytryptophan, increased the intensity of fluorescence, but there was no change after injecting the catecholamine precursor 3,4-dihydroxyphenylalanine.

Isolated cells were assayed on the heart of *Helix pomatia*, a preparation known to be remarkably sensitive to serotonin but insensitive to catecholamines[9]. Control extracts were prepared from the same number of neurone somata of a similar size, which did not fluoresce. Giant metacerebral cell extracts increased the amplitude and frequency of heart-beat in a manner indistinguishable to that produced by serotonin whereas control extracts had no effect (Fig. 1). Bromo-lysergic acid diethylamide antagonized the effect of the giant cell extract as well as that of serotonin. These experiments provided strong independent evidence for the presence of serotonin in the metacerebral cells. On the basis of other experiments made on closely related species[10] and on the sensitivity of *Helix* hearts to different agents[9], we concluded that there is no substance other than serotonin which could have accounted for the pharmacological effect, certainly not dopamine, noradrenaline or adrenaline. From the results of seven assay experiments, the serotonin content was estimated as 0·7 ng/cell. The total volume of the giant cell is about 1·3 nl, but about one-fifth of this is occupied by the nucleus, which does not fluoresce. Thus the concentration of serotonin in the cytoplasm of the cell soma is 4×10^{-3} M.

Electron microscopy of the cell revealed the presence of large numbers of vesicles with irregular granular cores (Fig. 2*A*), together with elongated mitochondria, particles resembling lysosomes and other inclusions previously reported in molluscan neurones[11]. Ganglia removed from slugs during the summer months and fixed and processed by the method of Wood[12] for detecting amines contained electron dense reaction products in the small granules of the serotonin-containing cell (Fig. 2*B*). Prior injection of reserpine greatly reduced the number of granules in the serotonin-containing cell. The histochemical method has been shown to be specific for amines in molluscan tissues[9]. We therefore concluded that serotonin is sequestered within the small granules. The granules have similar dimensions to those which were suggested to bind primary catecholamines and serotonin in the nerves of the snail heart[9] and catecholamines in bivalve ganglia[13] and also to the granules of the Retzius cell of one leech, which are considered to bind serotonin[14].

Tissue studied earlier in the early spring of this year provided evidence for an additional site of binding of serotonin. At this time, preparations stained for the ultrastructural localization of amines showed dense reaction products in certain irregular areas of the particles resembling lysosomes (Fig. 3). In post-osmicated tissue, areas of the "lysosomes" also appeared extremely electron dense. After treatment with reserpine, dense staining was only rarely observed in tissue processed by the two histochemical methods used. It therefore seems that in some conditions as yet insufficiently specified, but perhaps associated with the season, serotonin may also be associated with particles which resemble lysosomes in these neurones. In connexion with this observation we have repeatedly noted in tissue fixed and stained by standard methods the occurrence of small granulated vesicles in the "lysosomes" of the giant serotonin cells.

It is interesting to note that the serotonin-containing neurones described here are homologous to the *Helix* metacerebral neurones which exhibit marked anomalous rectification[16]. We have observed that these *Helix* cells also contain serotonin in about the same concentration as those of *Limax*.

We thank the Wellcome Trust for financial aid.

G. A. COTTRELL
N. N. OSBORNE

Wellcome Laboratories of Pharmacology,
Gatty Marine Laboratory,
University of St Andrews,
Scotland.

Received October 6, 1969.

[1] Cottrell, G. A., and Laverack, M. S., *Ann. Rev. Pharmacol.*, **8**, 273 (1968).
[2] Stefani, E., and Gerschenfeld, H. M., *J. Neurophysiol.*, **32**, 64 (1969).
[3] Cottrell, G. A., *Comp. Biochem. Physiol.*, **17**, 891 (1966).
[4] Cottrell, G. A., and Maser, M., *Comp. Biochem. Physiol.*, **20**, 901 (1967).
[5] Dahl, E., Falck, B., von Mecklenburg, C., Myhrberg, H., and Rosengren, E., *Z. Zellforsch.*, **71**, 489 (1966).
[6] Corrodi, H., and Jonsson, G., *J. Histochem. Cytochem.*, **15**, 65 (1967).
[7] Koe, B. K., and Weissman, A., *J. Pharmacol. Exp. Therap.*, **154**, 499 (1966).
[8] Spector, S., Sjoerdsma, A., and Udenfriend, S., *J. Pharmacol. Exp. Therap.*, **147**, 86 (1965).
[9] Cottrell, G. A., and Osborne, N. N., in *Comparative Physiology of the Heart: Recent Trends* (edit. by McCann, F. V.), *Experientia* Suppl., 15 (1969).
[10] Kerkut, G. A., and Cottrell, G. A., *Comp. Biochem. Physiol.*, **8**, 53 (1963).
[11] Amoroso, E. C., Baxter, M. I., Chiquoine, A. D., and Nisbet, R. H., *Proc. Roy. Soc.*, B, **160**, 167 (1964).
[12] Wood, J. G., *Nature*, **209**, 1131 (1966).
[13] Cottrell, G. A., in *Neurobiology of Invertebrates* (edit. by Salanki, J.), 353 (Plenum, 1968).
[14] Rude, S., Coggeshall, R. E., and Van Orden, L. S., *J. Cell. Biol.*, **41**, 832 (1969).
[15] Cannata, M. A., Chiocchio, S. R., and Tramezzani, J. H., *Histochemie*, **12**, 253 (1968).
[16] Kandel, E. R., and Tauc, L., *J. Physiol.*, **183**, 287 (1966).

Annual Production and Respiration in Animal Populations

ENGELMANN[1] has examined the relationship between annual production and annual respiration in kilocalories per m² per year for terrestrial animal populations. We have re-examined this relationship in the light of a large number of further studies, taking into account aquatic and marine animals not considered by Engelmann and correcting a number of minor errors in his article, and we have tried to explain several features of the relationship in terms of the life histories of the species investigated.

We have used the following terminology[2]: $R =$ annual population respiration, $P =$ annual population production (including somatic growth, eggs and exuvia), $R + P = A =$ annual population assimilation; all expressed as kilocalories per m² per year.

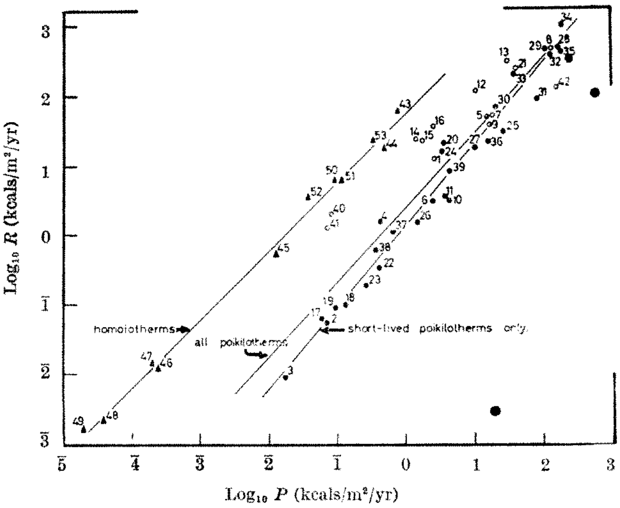


Fig. 1. The relationship between annual production (P) and annual respiration (R) (kcalories/m²/yr) in animal populations. The numbers associated with each point correspond to the numbers in the first column of Table 1. ▲, Homoiotherms; ○, long-lived poikilotherms; ●, short-lived poikilotherms.

The data reviewed are presented in Table 1. We have omitted those studies that assume *a priori* P/R ratios (for example, data on nematodes and *Prokelisia* included by Engelmann), and "old field" data on *Philaenus*[21] in which R was greatly inflated by immigration of adults; also omitted are data on the ant *Pogonomyrmex*[30] shown by Engelmann to be atypical, and data on the planarian *Phagocata*[19], which includes large quantities of mucus in the estimates of P. Data from Table 1 are plotted in Fig. 1. (Note that data on *Passerculus* and *Mustela*, plotted incorrectly by Engelmann, have been corrected.)

Because direct and independent field measurements of P and R are impossible, both these parameters are usually calculated by multiplying data on individual production and respiration respectively by estimates of population numbers or biomass. It may therefore be argued that P and R, when estimated in this way, are not statistically independent variables. Nevertheless, we believe that the empirical relationships between P and R obtained by normal regression techniques are of sufficient interest to justify their use.

Because respiration in the absence of living tissue is impossible, Fig. 1 shows $\log_{10} R$ as ordinate plotted against $\log_{10} P$ as abscissa. Strictly, however, R is a function of population biomass (that part of production which, temporarily, persists) rather than population production *per se*, and it is also practically useful and theoretically possible to consider P as a function of R. Consequently, in analysing the relationship between P and R, two equations are presented at each step, one with R and the other with P as the dependent variable.

The following least squares regression equations were calculated:

For poikilotherms, $\log R = 1{\cdot}0733 \log P + 0{\cdot}3757$ (1)

The slope of this line does not differ significantly from 1·0 (t_{40} 1·1899).

Alternatively, $\log P = 0{\cdot}8233 \log R - 0{\cdot}2367$ (2)

The slope of this line differs significantly from 1·0 (t_{40} 3·7405***).

For homoiotherms, $\log R = 0{\cdot}9812 \log P + 1{\cdot}7418$ (3)

The slope of this line does not differ significantly from 1·0 (t_9 0·7866).

Alternatively, $\log P = 1{\cdot}0137 \log R - 1{\cdot}7761$ (4)

The slope of this line does not differ significantly from 1·0 (t_9 0·5548).

Confidence intervals (95 per cent) were calculated for these regressions[31]. For poikilotherms (equation 1) between $\log P = -2\cdot0$ and $\log P = +2\cdot0$ these were $\pm0\cdot9136$ to $\pm0\cdot9698$; for poikilotherms (equation 2) between $\log R = -2\cdot0$ and $\log R = +3\cdot0$, $\pm0\cdot7997$ to $\pm0\cdot8507$; for homoiotherms (equation 3) between $\log P = -5\cdot0$ and $\log P = 0\cdot0$, $\pm0\cdot3150$ to $\pm0\cdot3338$; and for homoiotherms (equation 4) between $\log R = -3\cdot0$ and $\log R = +2\cdot0$, $\pm0\cdot3202$ to $\pm0\cdot3849$.

Part of the large scatter observed in the poikilotherm data (equations 1 and 2) may be explained in terms of life histories; further examination of the poikilotherm data suggests that populations of species known to be long lived can be distinguished from species with shorter generation times. An arbitrary age of two years was selected, and those poikilotherm populations in which a proportion of individuals were known to exceed two years of age (long-lived poikilotherms) were distinguished from those populations in which all the individuals were less than two years of age (comparatively short-lived poikilotherms) (Fig. 1). Two further regression lines were calculated for the comparatively short-lived poikilotherms

$$\log R = 1\cdot1740 \log P + 0\cdot1352 \qquad (5)$$

$$\text{and } \log P = 0\cdot8262 \log R - 0\cdot0948 \qquad (6)$$

The slope of both these lines now differs significantly from 1·0 (equation 5 t_{27} 4·3762*** and equation 6 t_{27} 6·2116***) and the 95 per cent confidence limits within the range of values of $\log P$ and $\log R$ given above all poikilotherms are reduced to $\pm0\cdot5140$ and $\pm0\cdot5544$ for equation 5 and $\pm0\cdot4312$ and $\pm0\cdot4598$ for equation 6; a considerable improvement.

The data do not justify similar regression calculations for long-lived poikilotherms, because less information is available and points 40 and 41 therefore have an unduly large influence on the regression lines. Nevertheless, it is clear from Fig. 1 that respiratory costs at any level of production tend to be higher in long-lived poikilotherms (where a standing crop of older individuals is maintained from year to year) than in short-lived poikilotherms. The inclusion of both groups in equations 1 and 2 greatly increases the scatter in the relationships between $\log P$ and $\log R$. It should be noted that our distinction between short and long-lived poikilotherms is purely arbitrary, and when further data become available it should be possible to quantify the influence of age in a more satisfactory manner. Several of the long-lived poikilotherms distinguished in this report, for example, are fish (points 12–16), individuals of which may be very long-lived and hence show the influence of age in an extreme form.

From the equations we have derived, it is possible to calculate mean net population production efficiencies,

$$\left(\frac{P \times 100}{A}\right)$$

Table 1. ANNUAL PRODUCTION AND RESPIRATION (KILOCALORIES PER SQUARE METRE) IN ANIMAL POPULATIONS

No. in Fig. 1	Species	Group or English name	Prod.	Resp.	Reference
			(kcalories per m² per year)		
	Poikilotherms				
1	* *Allolobophora rosea* (Sav.)	Lumbricidae	2·717	12·727	3
2	*Pteromalus puparum* (L.) larvae	Pteromalidae	0·074	0·056	4
3	*Pimpla instigator* (Fabr.) larvae	Ichneumonidae	0·017	0·009	
4	Oribatid mites	Acari	0·43	1·578	5
5	*Asellus aquaticus* L.	Isopoda	16·296	51·060	6
6	*Onychiurus procompatus* Gisin	Collembola	2·61	3·04	7
7	* *Scrobicularia plana* (da Costa)	Lamellibranchiata	17·5	54·6	8
8	* Ditto		124·0	476·0	
9	* *Modiolus demissus* Dillwyn	Lamellibranchiata	16·7	39·0	9
10	*Pyrrhosoma nymphula* (Sulz.) larvae	Odonata	4·436	3·169	10
11	Ditto		3·889	3·668	
12	* *Rutilus rutilus* (L.)	Roach	10·7	118·4	11
13	* *Alburnus alburnus* (L.)	Bleak	30·5	314·2	
14	* *Leuciscus leuciscus* (L.)	Dace	1·5	23·7	
15	* *Perca fluviatilis* L.	Perch	1·8	23·2	
16	* *Gobio gobio* (L.)	Gudgeon	2·6	37·0	
17	† *Leptoterna dolobrata* (L.)	Hemiptera	0·064	0·062	12
18	† Ditto		0·140	0·101	
19	† Ditto		0·096	0·088	
20	† 3 Orthoptera species	Orthoptera	4·0	21·6	13
21	* *Littorina irrorata* (Say)	Gastropoda	40·6	249·4	14
22	† *Chorthippus parallelus* Zett.	Orthoptera	0·420	0·338	15
23	† Ditto		0·273	0·190	
24	*Ligidium japonica*	Isopoda	3·5	16·0	16
25	*Japonaria laminata armigera*	Diplopoda	26·9	30·9	17
26	Ditto		1·5	1·51	
27	† *Orchelimum fidicinium* Rehn and Hebard	Orthoptera	10·8	18·6	18
28	*Limnodrilus hoffmeisteri* Claraparede	Tubificidae	160·5	483·6	19
29	*Asellus militaris* Hay	Isopoda	108·3	486·1	
30	† Trichoptera	Trichoptera	20·9	67·5	
31	*Pisidium virginicum* (Gmelin)	Lamellibranchiata	81·8	90·9	
32	† *Calopsectra dives* (Johannsen)	Chironomidae	130·7	389·6	
33	*Anatopynia dyari* (Coquillett)	Ditto	37·4	208·5	
34	*Gammarus pseudolimneus* Bousfield	Amphipoda	183·0	1,039·0	20
35	*Physa integra* Haldane	Gastropoda	170·6	440·7	
36	† *Philaenus spumarius* L.	Hemiptera	15·963	22·602	21
37	† Orthoptera: chiefly *Melanoplus* spp.	Orthoptera	0·638	1·120	22
38	† Ditto		0·373	0·596	
39	† Ditto		4·573	8·690	
40	* *Lithobius forficatus* (L.)	Chilopoda	0·085	1·998	23
41	* *Lithobius crassipes* (L. Koch)	Ditto	0·074	1·268	
42	* *Cherax albidus* Clark	Decapoda	158·0	132·0	24
	Homoiotherms				
43	*Adenota Kob thomasi* Neumann	Uganda Kob	0·81	61·68	25
44	*Microtus pennsylvanicus p.* Ord.	Meadow vole	0·517	16·99	26
45	*Mustela rixosa allegheniensis* Rhoads	Least weasel	0·013	0·5434	
46	*Clethrionomys rutilus dawsoni* Merriam	Red-backed vole	0·00024	0·01246	27
47	*Tamiasciurus hudsonicus preblei* Howell	"Red squirrel"	0·00020	0·0144	
48	*Microtus oeconomys macfarlani* Merriam	Tundra vole	0·00004	0·00216	
49	*Glaucomys sabrinus yukonensis* Osgood	Flying squirrel	0·00002	0·00168	
50	{ *Pitymys subterraneus* (De Selys Long.) *Microtus agrestis* (L.)	Pine vole Field vole	0·0955	6·0017	28
51	*Peromyscus polionotus* Osgood	Old-field mouse	0·12	6·57	13
52	*Passerculus sandwichensis* (Gmelin)	Savannah sparrow	0·04	3·55	
53	*Loxodonta africana*	African elephant	0·34	23·00	29

* Long-lived poikilotherms (see text).

† Comparatively short-lived poikilotherms with low respiratory cost resting stages (see text).

The remaining poikilotherm species listed are comparatively short-lived poikilotherms with high respiratory cost non-productive stages (see text).

For homoiotherms (equations 3 and 4), b does not differ significantly from 1·0 and mean net population production efficiencies are approximately constant for all levels of production, varying only between 1·4 and 1·8 per cent. For poikilotherms in general (equations 1 and 2) the situation is less clear. In equation 1, plotting log R against log P, b does not differ significantly from 1·0 but, in equation 2, plotting log P against log R (because of the greater variability in values of R relative to values of P), b does differ significantly from 1·0. Both slopes for comparatively short-lived poikilotherms (equations 5 and 6) differ significantly from 1·0, however, and give virtually identical regression lines. For these comparatively short-lived poikilotherms, mean net population production efficiencies therefore increase markedly at lower production levels (Table 2). Two possible explanations can be suggested, and these are not necessarily mutually exclusive: populations of comparatively short-lived poikilotherms, in which total annual production is for some reason restricted at a low level may tend to maximize production efficiencies, in an attempt to compensate for this restriction or, alternatively, the apparent variation in mean net population production efficiency may be caused by differing life histories. Most comparatively short-lived poikilotherms, below log $P = 0$, overwinter as eggs; respiration during such resting periods is therefore reduced to a minimum. Most of the comparatively short-lived poikilotherms, above log $P = 0$, overwinter as juveniles or adults, and therefore respiration continues at a relatively high level when production is virtually zero.

Table 2. MEAN NET POPULATION PRODUCTION EFFICIENCIES FOR SHORT-LIVED POIKILOTHERMS, CALCULATED FROM EQUATION 5

Production P	Respiration R	Assimilation A $(P+R)$	Net population production efficiency $\frac{P}{A} \times 100$
	(kcalories/m²/year)		(per cent)
0·01	0·006125	0·016125	62
0·1	0·09145	0·19145	52
1·0	1·366	2·366	42
10·0	20·38	30·38	33
100·0	304·2	404·2	25

It may therefore be possible to recognize three types of poikilotherm; comparatively short-lived poikilotherms, which show low respiratory cost resting stages, in which annual population production efficiencies are high; comparatively short-lived poikilotherms, which show high respiratory cost non-productive stages, in which annual population production efficiencies are intermediate; and long-lived poikilotherms where a proportion of the population is in excess of two years old and probably experiences at least two high respiratory cost, non-productive periods; here, annual population production efficiencies are low.

"Life history effects" are not apparent for the limited homoiotherm data reviewed, because such effects are probably masked by the high respiratory cost of homoiothermy.

Apart from attempting to explain several features of the relationship between annual production and annual respiration in animal populations, the results may also prove to be of considerable practical value. There is increasing need in ecology for short cut methods for analysing the trophic-dynamics of complex communities, and these relationships, despite their large confidence intervals, may prove useful in predicting total energy flow through populations in which either P or R is known.

We thank those authors who allowed us to quote unpublished thesis material.

S. McNeill

Imperial College Field Station,
Silwood Park,
Sunninghill, Ascot, Berkshire.

J. H. Lawton

Animal Ecology Research Group,
Botanic Garden,
High Street, Oxford.

Received September 15; revised October 22, 1969.

[1] Engelmann, M. D., *Advances in Ecological Research*, **3**, 73 (1966).
[2] Petrusewicz, K., *Secondary Productivity of Terrestrial Ecosystems* (edit. by Petrusewicz, K.), 17 (IBP-PT Warszawa-Kraków, 1967).
[3] Bolton, P., thesis, Univ. of Durham (1969).
[4] Chłony, J., *Ekol. Polska A.*, **16**, 505 (1968).
[5] Engelmann, M. D., *Ecol. Monog.*, **31**, 221 (1961).
[6] Fitzpatrick, C. M., thesis, Univ. of Durham (1968).
[7] Healey, I. N., *Secondary Productivity of Terrestrial Ecosystems* (edit. by Petrusewicz, K.), 695 (IBP-PT Warszawa-Kraków 1967).
[8] Hughes, R. N., *J. Anim. Ecol.* (in the press).
[9] Kuenzler, E. J., *Limnol. Oceanogr.*, **6**, 191 (1961).
[10] Lawton, J. H., thesis, Univ. of Durham (1969).
[11] Mann, K. H., *J. Anim. Ecol.*, **34**, 253 (1965).
[12] McNeill, S., thesis, Univ. of London (1969).
[13] Odum, E. P., Connel, C. E., and Davenport, L. B., *Ecology*, **43**, 88 (1962).
[14] Odum, E. P., and Smalley, A. E., *Proc. US Nat. Acad. Sci.*, **45**, 617 (1959).
[15] Qasrawi, H., thesis, Univ. of Exeter (1966).
[16] Saito, S., *Jap. J. Ecol.*, **15**, 47 (1965).
[17] Saito, S., *Res. Popul. Ecol.*, **9**, 153 (1967).
[18] Smalley, A. E., *Ecology*, **41**, 672 (1960).
[19] Teal, J. M., *Ecol. Monog.*, **27**, 283 (1957).
[20] Tilly, L. J., *Ecol. Monog.*, **38**, 169 (1968).
[21] Wiegert, R. G., *Ecol. Monog.*, **34**, 217 (1964).
[22] Wiegert, R. G., *Oikos*, **16**, 161 (1965).
[23] Wignarajah, S., thesis, Univ. of Durham (1968).
[24] Woodland, D. J., thesis, Univ. of New England, Armidale, NSW (1967).
[25] Buechner, H. K., and Golley, F. B., *Secondary Productivity of Terrestrial Ecosystems* (edit. by Petrusewicz, K.), 243 (IBP-PT Warszawa-Kraków, 1967).
[26] Golley, F. B., *Ecol. Monog.*, **30**, 187 (1960).
[27] Grodziński, W., *Lynx*, **6**, 47 (1966) quoted in *Secondary Productivity of Terrestrial Ecosystems* (edit. by Petrusewicz, K.), 145 (IBP-PT Warszawa-Kraków, 1967).
[28] Grodziński, W., Górecki, A., Janas, K., and Migula, P., *Acta Theriol.*, **11**, 419 (1966).
[29] Petrides, G. A., and Swank, W. G., *Proc. Ninth Internat. Grassland Cong., Sao Paulo, Brazil*, 831 (1966).
[30] Golley, F. B., and Gentry, J. D., *Ecology*, **45**, 217 (1964).
[31] Snedecor, G. W., and Cochran, W. G., *Statistical Methods* (sixth ed.), 135 (Iowa State Univ. Press, 1967).

Pleistocene South-east Asiatic Pygmy Stegodonts

During the past fifteen years a series of Pleistocene pygmy stegodont elephants have been described from the Malay Archipelago. These forms are equal in importance and evolutionary interest to the classic examples of dwarfed insular elephants of the Mediterranean that have been known for more than a century[1], and so a brief account of their characteristics may be useful.

The best known Asiatic species of the genus *Stegodon* (a Plio-Pleistocene elephantid from Asia and Africa) is *Stegodon trigonocephalus* Martin from Java. The wealth of material of this species collected by Dubois during the last decade of the nineteenth century shows to advantage the amount of individual variation within a single species[2]. The unworn molars of this species of *Stegodon* are from a half to two-thirds as high as wide.

Stegodon hypsilophus Hooijer[3], probably confined to the Early Mid-Pleistocene Djetis Beds of Eastern Java, is characterized by the great relative height of its molar crowns as well as by its small size; as Table 1 shows, the height of M^3 is not less than that in *Stegodon trigonocephalus* but the width is rather less, resulting in a high height/width index (height : width × 100).

Table 1. MEASUREMENTS AND INDICES OF VARIOUS SPECIES OF *Stegodon*

	S. trigonocephalus		*S. hypsilophus*	*S. timorensis*
M^3	Java	Flores	Java	Timor
Width	76–113	77	64	51
Height	49–59	55	57	48
H/w index	46–68	71	89	94
	trigonocephalus		*sompoensis*	*timorensis*
M_3	Java	Flores	Celebes	Timor
Width	68–98	72	60	42
Height	48–61	50	40	37
H/w index	56–66	69	67	88

Measurements are in mm.

The first dwarfed stegodont to be found in the island of Flores[4] is on average only slightly smaller than *Stegodon trigonocephalus* from Java, and its molar crowns are just a little higher. This form has been considered distinct from the Java form at the subspecies level only: *Stegodon trigonocephalus florensis* Hooijer.

Several years later[5] another pygmy stegodont was found in Flores, with milk molars half as long and wide as those in the Java *Stegodon trigonocephalus*. Meanwhile, a *Stegodon* from the Pleistocene of the island of Celebes, associated with a very primitive archidiskodont elephant, was named *Stegodon sompoensis* Hooijer after its type locality[6]. The Celebes pygmy stegodont M_3 is decidedly smaller than its counterpart in *Stegodon trigonocephalus* from Java, but has not developed the high molar crowns: its height–width index is only 67 (Table 1).

Finally, two forms of *Stegodon* have been recorded from Timor[7], one only half the linear dimensions of *Stegodon trigonocephalus* from Java but with a very high molar crown, *Stegodon timorensis* Sartono, and the other hardly smaller than the Java *Stegodon trigonocephalus* and apparently indistinguishable from the Flores race of this species. The smaller of the two Flores forms and the larger of the two Timor forms are based only on a few worn milk molars (Table 2 and Figs. 2 and 3).

Table 2. MEASUREMENTS OF MILK MOLARS OF VARIOUS SPECIES OF *Stegodon*

		S. trigonocephalus florensis		*S. timorensis*	
	Java	Flores	Timor	Flores	Timor
DM^3, length	51–58	—	44	—	—
width	34–45	—	32	—	—
DM_3, length	56–68	—	—	28	—
width (anterior)	23–28	20	—	14	—

Measurements are in mm.

It seems likely that the two Flores stegodonts will prove to be identical with the two Timor forms. This, then, would make for an interesting palaeozoogeographic conclusion: the islands of Flores and Timor may not have been sufficiently isolated from each other in the Pleistocene to prevent the dwarfing *Stegodon* populations from wandering back and forth. Otherwise, it is hard to see how the two evolutionary products on Flores and Timor could have been so similar. Celebes remained well isolated from these Lesser Sunda Islands, by a strait at least 50 miles wide even during Pleistocene low sea-level stages, and its *Stegodon* is very distinct: it did not develop high molar crowns.

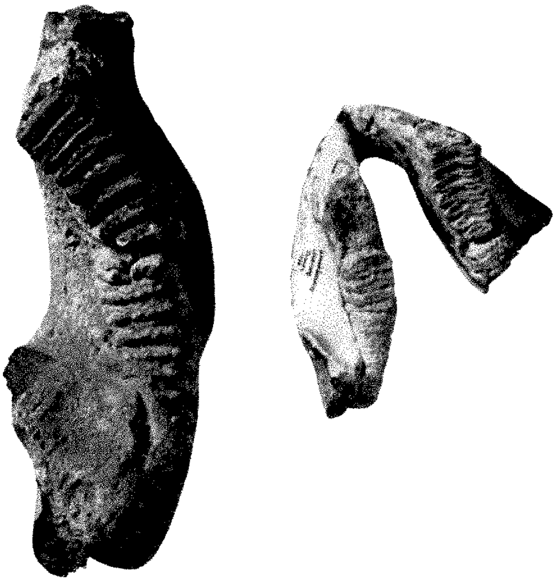

Java Timor

Fig. 1. Half-mandible of *Stegodon trigonocephalus* Martin from Java (left) compared with mandible of *Stegodon timorensis* Sartono from Timor (right). The left last molar is 24 cm long in the Java against 12 cm in the Timor specimen. (Both figures × 3/16.)

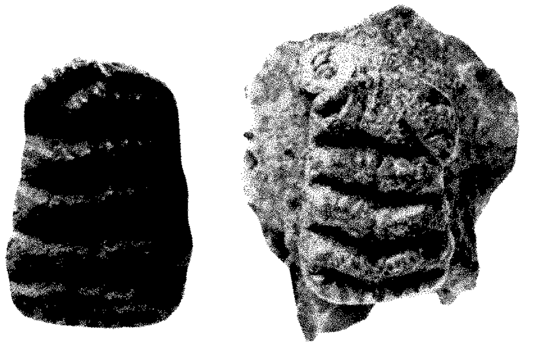

Java Timor

Fig. 2. Right DM^3 of *Stegodon trigonocephalus* Martin from Java (left) compared with left DM^3 of *Stegodon* cf. *trigonocephalus florensis* Hooijer from Timor (right). (Both figures × 2/3.)

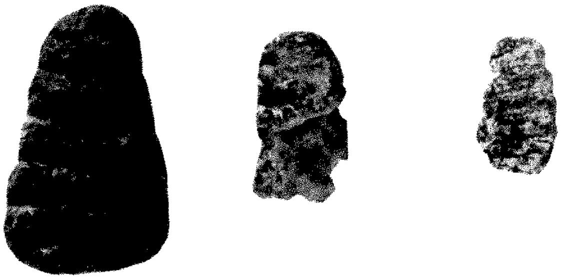

Java Flores Flores

Fig. 3. Right DM_3 of *Stegodon trigonocephalus* Martin from Java (left) compared with anterior portion of left DM_3 of *Stegodon trigonocephalus florensis* Hooijer from Flores (centre) and with left DM_3 of *Stegodon* cf. *timorensis* Sartono from Flores (right). (All figures × 5/8.)

The route of dispersal of the stegodonts from Flores and Timor was evidently along the Lesser Sunda Islands chain from Java; various recent Asiatic mammals have spread along this chain to Timor. It is only remarkable that among the extinct forms *Stegodon* alone should have reached Flores and Timor; no fossil remains of other mammals (apart from a jaw of a small murid rodent, *Papagomys*, in Flores) have been found along with the *Stegodon* fossils. The ancestral form of the Celebes *Stegodon sompoensis* may have migrated from the Asiatic mainland via Formosa and the Philippines.

The geology of the *Stegodon* sites in Celebes, Flores and Timor is largely unknown. It is possible that *Stegodon timorensis* is of more recent origin than *Stegodon trigonocephalus florensis*. If so, the former could have descended from the latter; the trends toward smaller size and higher molar crowns that distinguish *Stegodon trigonocephalus florensis* from its parent species of Java are merely carried on further in the Timor species. On the other hand, the two forms may prove to be contemporaneous; from the ratio in linear dimensions of teeth and bones, five to three, it follows that *Stegodon trigonocephalus florensis* had five times the body size of *Stegodon timorensis*, a difference probably large enough to permit coexistence.

D. A. HOOIJER

Rijksmuseum van Natuurlijke Historie,
Leiden.

Received October 8, 1969.

[1] Vaufrey, R., *Arch. Inst. Pal. Humaine*, Mém. 6 (1929); Leonardi, P., *Ann. Paléontol.*, 40 (1954).

[2] Hooijer, D. A., *Zool. Verh. Museum Leiden*, **28** (1955).

[3] Hooijer, D. A., *Zool. Med. Museum Leiden*, **33** (1954).

[4] Hooijer, D. A., *Treubia*, 24 (1957).

[5] Hooijer, D. A., *Bull. Geol. Surv. Indonesia*, **1** (1964).

[6] Hooijer, D. A., *Zool. Med. Museum Leiden*, **40** (1964).

[7] Sartono, S., *Proc. Kon. Ned. Akad. Wet. Amsterdam*, B, **72** (1969); Hooijer, D. A., *ibid*.

Chlorotic Dwarf of Eastern White Pine caused by an Ozone and Sulphur Dioxide Interaction

CHLOROTIC dwarf disease of eastern white pine (*Pinus strobus* L.) is a physiogenic condition characterized by stunted roots and tops, mottled needles and premature foliar abscission. Recent work has indicated that the disease is caused by gaseous pollutants that injure the needles of genetically susceptible white pines[1]. We have now shown that the action and interaction of ozone and sulphur dioxide cause characteristic symptoms on the foliage of white pine ramets that are susceptible to chlorotic dwarf.

Between June and September 1967, we took continuous measurements of ozone and samples of sulphur dioxide every 2 h from Monday to Friday. We worked in Ohio white pine plantations where there was a high incidence of chlorotic dwarf, aiming to assess the possible role of these toxicants in the induction of the disease. A Mast ozone meter, equipped with a chromium trioxide scrubber[2] to eliminate interference from sulphur dioxide, monitored concentrations of oxidant (ozone). Readings were corrected by a factor of 1·5 to give oxidant values in terms of neutral KI. Sulphur dioxide was collected with a sequential sampler, and concentrations were determined by the method of West and Gaeke[3].

Daily 2 h peak concentrations of sulphur dioxide varied from 0·0 to 7·8 p.p.h.m. Two hour averages of 5·0 p.p.h.m. of sulphur dioxide were recorded on 25 per cent of days. Maximum hourly averages for oxidant (ozone) registered greater than 5·5 p.p.h.m. for 60 per cent of days. The greatest hourly average was 21 p.p.h.m., and concentrations larger than 10 p.p.h.m. were common. The greatest combination was 12 p.p.h.m. of oxidant and 7·8 p.p.h.m. of sulphur dioxide. Concentrations of sulphur dioxide and oxidant in the field were both greater than 5 p.p.h.m. on 16 per cent of days. Measurements made throughout the summer of 1968 were similar.

Table 1. MOTTLING ON WHITE PINE AFTER EXPOSURE TO 10 P.P.H.M. OF SULPHUR DIOXIDE AND/OR 10 P.P.H.M. OF OZONE

Treatment	Concentration p.p.h.m.	Replications No.	Injury* per cent
Ozone	9·9		
Sensitive		9	3
Resistant		2	0
Sulphur dioxide	9·6		
Sensitive		6	4
Resistant		3	0
Ozone/sulphur dioxide	9·9/9·8		
Sensitive		9	16
Resistant		3	0
Control	0		
Sensitive		6	0
Resistant		5	0

* Injury was rated on the percentage area of needles showing the chlorotic mottling condition.

In our experiments with ozone and sulphur dioxide we fumigated some resistant and sensitive clonal material of white pine that had been grafted onto 2 yr rootstocks in 1964. White pine plants were placed in a cold storage room in the autumn of 1967, and groups were removed at intervals after 8 weeks. Dormancy was broken in a growth chamber in a regime of 16 h day, 2,000 foot candles of light, 26° C daytime temperature, 21° C night temperature and 60–74 per cent humidity. After the buds had broken and the candles had begun to elongate, but before the needles separated within the fascicles, the plants were placed in charcoal-filtered chambers[4].

Sensitive and resistant white pine ramets were exposed to 10 p.p.h.m. of sulphur dioxide, or to 10 p.p.h.m. of ozone, or to mixtures of the two (each in a concentration of 10 p.p.h.m.). Control plants remained in the charcoal-filter growth chambers. Other white pines were fumigated for 4–8 h each day, 5 days a week, for 4–8 weeks between January and April 1968 (Table 1).

Symptoms were not observed on any resistant ramets or sensitive controls. Chlorotic dwarf symptoms of flecking (mottling of current needles), occasionally accompanied by a shedding of older needles, were noted on most sensitive pines. There was little or no injury to plants treated with one of the gases, and moderate injury in those treated with the gaseous mixture. The percentage of mottling on current needles and the premature drop of older needles exposed to the mixture far exceeded the damage in those treated with one gas. This strongly suggests that the two pollutants act synergistically on sensitive white pine plants. A similar response has been reported in tobacco[5].

For a brief time during growth, needles were extremely susceptible to damage by pollution. Chlorotic mottling developed 2–4 weeks after exposure began (about the time that the needles separated in the fascicles). Subsequent exposure intensified the mottling only slightly.

We have found that ozone and sulphur dioxide, acting independently or synergistically, can promote the initial symptoms of current needle mottling and premature defoliation associated with the chlorotic dwarf syndrome. Sulphur dioxide seems to be much more reactive than ozone in producing the symptoms.

With continued breakdown of chlorophyll and loss of leaves, as observed in these long term exposures, growth could be reduced through the inability of the leaves to supply the normal photosynthetic needs of white pine plantations[6].

Other white pine needle blights that seem to be related to air pollution may be caused by a mixture of two or more pollutants. When recorded levels of single gases are not great enough to damage white pine, mixtures of gases may injure trees before either pollutant would itself cause any damage.

Environmental factors also directly influence the sensitivity of white pine to one or more pollutant. Further study of these interactions between the suscept and its environment should help to explain variations in the expression of symptoms.

L. S. DOCHINGER
F. W. BENDER

Northeastern Forest Experiment Station,
Forest Service,
US Department of Agriculture,
Forest Insect and Disease Laboratory,
Delaware, Ohio.

F. L. FOX
W. W. HECK*

National Air Pollution Control Administration,
Consumer Protection Environmental
Health Service,
US Department of Health,
Education and Welfare,
Cincinnati, Ohio.

Received July 7; revised September 29, 1969.

* Also at: Crops Research Division, Agricultural Research Service, US Department of Agriculture, Cincinnati, Ohio.

[1] Dochinger, L. S., Seliskar, C. E., and Bender, F. W., *Phytopathology*, **55**, 1055 (1965).
[2] Saltzman, B. E., and Wartburg, jun., A. F., *Anal. Chem.*, **37**, 779 (1965).
[3] West, P. V., and Gaeke, D. C., *Anal. Chem.*, **28**, 1816 (1956).
[4] Heck, W. W., Dunning, J. A., and Johnson, H., *Nat. Air Pollution Control, Admin. Pub. APTD*-68-6 (1968).
[5] Menser, H. A., and Heggested, H. E., *Science*, **153**, 424 (1966).
[6] Dochinger, L. S., *Forest Serv. Res. Note* CS-16 (1963).

Book Reviews

ORDERED ELEMENTS

The Periodic System of Chemical Elements

A History of the First Hundred Years. By J. W. van Spronsen. Pp. xv + 368. (Elsevier: Amsterdam, London and New York, 1969.) 120*s*.

THE importance of the periodic system in modern thought has never been adequately estimated or expounded. More than any other interpretation of physical nature except Newton's, it made clear the orderliness and potential comprehensibility of the material world. One reason why nobody has yet written an appreciation of its effect on the rest of science, let alone on modern philosophy, religion or literature, is that its history has been taken too much for granted. Chapters on it included in general histories of chemistry have usually repeated a few names and a few incidents, but have, by and large, neglected critical appraisal of its deeper consequences.

Now, van Spronsen has made an important and substantial contribution to the history of chemistry. The "Hundred Years" of his title is not to be taken literally: after a survey of the relevant features of the earlier history of chemistry, he begins his detailed study with the precursors of the modern classification system (for example, Döbereiner 1817) and goes on eventually to discuss the significance to classification of the quantum theory and the trans-uranium elements, covering more than a century and a half. In the period from Döbereiner's second essay (the triads of 1827) until Mendeléev's own second main paper of 1871, van Spronsen excels all previous writers on this subject in the completeness of his account. He shows how the Mendeléev system was the culmination of an irregular development, confidence among chemists that there was a system to be elucidated growing stronger decade by decade after 1827.

He draws attention to many important chemists whose work on classification has been forgotten, Dumas in particular. It is striking to see how the search for a grand design was frustrated again and again, paradoxically by a concentration on quantitative factors. As atomic weight determinations grew more precise and reliable, the arithmetic of the relation between their numerical values was pursued with such energy as to obscure the significance of mere sequence. One reason for this is that molecular weight determinations were also becoming more precise, so that the homologous series of organic compounds presented a very persuasive, but misleading, model for a theory of atomic weight relations. One incident in the long history of scientists failing to communicate is the proposal by Beguyer de Chancourtois of a helical distribution of atomic weights. He showed a periodicity but did so without clarity or force. He was well on the right track but his ideas were not published diagrammatically in a clear enough way. Van Spronsen does him justice as he does also to a virtually forgotten chemist, the Dane Hinrichs, who emigrated to become a good American and a good teacher. Hinrichs' flat spiral shows enough periodicity to be worth more than a mention.

The better known men, Odling, Newlands and Lothar Meyer, are fully dealt with, but this does not diminish the standing of Mendeléev. He sought a means of putting order into his teaching and in doing so produced not only a new foundation for the whole of chemical teaching, but also a unified theory and a stimulus to research, a very great achievement.

Van Spronsen examines the various predictions of new elements, the gradual solution of problems like the deviation from weight order, the assimilation to the system of the noble gases, and the inevitable debates on priority. He brings the story up to the point where it can be seen to be continuous with recent progress in physics and chemistry. Every section is well illustrated with original or adapted diagrams and tables.

This book can hardly fail to have a beneficial effect on the study not only of the periodic system but of chemistry as a whole. Perhaps it may even encourage somebody to attempt a general appreciation of the place of the periodic system in modern thought. FRANK GREENAWAY

PHENOMENOLOGICAL CLASSIC

Formal and Transcendental Logic

By Edmund Husserl. Translated by Dorion Cairns. Pp. xix + 340. (Nijhoff: The Hague, August 1969.) 30.60 guilders.

EDMUND HUSSERL began his life as a mathematician, but as a philosopher he moved steadily away from rigorous deductive procedures into a preoccupation with problems of the psychology of perception. Thus his intellectual development diverged widely from that of Bertrand Russell, and this divergence expressed the great cleavage which has developed during this century between continental European schools of philosophy and those of the English-speaking world. The same cleavage is reflected in the fact that 40 years elapsed between the original publication of this major work of a leading European philosopher and its first English translation, a labour of devotion by Professor Cairns.

Formal and Transcendental Logic is a very abstract and technical work which uses a somewhat esoteric vocabulary. Serious philosophers with an interest in phenomenology will welcome it and study it, but it is hardly a work which will be suited to the needs of the average scientist in search of a philosophical basis for his thinking. Husserl himself was interested in science, and he hoped that his phenomenology would provide a better basis for scientific thinking than other modern philosophies could. Oddly enough, the author of *Cartesian Meditations* tends to turn to Euclidean geometry when he wants an example of scientific method.

Formal logic is the kind that is classically formulated in Aristotle's *Analytics*, and the first part of Husserl's book is devoted to an examination of its nature and limitations, when looked at from the point of view of the psychology of perception. "Transcendental", in Husserl as in Kant and Hegel, means concerned with the intrinsic nature of existing things, rather than with mere perceptions or concepts; the Hegelian dialectic was proposed as a procedure of transcendental logic, though Husserl does not deal with it in this book. The latter half of it is an attempt to explain how transcendental logic differs from and transcends the formal variety, without invalidating or negating it. The book does not include, nor did Husserl ever formulate, a direct exposition of the principles of transcendental logic on the lines of the *Analytics*.

For scientists, without the philosophical training required by a reader of this book, there are two currently available works which provide in more readily understood form critiques of formal logic basically similar to that of Husserl and which both look forward to something like the transcendental logic which he advocated; they are Michael Polanyi's *Personal Knowledge* and Stephen Toulmin's *The Uses of Argument*. Those who have read and understood works like these might be ready to begin the study of Husserl's philosophy. R. A. CROWSON

THEORY OF BONDING

Elementary Molecular Bonding Theory

By Peter G. Perkins. (Methuen's Monographs on Chemical Subjects.) Pp. viii + 183. (Methuen: London, October 1969.) 28*s*.

Chemists of all types and sizes have always been interested in theories of bonding. In recent years, theoretical chemistry has advanced to a point where it is possible to claim that we understand the principles of atomic interaction. Current work is devoted to investigating quantitative aspects of the theory applied to particular molecules, and—all too rarely—to predicting new molecular structures and properties. The established approach is through molecular orbitals, which represent many-electron structures in terms of one-electron functions; electron correlation can be incorporated through "configuration interaction".

The aim of this little book is to present the modern theory of the chemical bond at an elementary level suitable for sixth-form pupils and for students at universities and technical colleges. This is a worthy but challenging objective. To reach it, an author needs to be the complete master of his subject and to possess that rare gift of clear exposition.

This book begins with a discussion of early ideas of bonding and then introduces Schrödinger's equation and atomic orbitals. The third chapter is on valence bond theory, which could well have been omitted. The fourth chapter is on molecular orbitals in diatomic molecules, and the fifth on molecular symmetry and orbitals in polyatomics. Finally, there is a short summing-up.

There are a number of obscurities and inaccuracies that mar the book. On page zero e is given with the wrong sign. Many of the figures are poor (Fig. 2.18 should be inverted, plane I in Fig. 5.6 fails to look anything like a mirror plane, and Fig. 3.15 is not easy to follow), and some have left-handed coordinate frames. Also, Mulliken's recommendation for the choice of axes to describe a molecule like H_2O (the two-fold rotation axis should be z, with x perpendicular to the molecular plane) has not been adopted. Nevertheless, the author has a pleasing style and his book is bound to be useful to critical students. It may not, however, possess the clarity required of a book that is to make an important contribution to sixth-form teaching. A. D. Buckingham

REFINING PROCESS

Electro-Slag Refining

By W. E. Duckworth and G. Hoyle. Pp. x + 178 + 44 plates. (Chapman and Hall: London, November 1969.) 80*s*.

The technology of steel manufacture has developed rapidly in recent years to meet the increasingly more stringent requirements for closer tolerance on composition and freedom from non-metallic inclusions. During the past decade, electro-slag refining (ESR) has progressed from the status of a laboratory technique to a manufacturing process for the production of high-grade steels, comparable in output to vacuum arc remelting (VAR).

The process was first exploited in the Soviet Union, but is now in use in many countries. In Great Britain, a development unit was set up, under the leadership of the two authors, by the British Iron and Steel Research Association, and this has now acquired an international reputation in electro-slag technology. The book gives a detailed and fascinating account of the evolution of the process to the present stage of development. A treatise, published in Russia in 1963, referred only to work carried out in the Soviet Union. This is the first book to appear in the English language and the authors have attempted to collect an accurate account of the process in all the countries in which it is being applied.

The first half of the book describes the fundamental principles of the process and reviews present knowledge of the physical and chemical properties of the various slag compositions which have been studied. The conditions controlling the gas–slag–metal reactions are described and data are presented to aid in the selection of the optimum operating conditions for specific purposes. The research worker may regret the absence of consideration of the mechanisms of inclusion removal, but the authors have concentrated on established facts and have avoided the areas which are still controversial.

The second half contains descriptions of typical plant installations and operating techniques. Twenty-one tables list changes in composition and mechanical properties resulting from electro-slag refining of various grades of steel. The economics of the process are honestly examined and it is shown that present costs of ESR are comparable with those for VAR.

The authors state that they have tried to "present a rounded account of the state of knowledge" of ESR and "make it more widely known than is the case at present". They have succeeded in the first objective and anybody who reads the book cannot fail to be impressed by the potential of the process.

The book is well written, illustrated and presented. A minor criticism is the frequent reiteration of statements on successive pages in chapter three. C. Bodsworth

CONTROLLED NUCLEAR FUSION

Plasma Physics and Controlled Nuclear Fusion Research

Vols. 1 and 2. (Proceedings of the Third International Conference on Plasma Physics and Controlled Nuclear Fusion Research held by the International Atomic Energy Agency at Novosibirsk, August 1–7, 1968.) Vol. 1: Pp. 998. Vol. 2: Pp. 836. (International Atomic Energy Agency: Vienna; HMSO: London, 1969.) Vol. 1: 191*s* 8*d* ($23); Vol. 2: 162*s* 6*d* ($10.50).

The 1968 conference at Novosibirsk may well be remembered as the conference where the ghost of Bohm diffusion was laid. Experimental evidence reported at the conference showed that containment times of 50 to 100 Bohm diffusion times could now be achieved in laboratory plasma. The quest for a thermonuclear reactor is on the up again.

The two volumes contain a total of 134 papers of which 80 are in English, 37 in Russian and 10 in French. The Russian and French papers have an English abstract. Translations of the Russian papers have already been published in *Nuclear Fusion*. It is a great pity, for Western readers at least, that the Russian and French papers could not have been translated before publication, more so because these volumes should remain a standard reference for a few years to come.

The first paper in the book is an extremely useful, but unfortunately rather short, review by Artsmovich of the present position in closed confinement systems. In another review, Buchsbaum argues for the continuation of study of mirror machines because of the relative ease of experimentation. This view is to some extent reflected by the number of papers presented at the conference on open ended machines. A feature common to most of the theoretical papers is that an effort is now being made to apply various theories to realistic geometries. Some of the experimental contributions already show that such a trend is most fruitful and will be even more so in the future.

These two volumes are obviously going to be standard reference books for those interested in controlled nuclear fusion. They also contain a large amount of information, particularly on the theoretical side, which will be of use to those engaged in more general studies of the plasma state. As is usual for IAEA publications, the printing and general layout are excellent. George Rowlands

NUCLEAR FUSION INTRODUCED

Nuclear Fusion

By H. R. Hulme and A. McB. Collieu. (The Wykeham Science Series for Schools and Universities.) Pp. vii + 153. (Wykeham: London and Winchester, 1969.) 20*s*.

THE "Wykeham Science Series" is intended to broaden the outlook of senior grammar school pupils and to introduce undergraduates to the present state of science, but it is difficult to see how *Nuclear Fusion* can be recommended to grammar school pupils or even to first-year undergraduates. The introduction is confused and its level is such that real understanding is likely to be achieved only by a young reader with a wide range of background knowledge or exceptional ability. The book might perhaps be useful in a school or undergraduate library as a reference book if it were not for the total lack of references or any guide to further reading.

The mature reader with a good knowledge of physics may, however, find considerable enjoyment in the later part of the book, which deals in some detail with thermonuclear reactions in the laboratory, the problems of plasma physics, and the technological and engineering problems associated with fusion reactors. For such a reader, an attractive feature of the book is the wide range of peripheral topics which are covered. These include the evolution and abundance of the elements, cosmological theory, neutrinos and superconductivity.

DAPHNE F. JACKSON

ATMOSPHERIC MOTION

Atmospheric Circulation Systems

Their Structure and Physical Interpretation. By E. Palmén and C. W. Newton. (International Geophysics Series, Vol. 13.) Pp. xvii + 603. (Academic Press: New York and London, July 1969.) 243*s*.

IN meteorology, as probably in most other rapidly advancing sciences, ideas need to be continually revised. There is so much new information that it deters even perfunctory review, while the work of digesting, clarifying and relating the gains over the whole field, for the benefit of philosophers and recruits, is perhaps passing beyond the powers and devotion of individuals. Consequently there is a dearth of good texts, and every new book published is inspected hopefully.

This book, by two authors whose contributions to the analysis of large-scale atmospheric phenomena are everywhere known and respected, has a disarming preface. "It is possible," they say, "that some readers will consider this book somewhat too qualitative or descriptive and perhaps not up to date in every respect." Well, yes, I am one of those readers, but I am grateful for the book for all that, and I commend it to others.

The atmospheric circulations which are discussed are those in the troposphere and of large scale; although attention is paid to the intense cumulonimbus, ordinary cumulus convection is represented only by transfer coefficients, and circulations of intermediate scale, such as those associated with sea breezes, are not considered. The authors choose to begin on the scale which is global, and then to reduce it, though not quite steadily. The chapters deal successively with the mean annual general circulation; its seasonal variation; air masses, fronts, jet streams and tropopauses; the polar front theory; "upper waves"; extratropical disturbances; fronts and jet streams (in more detail) and frontogenesis; three-dimensional motion in and the development of extratropical disturbances; their weather; cumulonimbus; tropical general circulation and disturbances, including hurricanes; and finally a review of the energetics of the various circulations.

The interposition of the discussion on fronts between those on the general circulation and the extratropical disturbances is symptomatic of a peculiar adherence to the so-called polar front "theory" (better regarded and respected as a philosophy and technique of analysis). The polar front had to be replaced by the broad baroclinic zone before a satisfactory theory of cyclones was evolved, and it is rather startling now to read of "the special importance of the polar-front theory for our present knowledge" (page vii), to find the name of Eady mentioned only once (separately from a single page given to the perturbation theory of cyclogenesis), and to read of the "general rule" that "the physical cause for the deepening of cyclones must be ultimately sought in an examination of the higher levels" (page 134). For a while, the impression grows that a cyclone is a pressure minimum on the surface weather map, although eventually (page 542) it seems "proper to consider a cyclone in the broad sense as including the neighbouring anticyclonic regions", and previously (page 338) it has been emphasized that a front is, "at least in some cases, a characteristic acquired by a cyclone during, rather than prior to, its development". Such old-fashioned attitudes are, however, usually later offset by references to recent studies, and do not affect the discussion of mean circulations and tropical disturbances.

Although the title of the book refers to physical interpretations, the relinquishment of the insight provided by perturbation theory and numerical solution of the equations of motion constrains it to detailed description without satisfactory understanding. Insistent compilation of fairly well balanced budgets of various properties, which certainly injects a backbone of quantitative and very interesting discussion into the book, is consistent with the view expressed on page 2 that the mean circulation and the disturbances "co-operate in a physically harmonious fashion to balance the energy and momentum budgets", but does not provide physical interpretation as I regard it.

Nevertheless, it would be absurd to complain that the book does not contain all one would like. It represents a great and valuable labour by the authors. It has more than 200 diagrams, of the kind one is glad to have collected together (although some suffer from the small page size), and several hundred references, work from practically all of which is sensibly incorporated into the text. It will stand as the most complete description of large-scale phenomena presented before the era of numerical analysis, satellite survey, and collaborative observational studies brought the need of surveys even more comprehensive, yet unified by physical reasoning. We will have to wait a long time before any appear, and in the meantime we can be well pleased to have the book which the authors have given us now.

F. H. LUDLAM

ALKALOID STRUCTURES

The Chemistry of the Isoquinoline Alkaloids

By Tetsuji Kametani. Pp. 265. (Hirokawa: Tokyo; Elsevier: Amsterdam, London and New York, 1969.) 145*s*.

THIS book is a compilation of data and references on isoquinoline alkaloids. All of the obviously isoquinoline structural types are included as well as those, like the colchicine and Amaryllidaceae alkaloids, which are related biosynthetically. The book thoroughly covers the literature to the end of 1967.

The tables of data are preceded by a short descriptive introduction to the field which in twenty-two pages can do no more than sketch in a very small sample of typical structural, synthetic and biosynthetic work on isoquinoline alkaloids. It is too brief to be of much value either to workers directly involved in the field or to the general reader, but would serve as a useful starting point for those newly entering on a study of this group of alkaloids.

The principal part of the book is divided into twenty-three sections, each dealing with a particular structural type. All the known alkaloids falling into the structural family are listed in order of increasing molecular weight. For each compound, the structural formula, melting point, specific rotation and plant sources are given; references to the original literature are provided for spectroscopic data and separately to relevant structural, synthetic and biosynthetic investigations.

The lay-out is good in general and the book is easy to use, though there is a unique system of abbreviations (for example, Ph = Physical data). Most of the structural formulae are rather inelegant and old fashioned in appearance, but they are rarely ambiguous. Curiously, occasional formulae are far clearer and modern in appearance (*cf.* adlumidine *versus* bicuculline on page 136).

This book, which has been compiled by a leading figure in isoquinoline alkaloid chemistry, will prove as useful a reference work to those engaged in this field as Hesse's *Indole Alkaloids* does to those studying this other large group of alkaloids. Unfortunately, as a result of the long time involved in its publication and the volume of work in this field in the past two years, the lists are already considerably out of date. It is to be hoped that Professor Kametani will, in due course, be able to produce further editions to update this one. A more rapidly published further volume would better suit the objectives of a book of this kind, particularly if the cost could also be reduced. J. A. JOULE

GENETICS FOR DOCTORS

An ABC of Medical Genetics

By C. O. Carter. (Reprinted from *The Lancet*, May 17 to June 28, 1969, with two additional chapters.) Pp. 94. (The Lancet: London, November 1969.) 15*s*.

THE title of this excellent little monograph could deceive if it is overlooked that it was first published in *The Lancet*. It is clearly intended primarily for doctors or medical research workers who want a guide to medical genetics which is short, clear and inexpensive but not entirely elementary; knowledge of Mendelian genetics and medical terminology is assumed. Bearing this in mind, the book fulfils its purpose admirably. The original chapters cover chromosome mutations, mutant genes of large effect, polygenic inheritance and common diseases, and genetic counselling. One additional chapter consists of "a formidable list of the most common single-gene-determined disorders which the paediatrician is likely to meet"; this is something not hitherto readily available cheaply, and which would alone justify purchase of this book (high marks to any doctor who can characterize a majority of the listed disorders; the overconfident might start on Stargardt's disease, Weill–Marchesani syndrome or leprechaunism).

The author writes with easy authority; depth is not sacrificed to brevity, and chapter references provide an excellent and up to date compendium of basic reading. There are one or two surprising omissions; for example, there is no mention of the Lyon hypothesis of random X-chromosome inactivation, although it is tacitly taken for granted. And there is no consideration of pharmacogenetics, which might be more relevant for practitioners than the theory of gene action (now adequately dealt with in most genetic textbooks). The book is, however, by contrast, particularly strong on the author's own interests, such as twin and familial studies and their bearing on polygenic inheritance.

The last chapter, on the control of genetically determined diseases, brings home the need for a large scale study of amniocentesis; now that this is clearly of value for the detection *in utero* both of chromosome abnormalities and of homozygosity for certain deleterious recessive conditions, the time seems to have come for a full assessment of the risks involved, and of its feasibility as a regular diagnostic technique for eugenicists.

E. H. R. FORD

REVERED EMBRYOLOGIST

Organization and Development of the Embryo

By Ross Granville Harrison. Edited by Sally Wilens. Pp. xxiv + 290. (Yale University Press: New Haven and London, November 1969.) 135*s*; $15.

ROSS HARRISON'S contributions to experimental morphology were of outstanding importance, but were offered with a diffident and, in some cases, almost a casual air. He is most widely remembered for having "invented" tissue culture in order to solve a simple, but cardinal, problem in neurogenesis. In fact, of course, he was not the inventor but the first to succeed in establishing a technique good enough to allow morphogenetic questions to be tackled *in vitro*. The method and the results were published in two preliminary papers and the whole story was only told a few years later, and with a minimum of fuss. His later work on limb polarity and on growth control was of equal intellectual importance, but its presentation was almost perversely modest. Nobody could have done more to invite under-estimation by posterity.

In 1949, towards the end of his working life, Harrison delivered the Silliman lectures but, perhaps predictably, deferred too long their writing up for publication. The present book is a posthumous substitute. It includes several of his earlier review papers (from the 1930s) on subjects cognate to the matter of the lectures. These are interspersed with, and interrupted by, additional material taken from his notes for the lectures and the pictures used to illustrate them. The result is a very handsomely produced memorial volume which contains the essence of his mature views on the major problems of animal development. One's only complaint is that the method of introducing the later material into the text is often confusing. Straight reprints of the papers with appendices would have been better.

The five principal chapters cover problems of cellular differentiation, the significance of tissue culture, neurogenesis, symmetry relations and the uses of heteroplastic grafting. A sixth chapter publishes, at last under his own name, the celebrated Harrison normal staging of *Ambystoma punctatum* which has been widely quoted in print for more than twenty years by other workers. The book ends with a brief summary chapter followed by a list of Harrison's publications.

Although this book will most please the older experimental embryologists for whom Harrison was, and rightly, a revered figure, it would be quite wrong to think that it has no present-day relevance. Most of the problems which the ingenuity of Harrison and his contemporaries exposed and clarified are with us still. D. R. NEWTH

MOVEMENTS IN PLANTS

Physiology of Plant Growth and Development

Edited by M. B. Wilkins. Pp. xxi + 695. (McGraw-Hill: Maidenhead, 1969.) 126*s*.

THIS volume describes itself as a textbook of whole plant physiology specifically aimed at advanced undergraduate and postgraduate students. Eighteen topics have been selected and appropriate authorities give a summary of the present state of thinking in these fields, as well as a background of the major experimentation that has led to the present views.

The first three chapters are devoted to a survey of auxins, gibberellins and kinins, giving the historical events leading to their discovery and isolation, together with the major effects of each of these types of hormones on different growing systems. The bias, as the title of the book implies, is towards physiological responses rather than to the biochemical implications of hormone regulation. The fourth chapter describes the phenomenon of polar transport in plant cells, and although there is little discussion or speculation on the mechanism by which molecules of hormones or other growth substances are moved through cells more easily in the downward than the upward direction, the author provides a comprehensive review of the facts as they are at present known.

There follows an exciting chapter on apical dominance in which the reader is led through the different theories which might account for suppression of lateral bud growth. The experiments are well described and the discussion ends with a set of clear summaries. The chapters on geotropism and phototropism follow in the same vein, and both define the major problems that still need clarification. Nastic responses are covered in an excellent chapter with a special emphasis on the rapid and so far unexplained movements of the sensitive plant *Mimosa pudica*.

A discussion of the factors governing the movement of stomata leads on to a clear chapter on photosynthetic processes and to another which sets out the problems and the theories of how carbohydrates synthesized in the leaves are translocated from one part of the plant to another. The evidence for long distance transport of metabolites within the highly specialized cells of the phloem is then summarized. The problem of how water is taken up and moved in a plant is described from a thermodynamic viewpoint. The pathway followed from root to leaf receives very adequate treatment, and an associated chapter deals with the mechanisms by which ions are taken up from aqueous solutions, and discusses how they too can be moved from one part of the plant to another.

The discovery and properties of the photosensitive pigment phytochrome are attractively told by one of the collaborators in this work. It precedes a comprehensive account of the part now seen for phytochrome in the regulation of plant morphogenesis in response to specific quanta and wavelengths of light. A discussion of photoperiodism and vernalization follows, in which the reader is acquainted with the effects of other environmental factors, day length and low temperatures, on the development and differentiation of plants. In a subsequent chapter the author discusses the problems of how and why and to what signals plants respond when they stop active growth and enter into the latent stage of dormancy, and what factors initiate the resumption of growth, as in the germination of a seed.

But the pattern of plant growth is not confined to a direct response to changes in the environment, for plants also measure time. In a final chapter on circadian rhythms the problem of why once a response to the environment is set in motion, it can be repeated at near 24 hour intervals under uniform conditions, serves as a good introduction to the study of biological clocks.

The errors in this volume are few, the references are comprehensive and are generally well chosen and the presentation is clear. Each chapter states the problems and the major facts and theories and seldom falls into the trap of supplying an unquestioned solution. The reader is left with ideas of how future research may develop and some of the experimental difficulties to be encountered. Most of all, this book encourages thought and achieves what it has set out to be—a very useful textbook for both undergraduates and postgraduates.

DAPHNE J. OSBORNE

COMMUNICATION UNDISPUTED

Communication

A Discussion at the Nobel Conference. (Organized by Gustavus Adolphus College, St Peter, Minnesota, 1969.) Edited by John D. Roslansky. Pp. ix + 131. (North-Holland: Amsterdam and London, 1969.) 42*s*.

ONCE again a group of people from widely diverse fields have been brought together to state their positions in terms which are clear to laymen and students alike. Communication is interpreted in the widest possible sense and the result is hardly the "discussion" that the subtitle claims. If you get people as diverse as these, and ask them to speak simply, they inevitably deal largely in generalizations making everything seem black or white. This is not to say that the participants did not say things which were interesting and often important. Augenstein considers the implications of our ability to change the "little black box called the mind"; whose ideals shall be inculcated ? To judge from this chapter, there must have been some splendid audience participation during the paper. The dangers of over-simplification are vividly demonstrated by the argument that Caryl Cheesman's beliefs enshrined in his memory were all that caused him to rape and murder; *ergo* change his memories and your criminal is reformed. Marler is on happier ground with his review of communication in the animal kingdom, pointing out clearly how all the design features of a language, proposed by Hockett and Altmann, can be exemplified somewhere in the animal kingdom, but appear together only in human language. Chomsky would find the essentially human character of man's language in its creativity which, he believes, reflects inherent properties of the human mind. Kaplan, speaking as a philosopher, underlines the importance of communion as distinct from communication; all too easily humans may communicate without communion.

But it is Lenneberg's contribution—as a psychologist—in which there appears the only real sign of the inter-disciplinary sparring which should be the real purpose of such gatherings. He criticizes the experiments by the Gardners on chimpanzees in which they taught the animals sign "language"; unlike Marler, he believes that the existence of this language is not proved, and he describes the critical experiments which he would like to see performed in order to settle the question.

There is therefore little real discussion and little comparison of the viewpoints of linguistics, philosophy, animal behaviour, psychology and so forth. Nevertheless, the papers make good reading as brief reviews of their authors' viewpoints.

J. D. CARTHY

Correspondence

NERC Support for University Research

SIR,—Your issue of December 6 last contained two constructive articles about my council's activities and policy arising from the publication of our annual report for 1968/69. Each raised, however, certain misgivings about the support by the council of research at universities. I would be grateful to have a little of your space to explain the position, and perhaps in so doing to assist your readers to distinguish between fact and hearsay in the article "Goldfinger no longer" of your issue of January 3, 1970.

There are perhaps three main points made in this series of articles concerning our policy for support of research in universities that deserve an answer. The first is that on the face of it NERC loaded itself with too many commitments when it was being encouraged to expand. I think the trouble here is partly due to a misunderstanding about our funding of university research arising from the difference between the total value of new grants awarded in a given year and the total expenditure on all grants, new or existing, in that year. The former figure is not expenditure but new commitments extending for several years ahead, depending on the duration of the grants. In steady conditions the figures for both new awards and annual expenditure will move together, but in practice these conditions have never arisen.

	1966/67	1967/68	1968/69	Estimated 1969/70
Total value of new awards (academic year, October–September)	1·8	1·2	0·9	(1·0)
Annual expenditure (financial year, April to March)	0·69	0·89	0·93	(1·2)

In particular, at the end of the last university quinquennium in 1967, a large proportion of existing grants terminated. It was necessary in that year, in order to sustain a steady growth in subsequent annual expenditure, to enter into an exceptionally large commitment on new awards, although this level of new commitment could not, and was never intended to be, sustained. The effect of the transition from one quinquennium to the next can be seen by comparing figures for value of new awards with those for annual expenditure on research grants, both given in millions of pounds.

These figures show that our annual expenditure on university research grants is likely to increase by about 75 per cent over the past 4 years from 1966 to the present time.

The second point concerns the fact that only 15 per cent of NERC's expenditure as shown in our financial statement for last year went to universities. These figures for university support are not, however, comparable as they stand with expenditure on research elsewhere by the council. In particular they exclude capital expenditure on buildings, equipment that is a regular part of the facilities of the department concerned, and supporting costs and services, all of which are provided at universities by the UGC but in our own institutes have to be included in the NERC expenditure. In the marine field the major part of the activities of our Research Vessel Unit, of the RRS John Murray and of the use of marine research equipment held at the Research Vessel Unit, are devoted to the needs of universities. Thus both in actual money and in research effort, our effective support of university research is a substantially larger fraction of our total expenditure than appears from the figures shown in the financial statement under that head for accounting purposes.

Our general policy towards support of university research in the environmental sciences has been dealt with at length in our current annual report. I need only say that we believe our support must be selective, should be guided (but not inhibited) by broad strategic objectives, and ought to link up with the programmes of our institutes whenever it is sensible and productive to do so. This brings me to the third point, namely, the use of the so-called "success rate", that is, the ratio of funds awarded to funds applied for (which last year was just over one-third overall), as a measure of this disappointment.

In fact, this ratio as it stands is a very unreliable index of the true extent to which our support is falling short of the total legitimate and deserving requests. Some of the applications we receive, including the largest ones, are either outside our remit or are for other reasons unsuitable for support. We believe it is right to bring all of them to the attention of one or other of our grant-awarding committees rather than to make the situation look more rosy by pruning them beforehand. Sometimes it is possible to find ways of achieving the desired research objectives more economically, particularly by sharing expensive equipment between universities or with council establishments. In some cases an award is made initially for a shorter period than is requested, with the possibility of an extension if promising results are achieved. Our grant-awarding committees would be failing in their task of distributing available funds in the best interests of science if they did not give careful and critical attention to such matters.

What my council is concerned to ascertain from our grant-awarding committees is whether, at the end of the day, they feel that a significant proportion of truly deserving applications has had to be turned down through lack of resources. So far, I am glad to say, this has not been the case.

Yours faithfully,
R. J. H. BEVERTON

Natural Environment Research Council,
Alhambra House,
London WC2.

Mild Thaw on Disarmament

SIR,—You state, sensibly enough (*Nature*, **225**, 211; 1970), that with regard to strategic arms limitation, "Relations with the nuclear pigmies—Britain, France and China—are also a complication, if only because nobody can at this stage commit himself to permanent restraint in nuclear weapons when there is no certainty that smaller powers will not seek to become top dogs". Certainly; but also vice versa: few among the "nuclear pigmies" (in esse or posse) can "at this stage commit [themselves] to permanent restraint in nuclear weapons when there is no certainty that" the nuclear giants "will not seek to become top dogs" in the nuclear condominium sense. This is the lesson of the non-proliferation treaty negotiations, and Americans and Russians had better not forget it. If, as *Die Welt* reported two weeks ago, there is a Russian–American plan not to consider Russian medium range strategic missiles until agreement has been reached and put into effect on intercontinental missiles, target states of those medium range strategic missiles—for example, France, United Kingdom, Japan, India, etc.—are unlikely to commit themselves to much "permanent restraint". This fact may well complicate negotiations at Vienna, but it will not go away merely because it is inconvenient.

Yours faithfully,
ELIZABETH YOUNG

100 Bayswater Road,
London W2.

Cover-to-cover Translation

SIR,—Abstracts as a form of journal publication from which subscribers would order full texts as needed are mentioned by your geomagnetism correspondent (*Nature*, **224**, 750; 1969) as a possible solution for meeting cover-to-cover translation problems in geophysics.

Such a scheme has been used for some twenty oriental vernacular journals at the Air Force Cambridge Research Laboratories (AFCRL) since 1962. Abstracts are made for each single number of each journal as received, and are circulated to AFCRL scientists. The individual scientist then requests full translation of those articles whose abstracts appear pertinent to his interests. Abstracts from any one journal are eventually bound together and classified by subject subdivisions in order to provide a desk tool for retrospective search. One such oriental abstract translation publication which is available to the public is *Acta Meteorologica Sinica; translated titles and abstracts from volume 27 (1956) through volume 35 (1965)*, published as AD 667 520. There are 360 articles abstracted in this publication, of which 69 (19 per cent) have been requested in full translation.

Your geomagnetism correspondent also suggests that cover-to-cover translation is not the most economical way of bringing Russian work to the attention of western scientists. However, AFCRL records of articles requested in xerographic copy show that the cover-to-cover translation of *Astronomicheskii Zhurnal* is the second most heavily used journal out of a total of 57 astronomical journals used during 1964–66. Of the 119 users of these 57 astronomical journals, 29 users (15 per cent) requested 106 articles from this fully translated journal. In this case, response time and costs would be greater for an abstract and translation cycle than for a cover-to-cover subscription, as the approximately $50 subscription cost would hardly cover the cost of one translation.

The current difficulties encountered by the American Geophysical Union in its cover-to-cover translation programme may therefore be difficulties specific to an individual discipline rather than to all disciplines. Among factors that appear relevant are reader population sizes among different disciplines and the related number of institutions carrying programmes in these disciplines. This raises some interesting questions about the relationship of disciplines to the need for subsidizing cover-to-cover translation programmes.

Yours faithfully,

OLE V. GROOS

AFCRL Research Library.

Numerical Notation: Decimalization

SIR,—The suggestion of Molyneux (*Nature*, **224**, 1237; 1969) that a number such as 12,300,000 could conveniently be written as "1·23 *D*7" and spoken as "one point two three deka seven" supports the earlier suggestion of Duffin as reported by Synge[1] and differs from it only in the use of "deka" and "deci" rather than "up" and "down" to indicate respectively positive or negative powers of ten.

Such systems, however unpalatable at first sight, lead to a great simplification in numerical notation avoiding both the need to devise[2] new prefixes to indicate multiplication by powers of ten higher than the current tera (10^{12}) or lower than atto (10^{-18}) and also the ever present and regrettable urge to invent new names for multiples or sub-multiples of the primary Système International (SI) units. Equally, the current ambiguities[3] of the terms billion and trillion could disappear from the international scene.

The indicators "up" and "down" are probably more graphic and therefore more readily assimilated than "deka" and "deci". On the other hand, the classical derivation of the latter would render them readily recognizable in other languages: but this is not likely to be material as the indicators "up" and "down" would always be better translated into the language used for the associated numerals in order to form a unified whole for the number expressed.

We are already accustomed to breaking down numbers into groups of three numerals and this facility could be retained by using powers of 10 in multiples of three only, thus

12,300,000 would be said as "twelve point three up six",

1,230,000 would be said as "one point two three up six",

but 123,000 would be said as "one hundred and twenty-three up three".

Yours faithfully,

F. W. DERWENT

National Coal Board,
Ashby Road,
Stanhope Bretby,
near Burton-upon-Trent.

---

[1] Synge, J. L., *Relativity: the General Theory*, 421 (North-Holland Publishing Co., 1960).

[2] Derwent, F. W., and Oakland, W. H., *Nature*, **220**, 311 (1968).

[3] Cuckow, F. W., *The Director*, **18**, 390 (1966).

University News

Mr Denis R. Towill has been appointed to the newly established chair in engineering production in the **University of Wales Institute of Science and Technology.**

Appointments

Philip E. Culbertson has been designated director of the **Advanced Manned Missions Program** in the National Aeronautics and Space Administration's Office of Manned Space Flight.

Announcements

The **Neill Prize of the Royal Society of Edinburgh** for 1967–69 has been awarded to **Dr Ann R. Sanderson** for her contributions to taxonomic zoology.

The president and council of the **Royal Astronomical Society** have made the following awards: the Gold Medal to **Dr Horace W. Babcock,** director of the Mount Wilson and Mount Palomar Observatories; the Eddington Medal to **Professor Chushiro Hayashi,** professor of physics, Kyoto University.

ERRATUM. In the paper "Genetics of NADP Isocitrate Dehydrogenase in *Paramecium aurelia*" (*Nature*, **225**, 181; 1970), line 16 on page 182 should read "heterozygote pattern (IDH$_m$ 1–2) is consistent with".

ERRATUM. In the article "The Moon at Houston" (*Nature*, **225**, 321; 1970), the second sentence under the sub-heading Chemical Composition on page 326 should read: "Of the ninety-two elements in the periodic table, results are now available for seventy-nine".

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Vacant: House in London, Ontario, Canada, for 12 months from mid-August 1970. Fully furnished, 4 bedrooms, 2 bathrooms, living room, dining room, kitchen, den, playroom, utility room. All necessary appliances, linen, dishes, etc. On 100 foot × 150 foot lot about 1 mile from University of Western Ontario. Please contact Professor Miles H. A. Keenleyside, Department of Zoology, University of Western Ontario, London, Canada.

Vacant: Large 3-bedroom, well furnished flat in Brooklyn, New York, with modern kitchen. Rapid transit time 30 minutes to mid-Manhattan. Opposite Park, Botanic Gardens, Public Library and Museum. For 9–12 months from September 1, 1970. $215/month. Please contact R. Pollack, 135 Eastern Parkway, Brooklyn, New York 11238.

Wanted: Furnished house or flat for New Zealand lecturer and family, 3 double bedrooms minimum, from beginning of March to mid-April, in Oxford, Wantage area. Please contact Dr J. Loutit, MRC Molecular Genetics Unit, Department of Molecular Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh EH9 3JR.

British Diary

Monday, February 2

Electronic Measurement Related to Fundamental Standards (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Ergonomics—Human Factors in Equipment Design (7 p.m.) Mr D. Beevis, Institution of Electrical Engineers, at the Royal Star Hotel, Maidstone, Kent.

Live-line Working on Distribution Systems (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Structure of the Chemical Industry by 1980 (6.30 p.m.) Mr M. C. Hyde, Society of Chemical Industry; and the Industrial Marketing Research Association, at 14 Belgrave Square, London SW1.

Theory of Emulsion Polymerization (7 p.m.) Mr D. Kinsler, Oil and Colour Chemists' Association, at Hull College of Technology.

Tuesday, February 3

Chimaeras in Mouse and Man (5.30 p.m.) The Hon. Dr Anne McLaren, University of London, at the Royal Veterinary College, Royal College Street, London NW1.

Developments in British Hydrography Since the Days of Captain Cook (5.15 p.m.) Rear-Admiral G. S. Ritchie, CB, DSC, Royal Society of Arts, Commonwealth Section, at John Adam Street, Adelphi, London WC2.

Dispersive Acoustic Devices (5.30 p.m.) Dr D. P. Morgan and Mr W. S. Mortley, Institution of Electrical Engineers, at Savoy Place, London WC2.

Expanded Polymers, Mr D. Pannell; **Microwave Heating Applied to the Rubber Industry,** Mr P. A. Wincott (6.30 p.m.) Institution of the Rubber Industry, at the Surrey Arms, Morden Road, Mitcham.

Experience with Area Control of Road Traffic in Glasgow and West London (5.30 p.m.) Mr J. A. Hillier and Mr B. M. Cobbe, Institution of Electrical Engineers; and the Automatic Control Group of the I.Mech.E., at Savoy Place, London WC2.

New Concepts of the Function of the Lymphatic System in the Swollen Leg (5.30 p.m.) Mr J. S. Calnan, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Eighth of fifteen lectures on "The Scientific Basis of Medicine".)

The Adsorption Recovery of Solvents from Moist Air (7 p.m.) Mr H. J. Abrams, Mr G. Jones and Mr C. H-T. Koo, Institution of Chemical Engineers, at the Oaklands Hotel, Hoole Road, Chester.

The Comfort of Unreason in Archaeology (8 p.m.) Dr Glyn Daniel, in the Middleton Hall, University of Hull.

Wednesday, February 4

Biochemistry of Bacterial Walls in Relation to the Membrane (4.30 p.m.) Professor Jean Marie Ghuysen, Medical Research Council, at the National Institute for Medical Research, Mill Hill, London NW7.

Colour in Situ (3 p.m.) Mrs P. Brooke, Mrs P. Pirie, Mr D. Phillips and Mr M. J. Thomas, Colour Group (Great Britain), in the Physics Building, Imperial College, London SW7.

Going Metric in the UK Petroleum Industry (5.30 p.m.) Mr A. Haddock, Institute of Petroleum, at 61 New Cavendish Street, London W1.

Medical Illustration (1 p.m.) Mr E. Gaskell, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Modern Techniques in High-voltage Cable Laying (7.30 p.m.) Mr D. J. Skipper and Mr E. P. C. Watson, at the CEGB, Guildford, Surrey.

Optical Communications through Glass Fibres (7 p.m.) Professor W. A. Gambling, Institution of Electronic and Radio Engineers, at Guildford Technical College.

Passive Satellite Communications (6 p.m.) Mr R. L. Harris, Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Recent Developments in the Fluid Transport of Granular Materials in Pipelines (5.30 p.m.) Dr R. A. Duckworth, University of London, at King's College, Strand, London WC2.

Science and Race (1.15 p.m.) Professor M. Banton, University of London, at Queen Elizabeth College, Campden Hill Road, London W8.

The Open University (6 p.m.) Dr Walter Perry, OBE, Royal Society of Arts, at John Adam Street, Adelphi, London WC2.

The Teaching of Hydrodynamic Lubrication Using Scaled Models, and Optical Elastohydrodynamics (6 p.m.) Mr A. Cameron, Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

The Testing of Servomechanisms for Development and Maintenance (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Thursday, February 5

Engineering and the Science Research Council (5.30 p.m.) Sir Brian Flowers, FRS, Institution of Electrical Engineers, at Savoy Place, London WC2.

Functional Coatings for the Packaging Industries (6.30 p.m.) Mr J. Howarth-Williams, Oil and Colour Chemists' Association, at the Royal Turks Head Hotel, Grey Street, Newcastle upon Tyne.

Have the Full Horrors of Metrication Dawned on the Steam Plant World ? (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Metabolism of Pyrrolizidine Alkaloids and Its Relation to their Biological Effects (5 p.m.) Mr A. R. Mattocks, University of London, at the Middlesex Hospital Medical School, Mortimer Street, London W1.

New and Traditional Refrigeration Systems for Air-Conditioning (6 p.m.) Mr J. H. Allen, Institute of Refrigeration, at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London SE1.

Physiological Measures in Psychiatry (5.30 p.m.) Dr M. H. Lader, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Ninth of fifteen lectures on "The Scientific Basis of Medicine".)

Pick-up Design (7 p.m.) Institution of Electronic and Radio Engineers; and the Institution of Electrical Engineers, at the Shrewsbury Arms Hotel, Market Street, Rugeley, Staffs.

Radiometric Standards (2.30 p.m.) Society for Analytical Chemistry, Radiochemical Methods Group, in the Lecture Room of the Laboratory of the Government Chemist, Cornwall House, Stamford Street, London SE1.

Reproduction in the Giant Pacific Octopus (4.30 p.m.) Professor T. R. R. Mann, FRS, Royal Society, at 6 Carlton House Terrace, London SW1.

The Making of Mountains (1.20 p.m.) Dr R. Mason, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Friday, February 6

Some Aspects of Direct Television Reception from Satellites (5.30 p.m.) Mr K. G. Freeman, Mr R. N. Jackson, Mr P. L. Mothersole and Mr S. J. Robinson, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Role of Excimers, Exciplexes and Electron Transfer in Photochemical Reactions (1 p.m.) Dr S. Davidson, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

Saturday, February 7

Appreciating the Countryside—Nature Trails and Field Museums in Britain and America (3.30 p.m.) Mr G. Stansfield, Inner London Education Authority, at the Horniman Museum, London Road, Forest Hill, London SE23.

Microcirculation of the Skin, Mr T. J. Ryan, Miss K. A. Grice, Mr C. A. Ramsay, Mr J. Morley, Mr T. J. Williams and Mr W. Hartson; **Other Microcirculation Topics,** Mr S. M. Blench, Mr A. L. Stalker, Mr L. J. Brown, Mr J. N. Mehrishi and Mr W. L. Whitehouse (2 p.m.) British Microcirculation Society, at Queen Mary's Hospital, Roehampton, London SW15.

Monday, February 9

A Volunteer at Masada (5.30 p.m.) Miss Katherine Blair, Royal Institution, at 21 Albemarle Street, London W1.

Colour Television Receiver Design and Maintenance (6 p.m.) Mr G. D. Barnes, Institution of Electronic and Radio Engineers; and the Institution of Electrical Engineers, at the University of Strathclyde, Glasgow.

Polymers in the Year 2000 (7.30 p.m.) Professor C. E. H. Bawn, CBE, FRS, Institution of the Rubber Industry, at the Donnan Laboratories, The University, Liverpool.

Techniques of Mask-making (2.30 p.m. colloquium) Institution of Electrical Engineers, at Savoy Place, London WC2.

HOW TO BUY NATURE

Volumes start in January, April, July and October, but subscriptions may begin with any issue.

The direct postal price per subscription for one copy of NATURE each week is:

12 MONTHS (52 issues and 4 indexes)

Great Britain and Eire.................................**£14.0.0** each
United States of America........(by air freight)............**$48** each
Canada..........................(by air freight)..........**$52** each
Elsewhere overseas by surface mail....................**£14.0.0** each

(Charge for delivery by air mail on application)

*Shorter periods *pro rata.* (Minimum three months.)

Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
4 LITTLE ESSEX STREET, LONDON WC2
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2
Subscription Department
MACMILLAN (JOURNALS) LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 5431
Advertisements only should be addressed to
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2
Telephones 01-242 6264/01-405 4743
Telegrams: Textualist London WC2
Registered as a newspaper at the Post Office

Copyright © Macmillan (Journals) Limited, January 31, 1970

UNIVERSITY COLLEGE OF NORTH WALES

BANGOR

DEPARTMENT OF PURE MATHEMATICS

Applications are invited for the post of LECTURER IN PURE MATHEMATICS.

Salary will be at a suitable point on the scale £1,240 by £115 to £2,850.

Further particulars of this post may be obtained from the Secretary and Registrar and applications (two copies), giving details of age, qualifications and experience together with the names and addresses of three referees, should be sent to reach the Secretary and Registrar by February 21, 1970. (386)

UNIVERSITY COLLEGE OF TOWNSVILLE

DEPARTMENT OF BIOLOGICAL SCIENCES

Applications are invited for the position of

TECHNICAL OFFICER GRADE I

(Curator of Microscopes)

An experienced microscope and optical instrument mechanic is required to establish a microscope curating service and to set up a small workshop for tool and precision instrument assembly and repair.

Present microscope stocks, manufactured by Zeiss, Leitz, Wild, Olympus, Vickers, Zeiss Jena, Nikon range from student monoculars to photomicroscopes.

Applicants must have served a recognized engineering apprenticeship, preferably with an optical instrument maker and must have recent experience in producing or servicing optical instruments.

Removal expenses and fares will be provided through the Australian Commonwealth Government Assistant Migrant Scheme.

The salary payable will be within the ranges $A1,138 to $A4,455 per annum. Persons holding appropriate Degree or Diploma receive an additional $A300 per annum.

Applications, giving full details of age, qualifications and experience, together with references or the names of referees, should be sent to:

The Staff Officer,
University College of Townsville,
P.O. Box 999,
TOWNSVILLE, Q. 4810,
AUSTRALIA.

with whom applications close on February 28, 1970. (370)

UNIVERSITY OF TORONTO

TORONTO, CANADA

The Institute for Aerospace Studies has a vacancy effective July 1, 1970, or earlier in the field of aerodynamic noise at the ASSISTANT or ASSOCIATE PROFESSOR level. Candidates should have a Ph.D. with strength in both experimental and theoretical aspects of the field. Duties would include teaching and research (with supervision of graduate students) to complement the existing programme on jet noise and sonic boom.

Applications should be sent to H. S. Ribner, Institute for Aerospace Studies, University of Toronto. A curriculum vitae, detailing background and publications, should be included. Letters of reference are desirable but not essential, and material will be held in confidence if requested. Interviews will be held by Dr. Ribner at Cumberland Hotel, Marble Arch, London, W.1, on February 9, 1970, between 10 a.m. and 4 p.m. (376)

SOUTH AFRICA

"geologists paradise"

is an epithet justified by large varieties of geological formations and rich mining deposits together with climate ideal for outdoor work.

The Geological Survey Department of the South African Government now offers the following opportunities for suitably qualified scientists:

GEOLOGISTS

holding at least a B.Sc. (Hons) degree (or equivalent four year degree) in geology, to deal with geological mapping, and field and laboratory investigation, on one or other specialised area.

GEOPHYSICISTS

with at least a B.Sc. (Hons) or equivalent four year degree in physics/geology, to work in the field on geophysical surveys in search of water and minerals, and on data reduction.

In addition to the indicated work ample opportunities will be available for research.

Initial appointment will be on an extendible three-year contract basis, while permanent pensionable positions could be available on the attainment of South African citizenship.

A salary in the region of £1,750–£2,625 will be offered, together with field and travelling allowances, for a five-day working week. Fringe benefits include generous vacation and sick leave, vacation bonus and medical benefit scheme. Full air or sea passage will be paid for successful applicants and their dependants.

Applications, or requests for further information, should be sent to the

Chief Migration Officer,
South African Embassy,
Trafalgar Square,
London W.C.2.

(415)

For further Classified advertisements see page iiib

Beecham Research Laboratories Walton Oaks Tadworth Surrey

Biochemists

to be employed in Lipid Metabolism Studies. These are new appointments and the successful applicants will work within a newly formed Project Group on investigations into nutritional and metabolic diseases in animals and humans. This will involve fundamental studies in the fields of lipid and carbohydrate metabolism.

Candidates should have a good honours degree in Biochemistry and either a further degree or experience in biochemical research. Some training in physiology and a background in nutritional research, although not essential, would be an advantage. The successful applicants will be joining a multidisciplinary team of scientists and will be given considerable scope to work on their own initiative.

A good starting salary will be offered and reviewed regularly. Beecham Research Laboratories is part of the Beecham Group, for whose generous non-contributory fringe benefits, including profit sharing worth an additional 10% to 11% of salary, and pension scheme with life coverage, the successful candidates will qualify. The laboratories are situated in a pleasant area of Surrey within easy reach of London, and consideration may be given to removal assistance.

Applications should be sent to: **Head of Personnel Department, Beecham Research Laboratories, Brockham Park, Betchworth, Surrey.** (436)

BIOCHEMICAL RESEARCH

TEAM LEADER

Biorex Laboratories Ltd. are seeking a Senior Biochemist with several years' postdoctoral experience who will recruit and lead a small research team in biochemistry. The team will co-operate with established teams of pharmacologists and chemists in the development of compounds of therapeutic importance and in the elucidation of their mode of action.

The research organization of Biorex Laboratories already collaborates extensively with various Universities and the successful applicant will be expected to foster and extend such collaboration.

Interested persons are invited, in the first instance, to write for additional information to **The Director of Research, Biorex Laboratories Ltd., Biorex House, Canonbury Villas, London, N.1.** (442)

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF PSYCHOLOGY

RESEARCH ASSISTANT

Applications are invited from suitably qualified persons for the post of RESEARCH ASSISTANT. The person appointed will work with Dr. J. S. Dimond on a project supported by the Medical Research Council on "The Role of the Cerebral Hemispheres in the Control of Human Performance." The post carries a salary of £1,000 by £100 to £1,200. The appointment will be initially for three years.

Letter of application, quoting Ref. No. ADV/N, together with a full curriculum vitae and the names and addresses of two referees should be submitted to the Registrar, University College, Cathays Park, Cardiff, from whom further particulars of the Department may be obtained, not later than February 28, 1970. (444)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BOTANY

Applications are invited for the post of LECTURER IN ECOLOGY in the Department of Botany. Preference will be given to candidates with interests in applied aspects of the ecology of terrestrial environments, particularly the effects of pollution by industrial activity or pesticides and herbicides or the problems of derelict land. The duties of the post will commence on 1st October, 1970. The initial salary will be within the range £1,240–£2,850 per annum.

Applications, stating age, qualifications and experience, together with the names of three referees, should be received not later than 9th March, 1970, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further details may be obtained. Please quote Ref.: RV/5680/N. (422)

UWIST CARDIFF

UNIVERSITY OF WALES INSTITUTE OF SCIENCE AND TECHNOLOGY

APPLIED PHYSICS DEPARTMENT

LECTURER IN ELECTRONICS

required to assist in teaching students preparing for the B.Sc.(Tech.) and M.Sc./Diploma in Electronics.

Applications would be particularly welcome from persons with experience of semiconductor physics of particular application to integrated circuits and with practical experience in this field.

Salary for initial appointment in the range £1,240 by £115 to £1,700. Full salary scale £1,240 to £2,045 (bar) to £2,850.

Application forms (to be returned by February 23 quoting Ref. N) and further particulars may be obtained from the Personnel Officer, UWIST, Cardiff, CF1 3NU. (410)

WEST HAM COLLEGE OF TECHNOLOGY

ROMFORD ROAD, LONDON, E.15

RESEARCH TECHNICIAN

required to assist project in association with the National Physical Laboratory and financed by the Ministry of Technology.

The work will involve evaporation of films in ultra-high vacuum.

Starting salary, £1,250 per annum.

Applications giving full details of age, qualifications and experience, together with the name of a referee, should be sent as soon as possible to the Head of the Physics Department. (426)

ROWETT RESEARCH INSTITUTE

BUCKSBURN, ABERDEEN, AB2 9SB

LARGE ANIMAL CALORIMETRY

The Calorimetry Section of the Physiology Department of the Institute is to be considerably expanded in size and staff and applications are invited from suitably qualified biochemists or physiologists for its Headship. The post is graded Principal Scientific Officer (£2,599 to £3,596) but for the exceptional candidate the Senior P.S.O. grading (£4,045 to £4,620) would be considered. Superannuation under F.S.S.U. Applicants will be expected to take part in the planning of the expansion as well as to undertake research into feed energy utilization by pigs, cattle and sheep.

Applications, which should include the names of three referees, should be sent to the Secretary of the Institute by February 28, 1970 (possible extension for overseas candidates). Further details may be obtained on request. (343)

MEMORIAL UNIVERSITY OF NEWFOUNDLAND

Invites applications for the position of

DEAN OF SCIENCE

Science Departments include Biochemistry, Biology, Chemistry, Geology, Mathematics, Physics and Psychology with an academic staff totalling 100. All Departments offer the M.Sc. and five have Ph.D. programmes. Special interests of the various Departments include: cell biochemistry, pollution-related biology, NMR and ESR spectrometry, solid earth geophysics, Appalachian geology, laser physics, and topology. Interdisciplinary studies are emphasized, particularly those bearing on the problems and resources of the Canadian Atlantic Region—and these have lead to the establishment of the Institute for Research in Human Abilities, the Centre for Environmental Biology and the Marine Sciences Research Laboratory.

A vigorous professional scientist is sought for this newly created post, preferably with administrative experience and an extensive background in teaching and research. The salary will be competitive.

Applications should include a full curriculum vitae and the names of three referees and should be addressed to: The President, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

A copy of applications should also be sent to the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London, W.C.1. from whom information about the University may be obtained. **(324)**

UNIVERSITY COLLEGE LONDON

Applications invited for two posts of LECTURER and TEMPORARY LECTURER (with prospects of permanent appointment) in Department of MATHEMATICS. Applicants should have a research interest in some branch of Pure Mathematics, to commence on October 1, 1970. Salary, according to age and experience, on scale £1,240 to £2,850, plus £60 London Allowance, p.a.; F.S.S.U.

Applications as soon as possible to the Secretary, University College London, Gower Street, W.C.1, from whom further particulars may be obtained. (433)

UNIVERSITY OF WALES

UNIVERSITY COLLEGE OF SWANSEA

SECOND CHAIR OF GEOGRAPHY

Applications are invited for the Second Chair of Geography in the department of Geography.

Further particulars, quoting reference No. B6, may be obtained from the Registrar, University College of Swansea, Singleton Park, Swansea, Glam. SA2 8PP, by February 21, 1970.

ANEURIN DAVIES,
Registrar.
(383)

CSIRO

RESEARCH APPOINTMENTS

AUSTRALIA

DIVISION OF PLANT INDUSTRY
TOBACCO RESEARCH INSTITUTE

GENERAL The Tobacco Research Institute at Mareeba is a unit of the Division of Plant Industry of the Commonwealth Scientific and Industrial Research Organization and is located 40 miles inland from Cairns, North Queensland, in the centre of a district in which tobacco growing under irrigation has expanded rapidly in recent years. Work at the Institute includes physiological, nutritional, and agronomic studies to determine factors responsible for the yield and quality of the cured tobacco leaf. The Institute has excellent facilities for laboratory and field research, including well-equipped laboratories, glass-houses, library and experiment station. In addition it has strong links with the headquarters of the Division in Canberra, A.C.T., which is engaged in research in many fields of plant biology.

Secondary education is available and housing will be provided at a nominal rental in the the case of married male appointees. Applications are invited for appointment to the following research positions.

SOIL CHEMIST (Reference No. 815/83) The growth of an exhaustive crop such as tobacco combined with the use of irrigation water to extend the growing season, has resulted in a decline in soil fertility with a consequent deterioration in the quality of the tobacco leaf being produced. The appointee will be required to investigate the possibility of preventing further deterioration of these sandy loams, of rehabilitating these soils and studying the contribution of rotation crops to tobacco nutrition under local conditions. The effect of irrigation on the activity of soil micro-organisms, would also be incorporated in the study.

CROP PHYSIOLOGIST—AGRONOMIST (Reference No. 815/82) Mainly because of disease and water availability, a pattern of tobacco production has been established involving field planting in the cooler months of the year, and harvesting in November-December. At this latter time the upper section of the plant is developing while the lower is senescing. These conditions require a delicate balance of nutrition to prevent deficiencies or excesses. With the introduction of disease resistant varieties and a possible shift in desired leaf types, changed planting times are being investigated with planting in late summer. An officer is required to participate in studies of the differences in leaf development under the two different regimes.

QUALIFICATIONS Applicants should have a Ph.D. degree in an appropriate field or postgraduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability.

SALARY Depending upon qualifications and experience, the appointments will be made within the salary range of Research Scientist, $A6,139–$A7,789 p.a., or Senior Research Scientist, $A8,105–$A9,448 p.a. Salary rates for women are $A428 p.a. less than the corresponding rates for men. In addition to salary a District Allowance of $A140 p.a. (Married) and $A70 p.a. (Single) is payable.

CONDITIONS The appointments will be initially for a fixed term of three years, after which, if mutually desired, either an extension of tenure or appointment for an indefinite period will be considered. The appointments will be conditional upon passing a medical examination and an initial probationary period of twelve months may be specified. An indefinite appointment carries with it Commonwealth Superannuation Fund or Provident Account privileges. Fares paid for the appointee and his dependent family.

Applications, quoting the appropriate reference number, and stating full name, place, date and year of birth, nationality, marital status, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant's academic and professional standing, should reach:

Mr. R. F. Turnbull,
Chief Scientific Liaison Officer,
Australian Scientific Liaison Office,
64 Kingsway, LONDON, W.C.2. by the *27th February, 1970.*

Applications in U.S.A. and Canada should be sent to The Scientific Attaché, Australian Embassy, 1601 Massachusetts Ave. NW, WASHINGTON DC 20036.

(396)

UNIVERSITY OF LEICESTER

LECTURER IN PHYSIOLOGY

Applications are invited for a LECTURESHIP in the DEPARTMENT OF PHYSIOLOGY in the School of Biological Sciences. Initial salary according to qualifications and experience on scale £1,240 to £2,850 a year, with F.S.S.U. membership. An appointment might be made in the Senior Lectureship grade (maximum £3,670 a year) if a suitable applicant presented himself.

Further particulars from the Registrar, to whom applications should be sent by February 7. (315)

BIOCHEMISTRY DEPARTMENT

ROYAL DENTAL HOSPITAL SCHOOL

LEICESTER SQUARE, LONDON, W.C.2

required to assist in full-time research. Experience in Auto-Analyser work, chromatography, fluorimetry or protein chemistry techniques an advantage. Appointment on Technician/Senior Technician scale according to age, experience and qualifications.

Apply to Head of Biochemistry Department. (342)

THE ROYAL HORTICULTURAL SOCIETY

A vacancy will shortly occur on the scientific staff of the Society at Wisley for which applications from graduates, men and women are invited. The duties of the post will be to assist the Plant Pathologist in advisory work and in carrying out scientific investigations on plant diseases and disorders. The successful applicant will be expected to take up the appointment as early as possible. The salary, reviewed annually, will be related to qualifications and experience. The post is pensionable.

Applications, with details of qualifications, experience and the names of two persons to whom reference may be made should reach the Secretary, The Royal Horticultural Society, Vincent Square, London, S.W.1, not later than Monday, March 16. (462)

UNIVERSITY OF QUEENSLAND

AUSTRALIA

POSTDOCTORAL RESEARCH FELLOW IN BIOCHEMISTRY

Applicants should have a Ph.D. with a First Class Honours degree in either Biochemistry or Chemistry. The successful applicant will work within the Enzymology group of the Department of Biochemistry, which is currently looking at the mechanism of action of a broad spectrum of hydrolytic enzymes.

A Postdoctoral Research Fellow is eligible for Superannuation similar to F.S.S.U., and salary will be at a rate of up to $A5,650 per annum.

Applications should include the names and addresses of three referees and state age, educational qualifications and experience and be forwarded to the Registrar, University of Queensland, St. Lucia, Brisbane, Queensland 4067, Australia by February 27, 1970. (413)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL

DENMARK HILL, S.E.5

Applications are invited for the following appointments in the Liver Research Unit, which includes the Medical Research Group on the Metabolism and Haemodynamics of Liver Disease:

(1) BIOCHEMIST (lecturer grade £1,645 to £2,105 per annum).

(2) BIOCHEMIST (Hospital biochemist scale £1,224 to £1,513 per annum).

Current research projects include studies of hepatic enzyme induction and bilirubin metabolism, factors controlling iron absorption and methods for treating acute liver failure, including transplantation. Further particulars may be obtained from Dr. Roger Williams.

Applications, stating age, qualifications and experience, with names of two referees, to the Secretary of the Medical School by February 7, 1970. (394)

UNIVERSITY OF ABERDEEN

LECTURER IN PHARMACOLOGY

Applications are invited for this newly created post. Experience in biochemical pharmacology or training in biochemistry required. Salary on scale: £1,240 to £2,850 with initial placing according to qualifications and experience. Superannuation (F.S.S.U.) and removal allowance.

Further particulars from the Secretary, the University, Aberdeen with whom applications (eight copies), should be lodged by February 28, 1970. (X403)

THE AGRICULTURAL RESEARCH COUNCIL has a vacancy for an Assistant Experimental Officer/Experimental Officer in the Unit of Invertebrate Chemistry and Physiology at the University of Sussex. The successful candidate will assist in the development of bioassays of insect hormones and the study of their mode of action in the adult insect. Work will involve the handling of insects, micro-assay and biochemical analyses, including the use of radio-isotopes. Detailed instruction will be given, but the candidate should be familiar with current biochemical techniques. Opportunities for independent research will arise. Qualifications. Candidates over 22 should have, or expect to obtain, a pass degree, H.N.C. or equivalent qualification. Candidates under 22 who have at least two G.C.E. "A" level passes in appropriate science subjects may also be considered. Salary. A.E.O., £683 per annum (at age 18); £940 per annum (at age 22); £1,208 per annum at age 26) rising to £1,454. E.O., £1,590 per annum to £2,006 per annum.—Applications should be sent to: Mr. S. E. Lewis, A.R.C. Unit of Invertebrate Chemistry and Physiology, Chemical Laboratory, University of Sussex, Falmer, BRIGHTON, BN1 9QJ, Sussex. (355)

UNIVERSITY OF ABERDEEN

EXPERIMENTAL PHYSICS

Applications are invited for post of RESEARCH FELLOW for work in BOTANY Department, supported by N.E.R.C. on transfer of energy and matter in forest canopies. Degree in Physics or Engineering or allied sciences with Ph.D. or other postgraduate experience; interest in aerodynamics or heat transfer and computing desirable.

Salary in range £1,240 to £2,850. Superannuation (F.S.S.U.). Appointment initially for two years.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (two copies), should be lodged by February 28, 1970. (457)

WELSH NATIONAL SCHOOL OF MEDICINE
(University of Wales)

RESEARCH SCIENTIST

Graduate, non-medical (e.g., physicist, chemist, physiologist), preferably with research experience, required to join a team studying the effects of automatic ventilation of the lungs in anaesthetized animals. Appointment up to three years and at a salary within the scale £1,240 to £2,045. Starting point according to qualifications and experience. Federated Superannuation System for Universities.

Further details from and applications to Department of Anaesthetics, Royal Infirmary, CARDIFF, CF2 1SZ. (452)

UNIVERSITY OF NATAL

DEPARTMENT OF CHEMISTRY, DURBAN

Applications are invited from suitably qualified and experienced persons for appointment to the post of LABORATORY TECHNICIAN (INSTRUMENTS). Applicants should hold a City and Guilds Science Laboratory Technicians' Advanced Certificate or equivalent trade certificate and should have an interest in modern chemical instrumentation particularly UV and IR spectrophotometers and gas chromatographs. The annual salary scale attached is R2,040 by R120 to R3,120 (£1,185 to £1,813). In addition a pensionable allowance of 10 per cent of salary is payable, this allowance will be increased to 15 per cent with effect from April 1, 1970.

Application forms are obtainable from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London, W.C.1, and application must be lodged not later than March 20, 1970. (446)

OXFORD UNIVERSITY

PHYSICAL CHEMISTRY LABORATORY

Applications are invited for a post-doctoral research assistant in chemical spectroscopy (mainly infra-red and photoelectron) in the salary range £1,200 to £1,600, starting October next (or earlier).

Applications, giving qualifications and the names of two referees, should be sent as soon as possible to Professor Sir Harold Thompson, Physical Chemistry Laboratory, South Parks Road, Oxford. (460)

LISTER INSTITUTE OF PREVENTIVE MEDICINE

(UNIVERSITY OF LONDON)

Required immediately—Post-doctoral Research Worker for studies on the metabolism, especially biosynthesis, of glycosphingolipids in mammalian tissues. Previous experience in lipid biochemistry desirable but not essential. The work is supported by the M.R.C. Salary up to £1,610, plus £60 London Allowance per annum and F.S.S.U. benefits.

Applications, with the names of two referees, should be sent to the Secretary, at Chelsea Bridge Road, London, S.W.1. (461)

POST-DOCTORAL RESEARCH FELLOW required in Department of Clinical Pharmacology to work on drug metabolism in man. Salary in range £1,480 to £1,690 per annum.—Applications to the School Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, London, W.12, quoting ref.: 2/458. (464)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF EARTH SCIENCES

M.Sc. courses in
GEOCHEMISTRY
GEOPHYSICS
ENGINEERING GEOLOGY AND GEOTECHNICS

Applications are invited for entry to the above postgraduate courses which lead to the degree of M.Sc. by examination. The courses are for 12 months, beginning on October 1, 197[illegible] and have been approved for the award of Advanced Course Studentships.

The entry requirements are a 1st or 2nd Class Honours degree in an appropriate subject: Geology or Earth Sciences, Chemistry, Physics, Mathematics, Civil Engineering or Mining, or a combined scheme of study which includes at least one of these subjects.

The course in Engineering Geology and Geotechnics is run jointly with the Department of Civil Engineering and the course in Geophysics is run in conjunction with the Department of Mining and Mineral Sciences.

Further details and application forms can be obtained from the **Secretary, Department of Earth Sciences, The University, Leeds LS2 9JT.** (465)

DALHOUSIE UNIVERSITY

DEPARTMENT OF GEOLOGY

HALIFAX, NOVA SCOTIA, CANADA

Applications are invited for an appointment in GEOPHYSICS and an appointment in GEOCHEMISTRY tenable in the Department of Geology. Successful candidates will be asked to teach at undergraduate and graduate levels, and develop graduate research programmes. If desirable, joint appointments in the Department of physics or the Institute of Oceanography may be arranged. Applicants with marine backgrounds may be interested in the oceanographic facilities in this area. The positions are tenable from July 1, 1970.

Write for further details, and send copies of curriculum vitae and names of three referees to: The Chairman, Department of Geology, Dalhousie University, Halifax, Nova Scotia, Canada. (405)

RESEARCH ASSOCIATE

B.Sc graduate wanted for research into aspects of coagulation and fibrinolysis, in Melbourne, Australia. Work could lead to Ph.D.

Apply to Dr. H. Ekert, Institute of Child Health, 30 Guildford Street, London, W.C.1. Tel. 242 9789. (381)

RADIOBIOLOGIST

A graduate research worker with interest or experience in mammalian radiobiology and its application to radiotherapy is required for a post in the Churchill Hospital Research Institute. The successful applicant will join an interdisciplinary group studying various aspects of tissue and tumour growth and the effects of ionizing radiation and drugs upon it. Salary according to age, qualifications and experience.

Replies to Dr. R. J. Berry, M.R.C. External Scientific Staff, Radiobiology Laboratory, Churchill Hospital, Oxford. (441)

TECHNICIAN/SENIOR TECHNICIAN I required in Department of Medicine. Some experience in basic immunological techniques, protein electrophoresis and column chromatography desirable. Salary according to qualifications and experience.—Applications in writing to the Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, London, W.12, quoting ref.: 2/466. (463)

SENIOR TECHNICIAN required to operate electron microscope in research department. Experience in maintenance and preparatory techniques essential.—Apply, with full details of age, qualifications and experience, to Dr. H. Stern, Virus Department, St. George's Hospital Medical School, London, S.W.1. (421)

NATURE

MACMILLAN (JOURNALS) LTD

No. 5232 SATURDAY FEBRUARY 7 1970 Vol. 225

CONTENTS

MORE FLEXIBILITY ON DRUGS—AIRPORT OUTCRY—SCIENCE POLICY NOW RESPECTABLE—100 Years Ago—University Entrance—Graduate Employment—Television Broadcasting—Parkinsonism—Enriched Uranium—CERN Accelerator—Fabian Society—London University—Wolfson Foundation—Infrared Astronomy—Instruments—Architecture—Conservation—Engineering—Parliament in Britain—Miscellaneous Intelligence 485

SELECT COMMITTEE GETS UNDER WAY 496

News and Views—150 YEARS OF ASTRONOMY—RNA Structure—Biochemistry—Nucleic Acids—Ichthyology—Enzymes—Stomata—Geomagnetic Field—Unusual Stars—Electron Beams 497

EXTRA-GALACTIC DISTANCE SCALE by Sidney van den Bergh 503

CAREER CHOICES BY SCIENCE STUDIES STUDENTS by D. O. Edge 506

COMPLEMENTARY OLIGONUCLEOTIDE BINDING TO THE ANTICODON LOOP OF fMET-TRANSFER RNA by Olke C. Uhlenbeck, Julie Baller and Paul Doty . . 508

DERIVATION OF THE SECONDARY STRUCTURE OF 5S RNA FROM ITS BINDING OF COMPLEMENTARY OLIGONUCLEOTIDES by James B. Lewis and Paul Doty . . 510

NEW APPROACH TO CANCER by P. R. J. Burch 512

Gigantopithecus AND THE ORIGINS OF HOMINIDAE by David Pilbeam . . . 516

TEICHOIC ACIDS AND MEMBRANE FUNCTION IN BACTERIA by S. Heptinstall, A. R. Archibald and J. Baddiley 519

PLATE TECTONICS, OROGENY AND CONTINENTAL GROWTH by J. F. Dewey and Brenda Horsfield 521

Letters to the Editor (see overleaf) 526

Book Reviews by G. M. Carstairs, D. H. Roscoe, M. M. Pickles, A. R. Perry, P. F. Wareing, J. P. Riley, W. J. G. Beynon, G. L. Sewell, J. G. Oldroyd and W. G. Richards 565

Obituaries—Professor E. H. F. Baldwin 569

Correspondence—Goldfinger no Longer by R. S. Glover 570

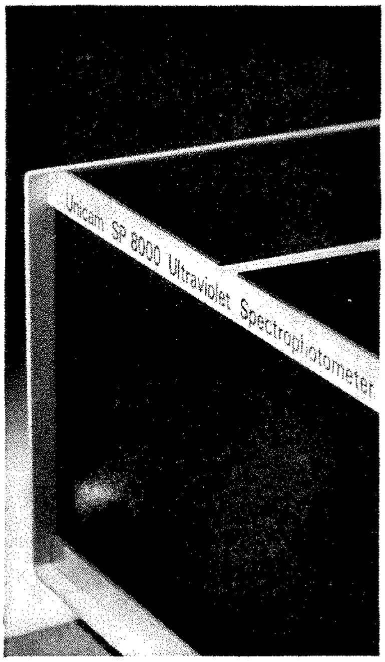
University News—Appointments—Announcements—International Meetings—Sabbatical Itinerants 570

Nature in North America: The *Nature* office in Washington DC opened last week. Contributors in the United States and Canada are invited to submit manuscripts for publication either to the address given below or to the present London office. The intention is that each office will be able to answer inquiries about all manuscripts and other matters, although a strictly symmetrical situation will not obtain until mid-February, from which point, for half a year, the editor of *Nature* will be based in Washington. This is one of several steps by which it is hoped to provide a better service for readers and contributors.

London Office
4 Little Essex Street,
London WC2.
01-826-6633

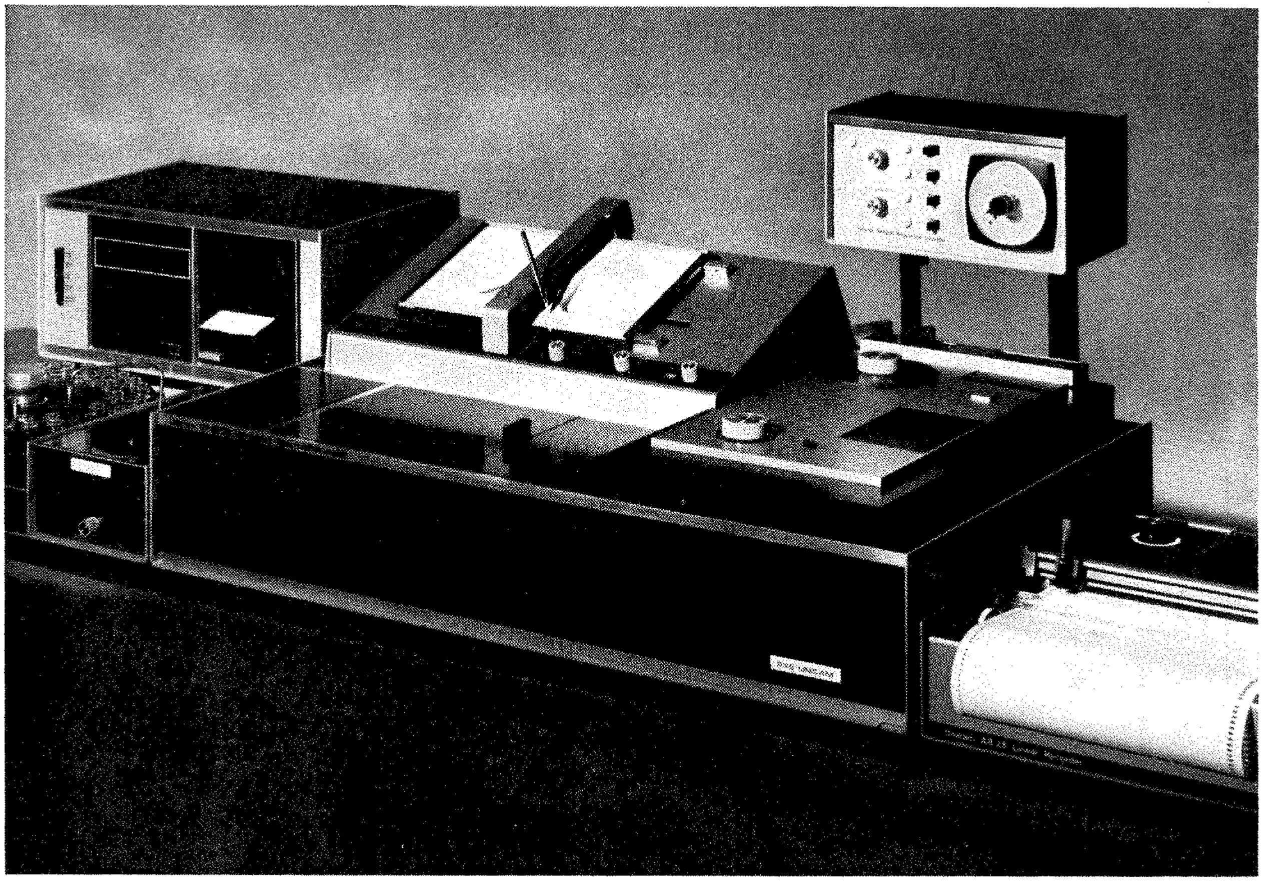
Washington Office
711 National Press
Building,
Washington DC 20004

(Continued overleaf)



This new Unicam UV Spectrophotometer has been made to meet your requirements

To meet, in fact, the requirements of every UV spectroscopist—whether engaged in research or in routine applications. And that means that the new Unicam SP8000 Ultraviolet and Visible Recording Spectrophotometer is just about the most versatile instrument available. To start with you get superb presentation and reproducibility of spectra on the integral flat-bed recorder. You get dual-absorbance scales of 0-1.0A and 0-0.2A with back-off. You get exceptional scale expansion. There is a large sample compartment and a unique second sample position. There is a whole range of modular accessories for automation. If you want digital print out, results in concentration units, automatic wavelength selection, programme control, automatic sample changing of up to 50 samples—you name it and the likelihood is that the Unicam SP8000 system can do it. The Unicam SP8000 is the latest addition to an outstanding range of UV instruments which includes the SP500 Series 2, the SP700, the SP800 and the award-winning SP3000. To learn more about any of these write to us today.



PYE UNICAM

1966 1969
THE QUEEN'S AWARD TO INDUSTRY

Precision Instruments

Pye Unicam Ltd
York Street Cambridge,
CB1 2PX, England
Telephone (0223) 58866 Telex 81215

LETTERS TO THE EDITOR

Page

PHYSICAL SCIENCES

NP 0532—Polarization measurements from Jodrell Bank—GRAHAM, LYNE and SMITH (Jodrell Bank) 526

—Polarization measurements from Arecibo—CAMPBELL, HEILES (Arecibo Observatory) and RANKIN (Iowa) 527

Interstellar medium—Size and motion of interstellar scintillation pattern—LANG (Arecibo Observatory) and RICKETT (Jodrell Bank) 528

CP 0329—Upper limit on size of emitting region—GALT (Dominion Radio Astrophysical Observatory), BROTEN, LEGG, LOCKE (National Research Council, Ottawa) and YEN (Toronto) 530

Interstellar extinction—First observations on Magellanic clouds—BRÜCK, LAWRENCE, NANDY (Royal Observatory, Edinburgh), THACKERAY and WOOD (Radcliffe Observatory, Pretoria) 531

Extensive air showers—Observation of structure in Čerenkov pulses—BOSIA, CASTAGNOLI, DARDO and MARANGONI (CNR, Turin) 532

Venus—How the solar wind affects the ionosphere—BAUER, HARTLE and HERMAN (NASA) . . 533

Electrodynamics—Remark on the Hoyle–Narlikar treatment of spontaneous emission—KATZ, ORNAN and SCHWARTZ (Weizmann Institute) . . . 534

Meteorology—Microbarograph observation of gravity waves—CLARKE (CSIRO) 535

Oscillating reactions—Concentration wave propagation—ZAIKIN and ZHABOTINSKY (USSR Academy of Sciences) 535

Colloid chemistry—Relation of colloid chemistry to solid state chemistry—HONIG (Philips, Eindhoven) 537

Polymers—Macro-lattice from segregated amorphous phases of a three block copolymer—KELLER, PEDEMONTE and WILLMOUTH (Bristol) . . . 538

Whiskers—Field emission from silicon carbide whiskers—BAKER (Explosives Research and Development Establishment) 539

Materials—Ribbed growth of graphite in Ni-C solutions containing boron—MINKOFF and LUX (Institut Battelle, Geneva) 540

NMR—Pitfalls in interpreting magnetic relaxation in ordered systems—HANSEN and LAWSON (Procter and Gamble) 542

Lasers—Tunable dye lasers in the ultraviolet—MYER, ITZKAN and KIERSTEAD (Avco Everett Research Laboratory) 544

BIOLOGICAL SCIENCES

Nucleosides—Conformational parameters in different molecular environments—WILSON (Dundee) . . 545

Page

Radiation chemistry—Anions from γ-irradiated cystamine and thymidine identified—LENHERR (Royal Military College of Science) and ORMEROD (Chester Beatty Research Institute) 546

Bradykinin—Correlation of structure with activity—MELROSE, MULLER and VAGG (New South Wales) 547

Proinsulin—Insulin biosynthesis in anglerfish islets—TRAKATELLIS and SCHWARTZ (The City University, New York) 548

L-Asparaginase—Carcinostatic action of preparation from *Aspergillus terreus*—CARTA DE-ANGELI, POCCHIARI, RUSSI, TONOLO, ZURITA (Rome), CIARANFI and PERIN (Milan) 549

—Effects on antibody producing cells—BERENBAUM (London) 550

Immunoglobulins—Identification at surface of mouse lymphoid cells—RAFF, STERNBERG and TAYLOR (MRC) 553

Nitrosamines—Gas chromatographic detection of N-alkyl derivatives—FOREMAN, PALFRAMAN and WALKER (Laboratory of the Government Chemist) . 554

Measles viruses—Adaptation of strains from human brains to growth in hamsters—BYINGTON, CASTRO and BURNSTEIN (Purdue) 554

Polymorphism—Mice are like men when tasting phenylthiocarbamate — KLEIN and DeFRIES (Colorado) 555

Pheromones—Has the pheromone of *Dendroctonus frontalis* really been identified ?—WOOD (California) and SILVERSTEIN (Stanford Research Institute) . 557

Brain "actomyosin"—Colchicine binding protein behaves like actin—PUSZKIN and BERL (Columbia) 558

Somatosensory function—Population analysis of thalamic neurone response to stimuli—HARRIS (Washington, Seattle) 559

Central nervous system—Attempt to determine the sites of GABA metabolism in the rat—CSILLIK and KNYIHÁR (Szeged) 562

Natural selection—Protein space model for protein evolution—MAYNARD SMITH (Sussex) . . . 563

The titles on this page are prepared by the staff of *Nature* on the basis of the titles submitted by authors which appear in full on the pages indicated

The new Monroe 820 Calculator

The Monroe 820 introduces a brand new generation in electronic calculators. Smaller, lighter and much more compact, thanks to integrated microcircuits. Simple to operate, priced to fit your budget. Using only four keys more than an adding machine to handle every business calculation in milliseconds.

You can do arithmetic operations in any sequence and there are a host of advanced features including automatic constant multiplier and divisor, automatic decimal point and roundoff, and automatic squaring.

Fourteen digit answers appear sharp and clear in large numerals on the non-glare display screen – plus the running total.

The 820 is ready for work the instant it's switched on and there's no need to clear the calculator.

It all happens in complete silence.

MONROE the new generation

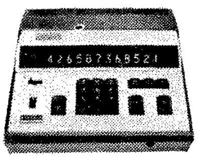
To: Monroe Calculators, Litton Business Systems Ltd.,
Litton House, Goswell Road, London E.C.1
Telephone 01-253 3090

PLEASE SEND me full information on the Monroe 820 Electronic Calculator.

Name

Address

N



NATURE Volume 225 FEBRUARY 7, 1970

More Flexibility on Drugs

THERE are signs that the Home Office is planning a more liberal revision of the British law on drugs than seemed probable a year ago, when the Home Secretary reacted like a Bourbon to the publication of the report of the committee under Baroness Wootton which recommended, among other things, lighter penalties for offences involving soft drugs such as cannabis. Then, Mr Callaghan went a long way to suggest that the committee's advice was not to be taken seriously. Now, it looks as if the Home Office will publish in the next few weeks proposals for new legislation on drugs which will direct the fullest rigours of the law at those who market illicit drugs, not at those who use them. By all accounts, there will be three categories of drugs in the new bill—hard, soft and softer—and penalties whose severity will be matched to the character of the drug concerned. The possession and presumed use of cannabis will apparently be dealt with more lightly than at present, when it is possible for people to be sent to prison for as much as ten years and fined as much as £1,000. (Cannabis offences under the Dangerous Drugs Act, 1965, led to close on 300 prison sentences a year in the mid-sixties, roughly half of them for periods of between three and six months.) Still less harmful drugs, pep pills, for example, will be dealt with more leniently still. So far, it is not clear how the Home Office will set about making life even harder for those who sell these drugs, but there is some awesome sabre-rattling in the wings. Everything should be clearer by the late spring, though it is only reasonable to wonder whether the Home Office can be right in hoping that its new legislation will be on the statute book before the end of the current parliamentary session in the autumn.

The coincidence of the talk about new British legislation and the somewhat fitful passage of Senator Thomas Dodd's bill through the United States Senate—it remains to be seen what the House of Representatives will make of it—is not conspicuously a marvel. Only a week ago, the United Nations Narcotics Commission in Geneva was putting the finishing touches to its new draft code intended to provide governments with guidance in the design of legislation. The essence of the commission's code is a catalogue of drugs of dependence—"psychotrophic drugs" as they are now called—which it may be sensible to deal with differently under the terms of national legislation. The next step will be consideration of the code by the Economic and Social Council of the United Nations, but long before that process is complete, it may well be that the United States and the United Kingdom will both be provided with legislation that deals differently, and more leniently, with users of cannabis than with users of heroin. That is at least something to be grateful for. The past few years have often made a mockery of the pharmacological characteristics of these and other substances, have dealt harshly with people deserving lighter penalties and have provided too many opportunities for arbitrary action by those who administer the law. The penchant of the British police for smelling out—in one case, at least, almost literally—pop musicians using cannabis, and the tendency for magistrates to regard the sentencing procedure which follows as an opportunity for delivering ill-conceived homilies on the iniquities of a younger generation, has only served further to undermine respect for the law on cannabis. This is probably the strongest argument for dealing differently with cannabis and heroin, as the Home Office now proposes.

Unfortunately, however, this cannot be an end of the process of bringing the law up to date. The revision now proposed may hold the line—as some will describe it—for a few years, but eventually there will have to be a much more radical reconsideration of the proper place of cannabis in modern society. The trouble, of course, is that the illicit cannabis habit has provided modern society with several important challenges so far largely ignored. Unlike many other drugs, especially the hard narcotics, cannabis seems to be comparatively benign. To be sure, there is still a good deal of dispute about the causal relationship between cannabis and hard drugs such as heroin—does the first lead to the second ? It is not, of course, surprising that most heroin addicts have previously smoked pot—poker players usually begin with snap. The possibility that cannabis causes a kind of pharmacological disposition towards heroin has been suggested, but the way in which the majority of cannabis users do not move on to dangerous narcotics suggests that it would be hard to distinguish between pharmacological and social acclimatization. In the past, it may well have been that the application of the same penalties to hard drugs such as heroin and softer drugs such as cannabis tended to lump them all together. Differential penalties should make the distinction more evident to the people most at risk, and may even thus help to dissuade potential addicts from the hard narcotics.

That is an immediate consideration. There remains the problem of how a society which is already well used to several forms of crippling self-indulgence shall justify its continuing refusal to put cannabis, by many tests a comparatively mild form of abuse compared with alcohol and tobacco, on general or even licensed sale. A year ago, Mr Quintin Hogg made the quite sensible if somewhat empirical point that it would be madness to admit yet another social scourge into common currency on the grounds that alcohol and tobacco are already familiar. This is good homespun, but unfortunately it cannot be expected to stand the test of time. The lighter penalties for cannabis usage now in prospect may for a short time ease the sense of conflict between those who use pot and the rest of society, but it will only be a few years before the reputation of cannabis is made to seem as anomalous as it is now. For there is very little likelihood that the new legislation will bring the peddling of pot entirely to a stop—it will merely increase the cost of breaking the law. In the circumstances, it is essential that the problems of cannabis should be regarded in a much more penetrating way than that which has now suggested new legislation.

What should be done? To begin with, there is a great need for more objective understanding of the effects of using cannabis, not merely in the pharmacological sense but in behavioural terms as well. Great though the interest in cannabis has been in recent years, only the surface has been scratched so far. Second, there is a great need of more public discussion of the problems of drugs of dependence in general and of cannabis in particular. One of the hallmarks of modern society is that it has found ways of bringing into intelligent discussion matters which were taboo only a few years ago. The time has now come when drug dependence, like attempted suicide, should be counted as a psychological problem and not a piece of criminality. To say this is not of course to pretend that drug dependence is a harmless condition, but simply to imply the hope that it is remediable. This is why the third urgent need is for more consistent and thorough means of treatment for those who are dependent on drugs like heroin—the scheme for setting up (in Britain) centres for the treatment of those who used to be known as addicts has been less successful than it might have been.

The most difficult problem remaining to be tackled, however, is that of knowing what should be done about the conventional opiates—alcohol and tobacco—not to mention those drugs such as barbiturates which are already so widely prescribed by doctors that many users must already be dependent on them. The first goal should be a much more consistent view of how damaging these materials may be. Is tobacco, for example, to be valued except for its side-effects? What of alcohol? Few would deny, by precept as well as words, that social drinking has some valuable, desirable or at least desired attributes. The snag is that many of the side-effects are unwelcome. So is it sensible to put alcohol on sale in the shops but to remain hard-faced about the supply of cannabis, the chief effects of which are said to have much in common with those of alcohol but which may have less damaging side-effects? This, of course, is a rhetorical question to which Mr Hogg's dictum is only partly a rhetorical answer. Among many of those who use cannabis, it is perhaps more relevant that their dispute with convention and the law seems to be a dispute between generations (which also implies, democracy being what it is, either that they have time on their side or that ageing pot-smokers can be relied on to give up the habit). But there are deep issues of principle involved in any attempt to prevent people from doing something which may not be harmful to themselves, which may not cause harm to other people and which may even be pleasurable. It will be a long time before the pot lobby has proved along these lines a case for licit cannabis, but long before then it is likely that the opiates of the middle-aged will have to be sold on licence, so many are the contradictions which have now accumulated.

Airport Outcry

ALONG with Rynemede and Crichel Down, the English name of Stansted will no doubt become one of the symbols of how sense can triumph over bumbledom. It is also well on the way to being a proof of how badly governments are advised on technical matters. The latest publication in the series put out by the Commission on the Third London Airport (*Papers and Proceedings*, Volume VII, HMSO, £4.20) is a final proof of that. Three years ago, the civil servants responsible for picking somewhere to land passenger aircraft settled on the sleepy village of Stansted in Essex because there was already a runway in operation and on a simple comparison of the capital costs of building there and elsewhere. The most immediate outcry came from those who said, quite rightly, that no attempt had been made to quantify the cost of keeping sleeping villages awake at night. The more serious indictment of the original decision was the falsity of the belief that in the planning of an important public project such as an airport, the initial capital cost is all that matters. In an ideal world, the objective should be to find a site that will maximize the benefit accruing to those who use airports and to those who never go near them. The chances are that the hidden costs are much more important than those of pouring a strip of concrete over virgin farmland.

This is what the Roskill Commission has discovered. The cost–benefit analysis now published as part of the latest instalment in the research programme shows that the cost of the airport itself—car parks and cafeterias included—works out at about £180 million for each of the four alternative sites considered in the study. (The sums of money are quite legitimately discounted to 1975.) This expenditure is entirely dwarfed by items such as "passenger user costs"—the notional but nevertheless real cost of moving people to and from the terminal buildings. For the three inland sites considered as part of the study, all of which lie north of London, surface travel of this kind is reckoned to work out at between £870 and £890 million, but the surface travel cost of the Foulness site in the Thames estuary is estimated at the equivalent of a capital sum of £1,040 million (in 1975), an understandable reflexion of the comparative remoteness of that place. In the same way, the cost of "airspace movement" is in all cases much larger than the cost of building the airport itself—the estimates range from £960 million to £990 million. One of the merits of this study is that some attempt has been made to cost the nuisance caused by noise and the disruption to various other kinds of services—agriculture and recreation at each of the four sites, for example—and nobody will be surprised that these factors go some way to compensate for the extra cost of getting people to and from the site in the Thames estuary. The result is that in terms of equivalent capital expenditure in 1975, the extra cost of the Thames estuary site at Foulness works out at £2,385 million, or something like five per cent more than the cheapest of the inland sites.

What then is the scandal? Did not the British Government always say that it would be too expensive to build the third London airport in the Thames estuary? The truth is, of course, that the basis of the analysis now being used is quite different from that on which the original decision was promulgated. Moreover, it is now apparent that the new basis is more valid than the old candle-counting procedure which the government's advisers first sought to use. Then, for example, in May 1967, the argument turned on the £15 million that would be spent on strengthening the terrain beneath the Thames estuary site then in fashion (on the south bank of the river, not the north). The result was an attempt to distinguish between the sites then available by taking account of items adding up to between five and ten per cent of the total cost of building and operating a modern airport. Three years ago, there seems to have been an almost total failure to appreciate that the third London airport would not be a simple concrete structure out in the fields or marshes but, rather, part of a closely integrated transport system which would have to be valued not in terms of how much it would cost to build but by the contribution it would make to the efficacy of air transport. And the neglect of discounted cash calculations in the original comparison is a kind of arithmetical boob for which students are now being—or should be—drummed out of first-year classes in economics. The minister who did most to defend the decision, Mr Douglas Jay, has already left the government on another issue, but nobody would pretend that he was personally responsible. The fault lies in the Civil Service, and it would help to restore public confidence if there were now some ostentatious sign that matters are being put right.

There remains the question of where the third London airport should be built. One of the most conspicuous features of the splendid study which the Roskill Commission is embarked on is that the variation from one site to another is a small percentage of the total cost. Given that the new airport must be close on fifty miles from Central London, it is inevitable that the mere cost of building the fixed installations will be a comparatively small part of the total cost. Moreover, it is clear that there are not merely considerable errors in the calculations which have been attempted but serious doubts about the basis on which some of the items in the calculation have been evaluated. Many people, for example, will say that the commission has underestimated the cost of the disturbance likely to be caused by aircraft noise. It is also possible that the comparatively high cost of surface movement to and from the Foulness site will serve as a spur for the design of better surface transport, thus helping to cancel out the present apparent disadvantage of building there. In short, the chief conclusion from the calculations so far made public is that there is very little to choose between one site and another. No doubt the Roskill Commission, in due course, will put its own gloss on the figures, and on the showing so far the result will probably be convincing. But it is only prudent at this early stage to acknowledge that the numerical comparison of the several sites available is likely, if taken seriously, to underline the importance of those considerations which cannot be quantified and which have not been fully counted in the calculations. In short, the airport will be best placed where it will be the least nuisance. Foulness, it would seem, is the best place.

Science Policy now Respectable

It has been announced that Mr Christopher Freeman, director of the Science Policy Research Unit at the University of Sussex, has been elected to the first university chair ever created specifically for science policy. The new chair has come as part of the requests made last November by Reginald M. Phillips of Brighton for science policy research at Sussex. The precedent will be of interest to all who have seen in the social studies of science an emerging field of activity, capable of pooling the talents and resources of many traditional disciplines. Coming at the end of a decade that has seen increased systematic academic and governmental interest in the relations between science, technology, economics and cultural development, this event augurs well for the field's future in this country.

As director of the SPRU since its creation in 1966, Christopher Freeman deserves much of the credit in science policy at Sussex. Previously he had pioneered the use of international statistical comparisons of research and development that have since become everyday instruments in governmental and intergovernmental decisions. Born in 1921, Freeman graduated with first class honours in economics from the London School of Economics in 1948. In 1959 he joined the staff of the National Institute of Economic and Social Research and shortly thereafter became involved in work in the OECD on international comparisons of research and development. This included preparation of a manual of proposed standard practices for surveys on R&D for the Frascati Conference on this subject in 1963. At the NIESR Freeman led research into the electronics, plastics and chemical process plant industries, and became a frequent consultant in Europe on questions concerning the economics of science. In 1965 he was invited by the University of Sussex to create the first British university centre in the science policy field.

Under Freeman's leadership and largely in response to requests from government and industry, the original group has grown in four years from two to over thirty research fellows, research students and staff. Its activities range from social and economic history of science to the relationship between science, industry and economic development, both in advanced and developing countries. Within the last two years fresh work has begun on the study of science in China and the Far East, on the economics of innovation and on safety in industry. Teaching duties of the unit's staff bring it into close contact with both science and arts schools of the university, while its research has made it a focus of international interest. Although the Phillips chair does not attempt to answer the unit's need for long term continuing support, it is a welcome witness to the increasing significances of science policy studies, which Christopher Freeman has helped to pioneer.

100 Years Ago

We referred last week to the "situation" at the Paris Observatory. The action of the French Government has been of the promptest and M. Le Verrier is no longer Director. This step indicates very clearly—too clearly we fear—the strength of the case put before the Minister of Public Instruction, in the memorial, of which a copy has been sent to us. This document, which is signed by all the *chefs de service*—Villarceau, Marié-Davy, Wolf, and Loewy—and the *astronomes adjoints* without exception, discusses all points connected with the administration of the Observatory, scientific and otherwise. It is to be sincerely hoped that M. Le Verrier may be able yet to do service to astronomy, in some other capacity, some position where his great talents alone will be called into play. His is a name that will never die, let us hope it is but momentarily eclipsed.

From Nature, **1**, 387, *February* 10, 1870. *The "situation" referred to was that the entire staff of the observatory had threatened to resign if Le Verrier were not dismissed, because of his uncompromising policies. He was reinstated at the observatory in* 1872, *but with reduced powers.* Nature's *comment on February* 3 *was that "the present régime at the Observatory of Paris has been rather more autocratic than could be patiently endured, even in a country subjected to eighteen years of personal government". Le Verrier predicted the existence of Neptune in* 1846, *without knowing that Adams had reached the same conclusion in England.*

UNIVERSITY ENTRANCE

Continuing the Swing

by our Education Correspondent

More than 1,300 places in the science, engineering and technology faculties of British universities were unfilled last October. In May 1969 the universities estimated that 10,447 engineering and technology places and 16,828 science places would be available this year. But in the event, only 9,984 and 15,978 students were admitted to these faculties, representing a shortfall of 463 and 850 respectively. This reduction in numbers, given in the report of the Universities Central Council on Admissions for 1968–69, seems to have been caused by a lack of suitably qualified applicants. In the sciences, the number of applicants was only 1·5 times greater than the number of places available, while the ratio for the arts was 1·8 : 1 and for the social sciences it was 2·5 : 1.

Because of the lack of competition for places, science, engineering and technology faculties seem to be willing to accept students with significantly lower qualifications than those accepted by arts or social science faculties. During the clearing procedure in September, when unfilled places are filled by suitably qualified candidates who were unsuccessful in the first round of the lottery, 38·5 per cent of the engineering and technology candidates and 37·4 per cent of the science candidates were accepted, compared with 14·9 per cent of the social science and 21·0 per cent of the arts candidates. Moreover, if A-level grades are awarded points—five for an A grade down to one for an E—282 candidates out of 922 with scores of five or less were awarded places in mechanical, electrical and civil engineering in the clearing procedure, compared with thirteen out of 343 in English.

The total number of admissions, at 61,139, was 3·1 per cent up on the total for October 1968, however, but the report says that "if suitably qualified candidates had applied in sufficient numbers, the increase in admissions could have approximated to 4·5 per cent". The number of applicants for university places was 114,289, which suggests that there are almost twice as many candidates as there are places available. But Dr Geoffrey Templeman, chairman of UCCA, suggests that this is misleading. He says in the introduction to the report that about one in five applicants do not achieve the minimum university entrance qualification, and that about 15 per cent of those who apply in any given year can be expected to have applied for admission in a previous year. He suggests, therefore, that about three out of every four qualified candidates were awarded places last October.

GRADUATE EMPLOYMENT

Industry's Share Growing

Graduates who go straight into employment in Britain now outnumber those who continue to study. The University Grants Committee's latest volume of statistics (*First Employment of University Graduates, 1967–68*, HMSO, 10*s*) shows that out of a total for the year of 42,615 graduates with first degrees, 17,508 took up a job in Britain and 17,047 carried on with their education. In 1966–67 the figures were 14,775 and 15,190 respectively. Industry seems at last to have reversed its declining attractiveness to graduates,

Table 1. DISTRIBUTION OF FIRST DEGREE GRADUATES BETWEEN EMPLOYMENT AND FURTHER EDUCATION

Percentages in:	1965–66	1966–67	1967–68
Further education	41·7	41·6	40·0
UK industry	25·1	25·1	25·7
Other UK employment	14·9	15·3	15·4
Others (including emigrants)	18·3	18·0	18·9

Table 2. EMIGRATION OF GRADUATES

First degrees	1965–66 (per cent)	1966–67 (per cent)	1967–68 (per cent)
Total emigrants	6·1	5·2	4·9
Pure scientists	4·3	3·7	3·8
Applied scientists	6·1	5·7	5·1
Permanent emigrants	1·6	1·4	1·2
Pure scientists	1·3	1·0	0·9
Applied scientists	3·4	3·1	2·6

Higher degrees	1965–66 (per cent)	1966–67 (per cent)	1967–68 (per cent)
Total emigrants	14·2	13·8	13·3
Pure scientists	19·5	19·1	18·0
Applied scientists	10·9	11·8	9·8
Permanent emigrants	6·7	6·9	6·2
Pure scientists	6·7	7·0	6·5
Applied scientists	8·6	9·5	6·8

"Total emigrants" and "permanent emigrants" are shown as percentage of the total number of graduates (42,615 for first degrees; 9,135 for higher degrees). "Pure scientists" as percentage of pure science graduates (12,993 for first degrees; 3,931 for higher degrees). "Applied scientists" as percentage of applied science graduates (8,198 for first degrees; 2,280 for higher degrees).

Permanent emigrants are "those who may reasonably be assumed to be lost to the British market at least for some years".

for its share rose from 25·1 to 25·7 per cent of the year's output (see Table 1). More encouraging still is a continuing fall in the proportion of emigrants—the figure of 4·9 per cent compares with 5·2 per cent for 1966–67 and 6·1 per cent for 1965–66.

The total number of first degree graduates includes 30,151 men and 12,464 women, and compares with the previous year's total of 36,528. Apart from the reversal of the trend away from industry, however, there has been little change in graduate career patterns. Teaching, for example, is still losing its popularity—the proportion that entered directly fell from 13·9 to 13·3 per cent of those taking up employment. The proportion that went into teacher training was unchanged.

A further 9,135 people graduated with higher degrees, comprising 8,150 men and 985 women and topping the numbers for 1966–67 by 1,758. Here, too, more have been looking to industry for a career—12·7 per cent in comparison with 11·8 per cent for 1966–67. The fall in the proportion of PhDs among the higher degrees awarded—from 61·4 per cent in 1963–64 to 47·4 per cent in 1966–67—has, however, been checked. In spite of the Swann report's recommendation that shorter periods of postgraduate study should be encouraged, the 1967–68 figure for PhDs was reduced only to 45·7 per cent.

With a little further analysis, the UGC's statistics reveal that between 1965 and 1968 the brain drain among newly graduated people was, in relative terms, significantly reduced, even though the numbers of emigrants continue to increase. Table 2 shows that 1·2 per cent of first degree graduates and 6·2 per cent of higher degree graduates went abroad "permanently" (the UGC's term for an indefinite length of time) in 1967–68. Two years earlier, the respective figures were 1·6 and 6·7 per cent. The fall for the total of emigrants is more marked. Although the extra graduates that the totals include intended to return to Britain after a period of overseas work or study, the experience of their predecessors suggests that they may not be able to find jobs when they want to come back (see *Nature*, **225**, 213). The net loss is thus likely to be somewhere between the two extremes that are given in Table 2.

Two features here call for further comment. First, perhaps surprisingly, relatively few pure scientists with first degrees go abroad—a total of 3·8 per cent in 1967–68, compared with 4·9 per cent as the proportion of all graduates. Against this, however, should be set the fact that 25·5 per cent of pure scientists continued academic study or went into research, compared with 18·6 per cent of all graduates. Second, applied scientists are more likely to go abroad permanently than pure scientists. While 18 per cent of pure scientists with higher degrees emigrated, only 6·5 per cent intended to stay abroad; among applied scientists, 9·8 per cent emigrated but the departure was permanent for 6·8 per cent.

Mr Enoch Powell has given widespread publicity to a survey by the Cambridge University Management Group, which reported that 27 per cent of an undergraduate sample were considering permanent emigration. Even allowing for subsequent emigration by some of those who start work in Britain, the UGC's figures suggest that many of the interviewees will have had second thoughts by the time they graduate.

TELEVISION BROADCASTING

Cable Talk

It is one thing to show, as Rediffusion's laboratories have in Britain, how cables can bring many more television channels into the home than can aerials on the roof. It is something else again to work out how cable television is to co-exist with conventional television broadcasting. In the United States, the broadcasting industry regards cable television as the dog in the manger. It steals programmes out of the air without paying for them, and transmits them to subscribers for a fee. And it threatens to grow into a national network, local cable companies joining up and perhaps receiving their signals from satellites, bypassing the conventional broadcasting networks altogether.

A report released last week by the Rand Corporation suggests how cable television might be encouraged to grow without running wild. Cable operators should be allowed to carry whatever programmes from whatever distance they choose (the Federal Communications Commission now places severe restrictions on their freedom to import signals), but they should pay for what they take. They should be allowed to originate programmes themselves (the first real hope that something like local television will ever come to pass) and they should be allowed to sell advertising—at least to pay for the programmes that they prepare themselves—and they should be allowed to interconnect into regional and national cable networks.

There is a threat in all this, in spite of the diversity implicit in the provision of 40 or 100 television channels to every television set, instead of two or three. The entire flow of information into the home would then be in the hands of a single operator. The best way round this, the Rand study suggests, is for certain channels to be given the status of "common carriers"—television service to be for sale to the public, just as telephone service is. The American Civil Liberties Union, incidentally, has also put its weight behind this idea.

All very well for the cable companies (who are having no trouble finding people willing to invest their money in what they see as tomorrow's goldmines), but what about protection of the broadcasters ? What they do offer is television, or radio, programmes free to the public. Cable companies charge a fee (about $5 a month in the United States) for wiring a home for television; people are willing to pay this because of the better reception or the increased choice of programme involved, but obviously there are many who, having scraped together the money for the set, could not afford the further cost. The Rand report concludes, perhaps too optimistically, that as long as cable television operators pay broadcasting stations for the signals that they take from the air, the broadcasters should find that what they lose in advertising revenue they would gain, roughly speaking, in royalties. The Rand theory is that just as radio broadcast has continued to grow in the face of competition from television, broadcast television should grow in spite of, or perhaps even because of, cable television. If legal restrictions were necessary, these might take the form of giving the broadcaster an exclusive period of time during which the cable operators might not show his programme.

Cable television has grown enormously in the United States, where it accounts for about six per cent of all the television reception. Its most spectacular growth, however, has been in Canada, where it is popular because of its ability to bring the signals of American broadcasting stations. Montreal has the largest concentration of cable television subscribers (100,000) in the world. In Britain, Rediffusion Ltd has just unveiled a system which could distribute twenty television programmes to entire towns or to a large housing estate and, moreover, is capable of being two-way; programmes could be injected from any part of the network.

PARKINSONISM

Scowen Committee Defied

The British company that intends to sell the scarce drug L-dopa, used for treating Parkinson's disease without submitting it to the Scowen Committee on the Safety of Drugs, has receive a sharp reproof from the Department of Health and Social Security. Mr Malcolm Sinclair, director of Chemica Laboratories Ltd, said last week that although he had offered the committee samples of his product, he was not prepared to divulge his manufacturing secrets. Because he felt unable to withhold his supplies from the home market any longer, he would by-pass the committee. The Department of Health, on behalf of the committee, quickly retorted that Chemica had ignored advice given to its representative last October, and had provided none of the required information about its preparation of L-dopa. Manufacturing procedures, the department points out, are treated in strict confidence, and no problem has arisen in dealing with nearly 600 other organizations.

The drug at the centre of this argument was first investigated as a possible cure for Parkinsonism after the discovery that drugs which cause a decrease in amounts of monoamines, particularly dopamine, in the brain can cause the disease (*Nature*, **223**, 889; 1969). Trials so far have shown that the L-form of this amine can relieve the symptoms—including rigid limbs and trembling hands and legs—in some but not all sufferers. It may also have side effects such as nausea and lowering of the blood pressure. In trials in the United States some patients suffered such ill effects that treatment had to stop. Only 5 per cent of patients experienced a real cure, although another 55 per cent were partly improved.

In Britain the very short supplies of L-dopa have been restricted to MRC clinical trials on the decision of the Department of Health. Until last week the only preparation passed by the Scowen committee for use in the trials was made by Roche in Switzerland. But now two more firms, whose names have not been revealed, have received clearance for clinical studies of their preparations in the treatment of Parkinsonism.

This should help to ease the shortage of supplies, which Mr Sinclair of Chemica says is one reason why he is going to sell his unscreened preparation in Britain. For fourteen months, he says, his company withheld supplies for ethical reasons, only to find that its exports were being imported back to Britain and sold at exorbitant prices. This, combined with the pressure of enquiries from doctors and patients, has given Chemica no choice but to sell its preparation direct to British doctors, who are, of course, free to prescribe the drug if they consider that it will benefit their patients.

Submission of drugs to the Scowen committee is at present entirely voluntary. A company wishing to market a drug approaches the committee with details of the preparation and trials carried out. If the committee is satisfied with the evidence, the drug is passed for sale; this apparently simple procedure is common with many of the preparations submitted. In the case of L-dopa, clearance has been restricted to the clinical trials, which are not expected to be completed for some months. Some people have been feeling impatient with the committee's slowness in making more supplies available. Among them is the Parkinson's Disease Society, which has been urging the Department of Health to improve the situation. Founded a year ago, the society is principally concerned with the social welfare of sufferers, but has made its voice felt on the question of L-dopa, which it believes to be a useful treatment, but not a cure. Although anxious to see more supplies made available, the society cannot support Chemica in flouting the Scowen committee.

ENRICHED URANIUM

Questions from Europe

When the news that the terms of the centrifuge agreement had been approved by Britain, the Netherlands and West Germany was announced just before Christmas, after long and hard negotiations, the Ministry of Technology made light of the condition that the agreement also had to be submitted to the European Commission for approval. It must have seemed that the commission was hardly likely to object, with the European Community's nuclear club—Euratom—still in something of a parlous state despite recent lifesaving efforts. It now seems that the ministry was over-optimistic about what seemed to be a formality.

Both the Netherlands and West Germany are members of Euratom, and under Article 103 of the Euratom treaty a member state has to submit draft agreements involving non-Euratom organizations to the European Commission. If the agreement contains clauses which go against the Euratom treaty, the commission has to communicate its observations to the Euratom states concerned within a month. This seems to be what has now happened. Although there is no official news of what the objections are, one possibility is that the commission is worried that Britain would be able to prevent members of the Community not in the centrifuge collaboration from access to the uranium. Under the draft agreement there is to be a joint committee with members from Britain, the Netherlands and West Germany to deal with relationships with other states, but the commission must feel that this is not enough to safeguard the interests of the Community as a whole.

The point of the centrifuge method is that it ought to be possible to produce enriched uranium at a price comparable with what the US Atomic Energy Commission charges—$26 per kg of separative work. Precisely what the selling price is likely to be has not been made known, and all that Mr Benn will say is that it will be competitive with the American product. At present, all the members of the European Community are supplied from the United States, with the exception of France which operates a gaseous diffusion plant. Part of the trouble seems to be that the Community is at present discussing arrangements for the supply of enriched uranium in the future and is bound to be disturbed with a *fait accompli* by two of its members.

Although the centrifuge has small beginnings—an aim of 50 tonnes of separative work in 1972—what the collaboration is working towards is the 15,000 tonnes which is the predicted European requirement by 1980. With this target in view, the commission is hardly likely to torpedo the project once there is assurance that it can be integrated into a European framework, and the Belgians and Italians have already been interested in joining. Once again, like the agreement to rescue Euratom early in December (see *Nature*, **224**, 1047; 1969), the move is as likely as not a political decision, and is another indication that Europe's nuclear policy is hardly likely to be settled until the question of British entry to the Community is decided one way or the other.

CERN ACCELERATOR

When is the Last Bus?

PROSPECTS for the proposed 300 GeV CERN accelerator are starting to look rather bleak. The West German Government finally called off the ministerial meeting scheduled to be held in Geneva last week after a period of tough talking between the German and Belgian Governments over the siting of the accelerator. Little progress seems to have been made in quelling the West German Government's conviction that it should now put pressure on its European partners for a fairer deal in joint scientific ventures, and there seems little chance that any of the other five participants to the project will allow the siting of the project to be determined by anything resembling strong-arm tactics.

One theory, which has made its way into print in the Belgian newspaper *Le Soir*, is that the West German Government has been re-evaluating the project and has found it too expensive. This would explain the odd timing of the announcement that the Federal Government will reconsider its participation if the West German site near Munster was not selected at the December meeting of the CERN Council. Some doubts have, in any case, been expressed about the suitability of the German site. Another view is that the French Government has decided to back the Belgian site in return for the purchase of Mirage aircraft by the Belgian Government, but in such a fluid situation the wildest speculations can easily gain a hold.

In one sense the British Government's decision to steer clear of the project may be held as the primary cause of the impasse, placing a heavier financial burden on each of the other governments and so elevating the importance of the location. In another sense, however, British participation would have added another arm to the multilateral tug of war, and the British Government may be feeling that it made the best decision after all.

FABIAN SOCIETY

Priorities in Education

by our Education Correspondent

THE Department of Education and Science should publish a green paper to stimulate discussion about the possibilities that exist at every level of education and their financial consequences. This, according to a pamphlet published by the Fabian Society, would provide a basis for informed discussion about priorities in education, and would help in the formulation of a national plan on the subject. The authors of the pamphlet strongly criticize the British Government for attacking the problem of expansion in education on a piecemeal basis and, in turn, they call for rapid expansion of nursery education and for more provision for further education of students leaving school at the age of sixteen.

The pamphlet is a brave attempt to assess priorities in education, and it also attempts to calculate the costs of alternative policies. The authors argue that if existing policies are continued, the share of the gross national product devoted to education will grow from the present 5·7 per cent to about 8 per cent in 1980, but if the policies that they recommend are adopted, then the figure would be about 9 per cent. This estimate is based on proposals for universal nursery education, the employment of teachers' aides in primary schools, as suggested in the Plowden Report, fully comprehensive education in secondary schools, a major expansion of further education and reorganization of higher education.

As far as higher education is concerned, the authors of the pamphlet argue that the Robbins committee grossly underestimated the likely demand for higher education. The major problem, they argue, is to increase the number of students and still maintain the existing standards of building and the staff/student ratio. The pamphlet calls for a greater variety of full and part time courses in the universities, and suggests that the main developments of this kind are at present occurring in the polytechnics. Boosting the polytechnics is therefore right in the short term, but "over a period of time, the separation of institutions of higher education into distinct classes could have most undesirable results". The authors argue that because the polytechnics devote a lower proportion of their resources to research, they offer fewer senior posts, and that the facilities provided for their students are inferior to those provided by the universities. If this situation persists, polytechnics will be nothing more than second class institutions, and the part they play will be devalued with them. The authors of the pamphlet therefore believe that "in due course federal structures should be developed, each of which should embrace all the institutions of higher education within its area".

The authors of the pamphlet were Professor H. D. Hughes, principal of Ruskin College, Oxford, Caroline Benn, editor of *Comprehensive Education*, Tessa Blackstone, David Downes and Howard Glennerster, lecturers at the London School of Economics, and Stephen Hatch from Brunel University.

LONDON UNIVERSITY

Taking the Strain

by our Education Correspondent

If the latest projections of student numbers in 1980 prove to be correct, the University of London external degree system will break down. The government and administration of the university will also be severely strained. These fears have prompted the setting up of a joint committee of the university and the Universities Grants Committee to look at the future of the University of London as a federal institution. The committee of inquiry will have sweeping terms of reference, but it will concentrate mainly on the organs of government of the university—the Senate and its standing committees, the convocation and the court—and it will also consider how staff and students can participate in the decision-making process.

The university Senate said last week that since 1962 its policy has been not to increase its external examining commitments, but despite the advent of the Council for National Academic Awards, the number of external degree students has increased by almost 50 per cent during the last eight years, and the external examining resources of the university are already severely strained. The committee of enquiry will therefore look at the relationship between the internal and external degree systems.

Such a committee is not without precedent. In 1963, the Robbins Committee said that the University of London has problems which call for investigation and remedy, and, if those problems could not be resolved internally, they "should be the subject of independent inquiry". The university set up a steering committee to consider the implications of the report, and since then, the Senate says, the academic organization of the university has been completely reshaped. But there are still problems which require outside help, and the University Grants Committee has agreed to cooperate in establishing the committee of inquiry.

WOLFSON FOUNDATION

More Grants to Universities

The Wolfson Foundation is to make further grants for university research projects likely to benefit British industry. In November 1968, the foundation supported fifteen projects to the tune of about £1 million (*Nature*, **220**, 1165; December 21, 1968) and General A. R. Leakey, the foundation's director, has indicated that a similar sum may be made available at the end of this year. The total value of the grants will, however, depend on the applications received from the universities.

The foundation's last spending spree in the universities attracted more than 150 applicants, and the fifteen chosen for support make an impressive list. It seems that the Wolfson Foundation has not found itself inundated with applications which have been turned down by other grant awarding bodies and, indeed, many academics may even apply to the foundation in preference to the research councils because it places less restriction on the way the grant is spent. Among the projects already being supported are several liaison units between university and industry. For example, the Wolfson Microelectronics Liaison Unit at Edinburgh, the Wolfson Industrial Unit at Southampton and the Wolfson Bioanalytical Centre at the University of Surrey are all hoping to undertake direct consultancy work for industry, the intention being to earn money for research which may have immediate industrial application. Other projects chosen for support also have potential industrial applications; for example, Birmingham is developing a high-speed press driven by combustion of hydrocarbon fuels and the University of Wales Institute of Science and Technology is setting up a centre for the technology of soft magnetic materials. Grants which will be awarded this year will probably support similar projects, and applications must be made before July.

INFRARED ASTRONOMY

Latest Light Bucket Ready

by our Astronomy Correspondent

One problem facing Professor Jim Ring this week has been how to get a 40-inch infrared telescope out of the cellar of 10 Prince's Gardens, the terrace-house home of the infrared astronomy group at Imperial College, London. The telescope is the latest step in a progression which people are hoping will lead one day to the building of a 120-inch infrared telescope for the consortium of British universities which have banded together to promote infrared astronomy. But the 40-inch telescope is an Imperial College venture, built on a shoe-string budget of £2,500 and started before the Science Research Council a year ago announced the award of £27,000 to the Imperial College group. This grant is earmarked for a 60-inch telescope, the next step in the path to the 120-inch, now past the design stage and awaiting detailed drawings before the parts go out to tender in April or May.

Infrared telescopes, more properly called flux collectors—light buckets in the language of astronomy—are cheap compared with similar equipment for the visible spectrum because the optics do not have to be so accurate. This leaves the way open for unusual optical systems, and the 40-inch has a novel design even for infrared telescopes. Light is directed on to the 40-inch paraboloid by a glass flat about 60 inches across. Because of the problem of supporting a sheet of glass this size, the flat is made up of a circle of six hexagons with a hole where the seventh hexagon in the pattern ought to be. The paraboloid converges the beam to a focus through the hole, and this is where the detectors will be fixed.

The next step is to transfer the telescope from the basement workshop at Prince's Gardens, where it was built because of problems of space and floor strength, to the Imperial College out-station at Silwood Park, Ascot. Here it will be used chiefly to test infrared systems, but Professor Ring hopes it will be possible to do some real astronomy through the moist air of Ascot. Although the important wavelengths at 10 microns are badly affected by water vapour, the band at 1 to 3 microns ought to be clearer and Professor Ring wants to record the infrared emission from the Orion Nebula. To get it out of the cellar, the telescope will probably have to be broken down into three components, and the Imperial College group is happy that the transfer will be accomplished safely.

Meanwhile the search for a permanent site for the 60-inch flux collector with the requisite minute amount of water vapour in the atmosphere has narrowed down to the Sierra Nevada Mountains in Spain and, rather pleasantly, Tenerife in the Canaries, where an inversion makes the atmosphere drier than expected for an island site. Testing is to go on at both places for most of the year, when the decision on the site should coincide with the completion of the 60-inch telescope expected by the end of this year. The mirror blank is at present being polished at Grubb Parsons optical works. Compared with an image size of 30″ of arc for the 40-inch telescope, the 60-inch telescope is expected to produce an image of 1″ of arc or so.

What form the 120-inch flux collector is to take—if it is built—is still under discussion, and much revolves around what wavelengths people most want to observe. One school of thought favours adding to the world's arsenal of 60-inch flux collectors before going to bigger instruments. These are questions which will have to be discussed before the British family of flux collectors grows further.

INSTRUMENTS

Microscopic Microanalysis

A POWERFUL new instrument which combines the facilities of an electron microscope and an X-ray microanalyser has been jointly developed by Tube Investment Research Laboratories near Cambridge and AEI Instruments at Harlow. The electron microscope microanalyser (EMMA) can simultaneously magnify and make an X-ray analysis of minute areas of material one tenth the diameter of the smallest objects visible in conventional electron microscopes. There are likely to be many uses for the instrument both in metallurgy, for which it was originally designed, and in biological and pollution research.

The advance which made EMMA possible was the invention of a miniature focusing lens to replace the bulky lens usually used in electron microscopes. The lens allows X-rays emitted at the surface of the specimen to be analysed by a series of spectrometers without interference from the lens. By removing the need to transfer a specimen constantly between a microanalyser and a microscope the new instrument makes possible precise analyses of small regions of the sample which are inaccessible by techniques involving two instruments. The electron beam in EMMA can be focused down to a diameter of about 1000 Å, and samples, which are in the form of thin films, can be magnified up to 160,000 times with a resolution of about 10 Å. The electron microscope is designed to operate at up to 100 keV, and in this range all elements with atomic number greater than about ten can be picked out by the spectrometers.

An interesting series of trials have already been carried out with EMMA. Surface cracks on steel tubing have been analysed and shown to contain particles of aluminium nitride with a diameter of 1500 Å, well below that resolvable in a conventional electron microscope. Certain steels are known to owe their creep resisting properties to plates in the structure, commonly thought to be silicon nitride. Analysis with EMMA revealed that some of the silicon sites were in fact occupied by manganese atoms, the presence of which had been concealed in the simple diffraction pattern.

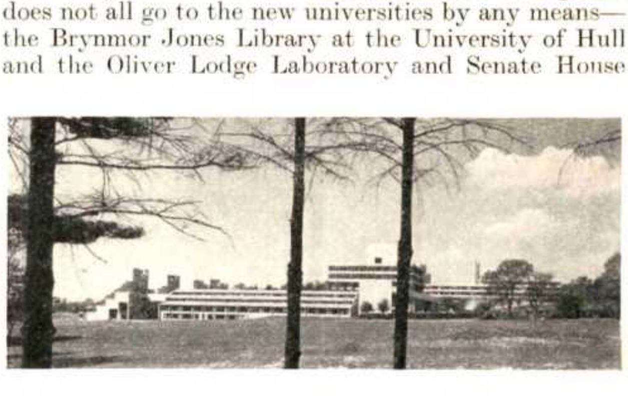
Asbestos fibres extracted from a human lung have been identified in EMMA as coming from a particular asbestos known as Amosite. By studying these fibres, which are often only 1000 Å thick, it may be possible to find out more about the disease known as asbestosis. In another test, the eyebrow of a chick has been studied. The cells were found to contain more phosphorus and less calcium than did the extra-cellular space, in which there was an excess of calcium. Dust particles collected in the upper atmosphere by an American rocket have also been analysed, and the absence of nickel has precluded the possibility of their being micrometeorites. The tests suggested that the particles probably came from a steel works in Sweden, showing the great potential of the instrument for studying problems associated with pollution.

Sales for the new instrument, which will cost between £35,000 and £45,000, are likely to be at least fifty a year according to AEI. The chief sales problem is expected to be in making people aware of the range of research tasks that can be undertaken with the instrument.

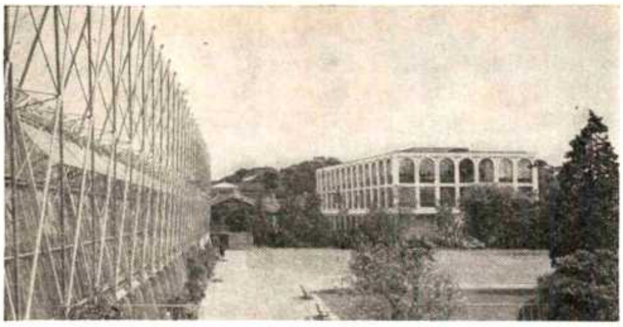
ARCHITECTURE

Prize Universities

THE encouragement given by British universities to good architecture in the past few years is reflected once again in the new crop of Civic Trust Awards. Among the 198 schemes for new buildings, restorations or landscape projects that have been recognized by the trust for the top accolade, an award or for a commendation are twenty-six from universities. The trust says that the challenge of the pace of building programmes to meet the huge expansion in university expansion in the past ten years has been met "dramatically" by the university administrators and academics. Its praise does not all go to the new universities by any means—the Brynmor Jones Library at the University of Hull and the Oliver Lodge Laboratory and Senate House



University of East Anglia.



Exhibition Plant Houses, Royal Botanic Garden, Edinburgh.

at the University of Liverpool (see *Nature*, **222**, 613; 1969) each receive one of the new awards.

On the new universities, the trust describes the designs for the University of East Anglia as the "boldest" of the master plans for the twenty-two new universities established in the past ten years. The first stage, which has been designed by Denys Lasdun and Partners, is set on the south-facing slopes of an open valley. Much of the credit for the high standard of university building, the trust says, is "due to the enlightened commissions" from the universities. "Why," it asks, "should not every government department and nationalized industry be given an equal lead in every new building for which they are responsible?" This is not to say that government departments do not win awards from time to time—indeed, the trust notes one or two schemes in the latest batch of awards—but, compared with the universities and with office buildings, government sponsored building is, as the trust says, not impressive. One that has made the grade is the Exhibition Plant Houses which have been built by the Ministry of Public Building and Works for the Royal Botanic Garden in Edinburgh. The buildings, which house subtropical and tropical plants, have supporting structures outside the glazing to protect them from the corrosive conditions normally found in plant houses. The assessor describes them as fitting in admirably with the adjacent herbarium (itself an award winner in 1966) and with their long horizontal lines and delicate and decorative pattern of external framing, they are "both gay and formal, an entirely appropriate character for their environment and one very difficult to achieve".

CONSERVATION

Italy's Obscene Harvest

The Italian Government has ushered in what was to be known as European Conservation Year with a measure that could be described as inauspicious. It has passed a bill which permits the netting of small birds, a practice which was banned in most of its aspects three years ago, as a result of which some 10 million migrants crossing between Africa and northern Europe are expected to perish each season, beginning from this autumn.

The bill, which has been passed on the initiative of the Italian Minister of Agriculture and Forests, and with the zealous support of the Under-secretary at the ministry, Signor Arnaldo Coleselli, is said to be necessary because the 1967 law impeded the capture of birds for scientific research. This has been denied by the Italian National Research Council.

An article in the Milan newspaper *Corriere della Sera* refers to the bird netting as an "obscene harvest" which will lose Italy friends abroad and even engender enough resentment to harm the tourist trade. Italy is the only European country in which small birds are netted for sport and eaten. A bill passed in 1967 which forbade the shooting of birds was intended to be the first step towards the total abolition of bird hunting. But the Ministry of Agriculture has now put the clock back against the appeals of the National Research Council and the protests of conservation societies.

ENGINEERING

Mr Howden's Ingenious Machine

An engine that can convert the thermal expansion of solids or liquids into useful mechanical work seems, on the face of it, not to be the most workable of ideas. Mr Patrick Howden thinks otherwise. He has not only developed his thoughts far enough to get several universities interested, but has built a working model to prove his point. Although what has emerged so far is clearly primitive, Mr Howden—a London physicist—claims that his engine promises in theory to be more efficient than the internal combustion engine.

In its simplest form, the Howden engine consists of two long metal tubes forming an elongated V shape and mounted on a common pivot. One is heated while the other is cooled—in the model this is done by passing hot and cold water through the tubes—and the effect of the expansion is to move the base of the V horizontally. The cycle is completed by cooling the first tube and heating the second, whereupon the base moves in the opposite direction. A ratchet converts the oscillations into continuous rotation. In a televised demonstration this set-up achieved a speed of one third of a cycle per second, but six cycles per second are apparently feasible.

The difficulties to be overcome are both theoretical and mechanical. The industrial development unit at the University College of North Wales, Bangor, has performed a thermodynamic analysis which reveals that, to obtain an efficiency of 50 per cent, pressures of a million atmospheres would be required within the expanding metals. In spite of this result the unit is continuing to investigate the engine's possibilities, for it may be that given the basic idea someone will be able to envisage a more practical way of applying it. Mr Howden, undeterred, has higher hopes for a version that would use a liquid of large expansion coefficient as its working substance instead of a metal; and obviously higher temperatures would be needed than can be attained with water.

On the practical side, the present form of the Howden engine makes heavy demands on the metal from which the expansion element is made. The model uses aluminium, but the desired combination of a large coefficient of expansion with high thermal conductivity might lead to a search for new materials if the engine is ever developed. Its advantages would include cheapness and reliability, and it could run on absolutely any fuel. But at the moment the Bangor unit finds that the problems arising from theoretical work on the engine are somewhat discouraging.

Parliament in Britain

CEGB. Generating Capacity

THE Central Electricity Generating Board estimates that this year it will spend about £850,000 on research connected with 500 MW generating units, and about £1 million next year. The work will be concentrated primarily on turbines, alternators, lubrication and bearings. This information was given by Dr Ernest A. Davies, joint Parliamentary Secretary to the Ministry of Technology.

Earlier, a number of questions about the CEGB's lack of spare capacity in the event of severe demand were addressed to the Minister of Technology. Mr Wedgwood Benn stated that apart from voltage reductions amounting to between 3 and 6 per cent for eleven days in November, the board has been able to meet demand. He said that faults in the 500 MW generator sets were responsible for the lack of spare capacity, but he gave no undertaking that the board would be able to meet demand during another cold spell, or when the sets would be back in service. Sir John Eden suggested that perhaps the CEGB should have decided on a more flexible plant mix, and Mr Eric Lubbock suggested that at least some of the criticism should be directed at private industry which supplied the faulty equipment. Mr Benn would not comment on the technical aspects of the failures, however, and he did not want to emphasize the part played by private industry because of the effect such a criticism may have on British exports. (Oral answers, January 26.)

Teacher Training Grants

MR TAM DALYELL asked the Secretary of State for Education and Science what consideration he is giving to the recommendation of the report of the working party of the Royal Society that graduates taking teacher training courses should receive postgraduate grants. Mr Gerald Fowler said, however, that grant arrangements for graduates training as teachers apply to graduates training for a number of other professions, and that he could not consider implementing the working party's recommendation without taking into account many other deserving claims on the funds available for student support. (Written answers, January 29.)

Experiments on Animals

LORD SILKIN called on the Government to introduce legislation to implement the Littlewood Report on experiments on animals, and to control the export of animals for research. No legislation has been made on this subject since 1876, he said, and the Littlewood Report was completed five years ago. Lord Platt said that although he does not like experiments on live animals, they are necessary for medical research, and that people who call for a complete end to animal experiments should refuse to take antibiotics, insulin, hormones and treatment of that kind. Replying to the debate, Baroness Llewelyn-Davies of Hastoe said that, by law, drugs must be tested on animals before they are marketed, and that if international exchange in special animal strains were restricted, the chances of another disaster on the scale of the thalidomide tragedy could occur. The welfare of animals is safeguarded under the act of 1876, she said, and the government sees no reason for giving new legislation a high priority. (Debate, Lords, January 27.)

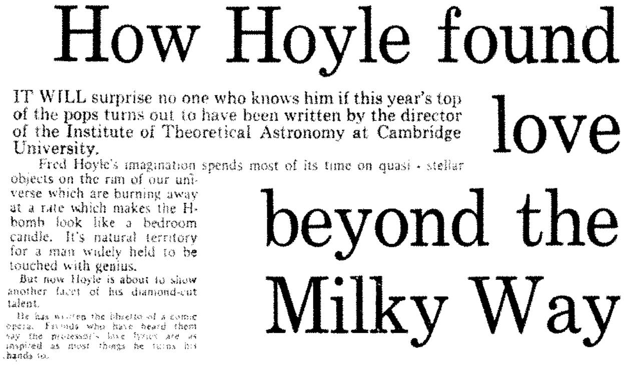
Miscellaneous Intelligence

RUMOURS that the Committee on Safety of Drugs is to recommend the withdrawal of king size cigarettes must be firmly stubbed out. The committee considers that cigarettes do not fall within its purlieu because they are not therapeutic agents and, unlike contraceptive pills, they are not "a drug administered for the modification of a physiological function". Those naive enough to think the committee is shirking its responsibilities should realize that more fundamental differences underlie this useful distinction between cigarettes and the pill. To start with, the Exchequer's revenue from cigarette duty this year was £980 million, whereas the pill is not taxed. Moreover, the pill undermines the social fabric by encouraging the sexual independence of women whereas tobacco is a social stimulant hallowed by tradition. It is true that 25,952 people in Britain died of lung cancer in 1968, compared with possibly 50 or so from thromboembolism associated with the pill, but that is no concern of the committee because nicotine is not a drug, or if it is, it is not administered to modify a physiological function, and if smokers think otherwise they must be deluding themselves.

THE discrepant estimates of the world's wild tiger population that were provoked by the recent arrival in London of Miss Gina Lollobrigida and her tiger skin maxi-coat (*Nature*, **225**, 215; 1970)—the World Wildlife Fund claimed 600 tigers and the Fauna Preservation Society 2,500—are believed to owe their difference to the old safari saying that for every tiger you see, five tigers see you.

THE United States is well on the way to becoming a Grove of Academe. In 1969, 61·4 million people, about 31 per cent of the population, were either teachers or students. Needless to say, production on this scale leads to an occasional glut; at the annual meeting of the American Physical Society in Chicago last month, only 100 employers arrived to interview some 1,500 applicants with doctoral degrees in physics.

A cow is a cow is a one-bar fire and a desalination machine; according to a handout from the Ministry of Agriculture, twenty cows give off the equivalent of 20 kilowatts of heat an hour, which equals the output of 20 one-bar electric fires. In the same interval the twenty bovids perspire 10 pints of moisture between them. Why the ministry does its sums in scores of cows instead of units is not clear unless it hopes thereby to woo farmers gently to decimalization.



How Hoyle found love beyond the Milky Way

IT WILL surprise no one who knows him if this year's top of the pops turns out to have been written by the director of the Institute of Theoretical Astronomy at Cambridge University.

Fred Hoyle's imagination spends most of its time on quasi-stellar objects on the rim of our universe which are burning away at a rate which makes the H-bomb look like a bedroom candle. It's natural territory for a man widely held to be touched with genius.

But now Hoyle is about to show another facet of his diamond-cut talent.

He has written the libretto of a comic opera. Friends who have heard them say the professor's love lyrics are as inspired as most things he turns his hands to.

From an article in the *Daily Mail* (February 2) describing how Professor Hoyle has written the libretto to *The Alchemy of Love*, an opera that will be performed by the Manhattan School of Music in New York next month.

Select Committee gets Under Way

by our Parliamentary Correspondent

THE Select Committee on Science and Technology has begun the first round of this session's main inquiries. Last week, the Registrar General and the Government Chief Actuary were the first to give evidence in the inquiry on population growth, and Sir Stanley Brown and Mr Eric Booth, chairman of the Central Electricity Generating Board and the board's member for engineering, respectively, appeared before the general purposes subcommittee which is investigating the recent failures in electricity generation. This week, representatives from International Computers, Ltd, gave evidence to the subcommittee looking into the UK computer industry.

Sir Stanley Brown said that the failure of the 500 MW generator units, which was responsible for the voltage reductions (or load-shedding) last November, was entirely unexpected, and Mr Booth suggested that the main cause of the failures was lack of attention to design detail. Both agreed, however, that if the generating sets had not been introduced quickly, Britain would have been frozen out of the world market for such equipment.

Responsibility for the failures seems to fall largely on the manufacturers, and Sir Stanley said that the board would not hesitate to order power station plant from abroad if British manufacturers failed to come up to scratch. The CEGB can, of course, impose sanctions on manufacturers in the event of shortfall in performance or lateness, but Sir Stanley denied that the CEGB had not been tough enough with the manufacturers. One turbine company and one boiler company have already been put out of business because the CEGB withheld further orders for plant but, because there are only two suppliers of turbo generators, Sir Stanley agreed that the capacity of both is needed, and that legal sanctions must be made more real.

As far as the corrosion problems of the Magnox reactors are concerned, these seem to be confined to small bolts, clips and straps made from mild steel, and Sir Stanley suggested that they are not a major cause for alarm. The corrosion has, however, reduced the output from the reactors by about 500 MW, because to run them on peak power now would shorten the life of the reactors. The first warnings of the trouble came from research in 1965, but Mr Booth said that the extent of the problem was not realized until 1968, when it was found that the interfaces of bolted joints were becoming corroded.

Commenting on the suggestion from the Select Committee's chairman, Mr Arthur Palmer, that the CEGB seems now to be concentrating more on reducing costs than on striving to maintain voltages, Sir Stanley said that he had hoped that the days of load-shedding were over. Failure of six 500 MW sets cannot pass without notice, however, and Sir Stanley indicated that further voltage reductions may be necessary this winter. That, he said, depends on the weather, and on how much plant is out of action at any given time.

The Select Committee's inquiry into the British computer industry will be undertaken by a subcommittee under the chairmanship of Mr Airey Neave and will concentrate on the possibilities of international collaboration and on the functions of the government, both as policy maker and as a user of computers. It is likely that the Minister of Technology, Mr Wedgwood Benn, will be invited to give evidence personally and, in addition to ICL, IBM and Honeywell will be appearing before the committee. Mr Neave said last week that other interested organizations, including the Atomic Energy Authority, which is a major user of computers, will give evidence later, but that written comments from any source will be welcome.

Professor Douglas, from the School of Computational Method at the London School of Economics, has been appointed special adviser to Mr Neave's subcommittee, and members of the subcommittee all attended a computer appreciation course at Imperial College earlier this month. The subcommittee hopes to complete its investigation by the end of this session.

The other main inquiry, on population growth, is being conducted by a subcommittee under the chairmanship of Mr Arthur Palmer. It kicked off last week with evidence from Sir Herbert Tetley, the government actuary, Mr P. R. Cox, deputy government actuary, Mr M. Reed, registrar general, and Miss J. H. Thompson, chief statistician in the Registrar's Department. In a memorandum to the committee, the witnesses said that the latest estimate for population in the UK in the year 2000 is 68·2 million, and in the next thirty years the average age of the population will decrease. These estimates, are, however, based on many assumptions which, if proved wrong, could significantly affect the figures.

The average number of children per family has remained fairly steady at between 2 and 2·5 between 1931 and 1961, and the estimates for the year 2000 are based on an average family size of 2·5 children. Mr Reed pointed out, however, that a change in family size from 2·5 to 2·25 would reduce the estimate by two or three million. Another factor likely to affect the predictions is a change in the birth rate. This has been far from constant this century. In the 1930s, the birth rate was comparatively low. It rose during the war years and immediately after, but started to fall in the early 1950s. It then rose again in the late fifties and early sixties, but now seems to have levelled off. Miss Thompson suggested that the recovery in the birth rate may be caused by the tendency towards earlier marriage and the increasing percentage of women who get married. Immigration, on the other hand, does not seem to be a significant factor in the projections for the year 2000.

These variations in family size and birth rate are the reason why the estimates of the population in the year 2000 have differed by almost 11 million between 1961 and 1968. In 1961, the Registrar estimated that the population of the UK would reach 63·8 million by the year 2000, but by 1964, this estimate had been revised to 74·7 million, and in 1968 estimates had dropped again to 68·2 million.

NEWS AND VIEWS

150 Years of Astronomy

Astrophilatelists will be delighted that the 150th birthday of the Royal Astronomical Society has not gone unnoticed by the British Post Office. A commemorative stamp is to be issued on April 1, believed to be at a value of 1*s* 9*d*. What the design will be remains, by Post Office tradition, a closely guarded secret until about a month in advance, but those guessing which astronomers or what telescopes are likely to get star billing will remember that the photogenic Mark 1 telescope at Jodrell Bank has already featured on a special issue. At a value of 1*s* 9*d* the stamp will not be publicizing the society on letters within Britain, but it will have a select circulation on first-class parcels weighing 1 lb and air-mailed letters to the United States. Thus the anniversary rates with Sir Francis Chichester's voyage round the world—the society could not have hoped for more.

Astronomers are fond of saying that they are the last gentlemen scientists, and it is true that the monthly meetings of the Royal Astronomical Society provide some of the more delightful moments of the London scientific scene. The gathering of members and fellows for tea in Burlington House beforehand is one of science's social occasions, and often rivals the meeting itself for interest, although this is not likely to be so at the next meeting. This is on February 13 (a Friday, but astronomers are not the type to worry about that) and is to be the official anniversary meeting. As well as a sentimental retrospective of the society's history, there will be the presidential address by Sir Bernard Lovell. The anniversary could hardly have happened at a more exciting time for British astronomy, and no doubt Sir Bernard will have a lot of interesting things to say about the future in his address on the prospects for British astronomy. As well as the upheavals which are going on in our understanding of the universe—the way the theory of supernovae has been stood on its head by the discovery of pulsars, for example—astronomy in Britain is going through an appraisal of priorities which will decide how the subject is to develop for the next few years at least. This is the review of astronomy in the northern hemisphere which the Science Research Council is conducting, and it is pleasing that the Royal Astronomical Society is now taking part. At the pace at which astronomy is going there seems good reason for making the review a frequent event.

Now that the old lecture room with its rogues' gallery of past presidents is being partitioned into smaller rooms, the society meets in the opposite corner of the courtyard of Burlington House in the rooms of the British Academy. There is no denying that this is far from ideal. For one thing, it is now clear that the British Academy room does not make a good lecture theatre. What is more, members are attending meetings without stepping foot inside the society's cramped quarters, and this is a problem which is no doubt exercising the society's council. Unfortunately there is not likely to be a solution until the societies which still inhabit the Burlington House warren are better housed. It is also a shame that although the financial position of the society has been in an encouraging state it is apparently not strong enough to see through the alterations at Burlington House as quickly as might have been hoped. An astronomy society is not the sort of venture which attracts money from industry unfortunately.

With astronomy advancing rapidly on diverse fronts, the Royal Astronomical Society has an important job to do. Astronomy has somehow become fragmented into classifications depending on what techniques are used—there are microwave astronomers, gamma ray astronomers and so on—and the society could be invaluable in bringing them together. The same goes for the new band of lunar scientists, dispersed among departments of physics, chemistry and geology, and waiting for new paths of communication to be established. Clearly there is a lot to be said for more meetings devoted to a particular topic, such as the recent meeting on Mars, where the different disciplines would rub shoulders. Astronomers might well find meetings along these lines more useful than the usual format—generally a hotchpotch of readings from papers accepted for *Monthly Notices*. In any case, with the General Assembly of the International Astronomical Union meeting in Britain this summer for the first time in forty-five years, and with British astronomy booming, the society has a lot to do.

RNA STRUCTURE

Codon and Nodoc

from our Molecular Biology Correspondent

Whereas several spectroscopic methods are available which will give the proportion of paired bases in an RNA chain, and with luck also a good estimate of the relative amounts of A·U and G·C pairs, there has been no dependable way of determining the distribution of paired and unpaired segments along a chain of known sequence. Chemical methods based on the expectation that the paired bases will in general be less reactive than the unpaired have given fragmentary information, but most activity has centred on tortured attempts to deduce unique pairing schemes by earnest contemplation of the sequence, by computer analysis and by some rather desperate model building.

On page 508 of this issue of *Nature* is recorded the birth of a new technique, evolved by Doty and his associates, and based on their studies of base-pair formation between complementary oligonucleotides. The principle is that even complementary sequences of as few as three base pairs will associate to a detectable extent at 0°, so long as at least one pair is G·C. If then tri and tetranucleotides are added to an RNA molecule, they will bind if the chain contains a sequence complementary to the oligomer, and not already involved in intramolecular pairing. The association of each oligomer is measured by equilibrium dialysis, and can be placed into one of three categories, according to its binding propensity—yes, no and perhaps. The last of these one supposes to comprise those oligomers with complementary sequences which, though unpaired, are not sterically favourably placed for the interaction, or are available only in conformational isomers of the native RNA present in low concentration. At all events, the range of binding constants is very large, and at the upper end of the range there is no doubt about the interpretation. To eliminate ambiguities and chart the RNA chain, a range of oligomers with overlapping sequences must be used. The particular beauty of the method lies in the low binding energies that are involved, and make it almost inconceivable that any structures of significant stability in the native RNA could be disturbed by the binding.

The rout of the model builders commences in the first article by Uhlenbeck, Baller and Doty (page 508), which concerns the anticodon loop of formylmethionine tRNA. This has the sequence AAUACUC, with UAC as the anticodon triplet. The latter binds the codon trimer with high efficiency, and the tetramer, AUGA, ten times more strongly yet, showing that the adjoining base on the 5′ side of the anticodon is also available. The tetramer, which overlaps on the 3′ side, UAUG, binds poorly. With a series of trimers and tetramers encompassing the loop sequence and beyond, it is found that only the anticodon triplet and the next two bases on the 5′ side, U and C, are essentially free, the rest not.

Formylmethionine tRNA is known to bind to the ribosome in the presence not only of AUG, but also of GUG, and indeed it turns out that GUG will bind well to the RNA alone. The binding of GUGA, as well as competition between GUG and AUG, shows that the GUG indeed pairs with the anticodon, and this must be seen as the first evidence, beyond the merely circumstantial, that stable G·U pairs really exist. This result also eliminates the notion that GUG binding is a unique aspect of the association of the initiator tRNA with the ribosome. It does, however, point to a special property of the "wobble" position in this sequence, for no other parts of the anticodon loop can be persuaded to participate in G·U pairs.

Lewis and Doty (page 510) have also studied the structure of 5S RNA of *E. coli*, which has a known sequence of 120 nucleotides, by binding experiments with forty-one tri and fifty-seven tetranucleotides. Fifteen of the former and seven of the latter were judged to bind strongly, whereas fourteen and nineteen respectively showed negligible binding, the rest being intermediate. The upshot is that there are four regions, centred around positions 10, 28, 61 and 97, comprising a minimum of twenty-five bases, which are unequivocally unpaired.

The limitations of the method are evident enough: if loops of unpaired bases are constrained by stacking, for example, and packed into a tight tertiary structure, some of the unpaired bases may not be fully available for pairing. On the other hand, there can be no reasonable doubt that the regions at which the oligomers do bind are indeed unpaired in the native structure. This is enough to demolish three different models proposed in the literature for the secondary structure of 5S RNA. Although when the loops have been delineated considerable stretches of complementary sequences can be discerned in the rest of the chain, there is still insufficient information to define the pairing scheme uniquely. Lewis and Doty surmise that it is a complicated affair, which cannot be expressed in two dimensions.

BIOCHEMISTRY

Running into Evolution

from a Correspondent

"You can't work long in biology without running into evolution—it's something that happens to you," said Professor G. Wald (Harvard University) when he gave the opening Feodor Lynen Lecture at the second annual winter symposium of the Department of Biochemistry of the University of Miami and the Papanicolaou Cancer Research Institute in Miami held from January 19 to 23. The title of the first meeting—homologies in enzymes and metabolic pathways—might have promised a hotchpotch of unrelated biochemical observations with a vague evolutionary leaning, but Wald's words provided a theme which was picked up by subsequent speakers. He spoke of classic work ("wrong—but old enough to be forgiven") which identified creatine phosphate or arginine phosphate as a marker differentiating vertebrate from invertebrate metabolism, or of vitamin A_1 or A_2 as distinguishing seawater or freshwater fish. The philosophical arguments between "analogy" and "homology" and the attendant story of "biochemical recapitulation" were themes which every subsequent speaker was obliged to consider.

Sequence differences in species of cytochrome *c*, analysed by Dr E. Margoliash (Abbott Laboratories, Chicago), suggested that although 30 per cent of the residues must always be invariant, any one species can tolerate changes in only 10 per cent of the chain at any one time. One feels that such statistical models of evolution will carry even greater weight when features of the tertiary structures of the proteins can be taken into account.

The selective pressure for sequence changes must lie in the metabolic environment. Phosphoenol pyruvate stands at the gateway of gluconeogenesis, and Professor H. L. Kornberg (University of Leicester) described how bacteria, plants and higher organisms achieve this important metabolic link. Dr W. J. Whelan (University of Miami) showed how animals and plants use different but analogous pathways to break down glycogen or starch, and Dr J. Preiss (University of California, Davis) discussed control of glycogen and starch synthesis. Small variations in mechanisms of fatty acid synthesis were shown by Dr F. Lynen (Munich) to account satisfactorily for the specific fatty acids of various species, and even the bewildering variety of steroid hormones was organized by Dr K. Savard (University of Miami) into a neat set of metabolic compartments. In all these talks it was clear that alternative routes could arise to meet specific metabolic needs of different organisms.

How could such metabolic pathways evolve? Dr E. C. C. Lin (Harvard University) showed how *Aerobacter* "borrowed" a permease from one pathway and a dehydrogenase from another when "learning" to grow on xylitol. Subsequent gene-doubling events would allow selection for greater specificity while preserving the parent pathway. One should expect to find families of enzymes with common mechanisms but different specificities. Dr N. O. Kaplan (University of California, San Diego) gave reason to believe that some dehydrogenases may form such a family, and Dr K. Muench (University of Miami) hinted that amino acyl tRNA synthetases may be another.

Dr B. S. Hartley (University of Cambridge) reminded the participants that the pancreatic serine proteinases are a family with identical conformations of the polypeptide chain, though the surface of these enzymes varies considerably. Yet a single amino-acid change is sufficient to explain the difference in specificity of trypsin and chymotrypsin. Bovine thrombin is similarly homologous with the pancreatic enzymes, but a bacterial proteinase, subtilisin, differs in both primary and tertiary structure. Nevertheless convergent evolution has produced a similar arrangement of catalytic residues in its active site. Dr D. R. Whitaker (University of Ottawa) also told of other serine proteinases from a bacterium and a mould which resemble chymotrypsin rather than subtilisin. Wald's "analogies" and "homologies" appeared as three dimensional molecular models in this symposium.

NUCLEIC ACIDS

Circles not Rolling

from our Cell Biology Correspondent

THE rolling circle model of bacterial and bacteriophage chromosome replication as proposed by Gilbert and Dressler was one of the chief talking points at the 1968 Cold Spring Harbor Symposium on DNA replication (see *Nature*, **219**, 17; 1968). But since then evidence on balance seems to have been running against the rolling circle model as a unitary explanation of the mechanism of chromosome replication in all phages and bacteria.

The model was originally proposed to explain the replication of the single stranded circular chromosome of øX174, and for this phage, and perhaps all phages with similar chromosomes, it still seems valid. Kiger and Sinsheimer (*J. Mol. Biol.*, **40**, 467; 1969) as well as Gilbert and Dressler have detected recently DNA strands longer than complete øX174 chromosomes in infected *E. coli*—the occurrence of such DNA strands is one of the chief predictions of the rolling circle model. On the other hand, attempts to extend the model to the replication of the double stranded circular chromosomes of bacteria, phages and viruses, such as polyoma, have not been convincing. The burden of existing and new experimental evidence, accumulated since 1968, has supported Cairns's model of replication of these chromosomes, which envisages a replicating point moving round a circular DNA unwinding and replicating the complementary strands as it progresses. This model, of course, predicts that the replicating point will be a Y shaped fork and DNA molecules longer than one genome's length should not occur.

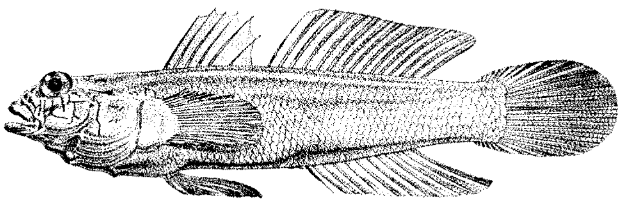
Cairns originally proposed this model to explain his autoradiographs of replicating *E. coli* chromosomes and lately electron micrographs of structures presumed to be replicating λ phage and polyoma chromosomes have been obtained, which are, in essentials, identical to Cairns's autoradiographs. Electron micrographs are, of course, always open to familiar criticisms, but recently Bourgaux, Bourgaux-Ramoisy and Dulbecco (*Proc. US Nat. Acad. Sci.*, **64**, 701; 1969) have reported a centrifugation analysis of replicating polyoma virus chromosomes which is completely consistent with the Cairns model. They have identified a replicative intermediate which is ring shaped, forms a heterogeneous population containing strands of variable length, has nicked DNA, and is larger than the relaxed circular DNA that can be obtained from virions. Moreover, label chases from this intermediate into the superhelical DNA of the virions. All the properties of this replicative intermediate fulfil the requirements of Cairns's model of replication; on the other hand, Bourgaux *et al.* found no evidence of single DNA strands longer than those found in the virus, which should, of course, be present if the polyoma chromosome replicated as a rolling circle.

Some of the conflicting ideas about the replication of λ phage chromosomes have also been resolved recently by Carter, Shaw and Smith (*Biochim. Biophys. Acta*, **195**, 494; 1969). In the past there has been argument as to whether λ DNA replication involves circular molecules or linear concatemers, and the answer seems to be that both forms occur at different stages in the replicative cycle. During the first phase, completed within the first 15 minutes, circular λ chromosomes are replicated, apparently by the Cairns mechanism, to yield covalently closed twisted circular progeny DNA, but these molecules according to Carter *et al.* are never packaged into progeny phage. Indeed, their role is a complete mystery. By 15 minutes after infection linear concatemers up to eight times as long as the λ genome appear and this DNA is fragmented and packaged into progeny phage. Unfortunately the experiments tell nothing about the origin of the concatemers; what data there are are equally consistent with the rolling circle or Cairns model of replication. This means, of course, that the rolling circle model cannot yet be dismissed as irrelevant to replication of λ chromosomes, and the electron micrographs of Cairns type structures isolated from λ infected *E. coli* may be irrelevant to the replication of DNA destined to be packaged into progeny phage. They may simply be pictures of molecules in the first, and apparently abortive, phase of replication.

ICHTHYOLOGY

Another Goby in British Waters

THE discovery that a fish presumed to be absent from British waters has actually been lurking in the shallows of south-west Ireland for at least thirty years is a blow to the complacency of those who like to think that the British fish fauna is thoroughly well known. Gobies are familiar members of the British sea shore fauna and commonly caught by holidaymakers with shrimping nets. But nobody knew that *Gobius cruentatus* came so far north until Dr A. Wheeler of the British Museum (Natural History) found a neglected specimen in the National Museum of Ireland. It had been



Gobius cruentatus from Barlogue Creek, Lough Ine, collected by L. P. W. Renouf in 1930.

there since being found in Lough Ine, County Cork, in 1930 (*J. Fish. Biol.*, **2**, 59; 1970).

Returning to his own museum in London, Wheeler was amazed to find another specimen of *G. cruentatus* hidden away under the name *Gobius niger*. This too dated from 1930, when it was collected in Bantry Bay, County Cork. Wheeler then contacted Professor J. A. Kitching of the University of East Anglia, who had been investigating the ecology of Lough Ine. He produced a collection of gobies, among which Wheeler identified another specimen of *G. cruentatus*.

This species, shown in the illustration, is large, at 166 mm long, compared with most other gobies, and is also distinguished by its large eyes, reddish head and black body. It belongs to the so-called Lusitanian element among fish, being most common in the southern waters around North Africa, Spain and Portugal (which country is roughly equivalent to the ancient Lusitania). The most northerly report of *G. cruentatus* was previously from Arcachon on the French Biscay coast in 1872. It is not altogether surprising that this fish should turn up in Irish waters, where the comparatively warm climate of the southern and western regions provides the northerly limit of distribution of several Lusitanian species.

Three specimens are obviously too few to form the basis of any theory as to how *G. cruentatus* came to Ireland, but the thirty years interval between the capture of Kitching's specimen and the two museum pieces suggests to Wheeler that there is a maintained population in the area. It seems unlikely that specimens could have been carried to the Irish coast by water currents during the planktonic larval stage, for their larval life is rather too short. In any case, currents from the north Spanish coast would not take them to Ireland.

There remains the question of why these gobies have not been found before in the sea around Ireland. The answer seems to lie in their habit of staying concealed just below tide level. Maybe many more are waiting to be found in northerly waters.

ENZYMES

Proline Hydroxylase and Liver

from our Medical Biochemistry Correspondent

The activity of protocollagen proline hydroxylase is increased in healing wounds, fibrotic liver and the skin of growing animals, as a result of the increased rate of synthesis of collagen. The activity of the enzyme might therefore be expected to be intimately connected with the activity of connective tissue. But a recent survey has indicated that it is the liver that is the vital tissue in this respect.

Stein *et al.* (*Lancet*, i, 106; 1970) have developed a technique for assaying proline hydroxylase activity in human serum, and measured the enzyme in twenty healthy volunteers and 124 patients with different conditions. The "normal" range found was from 79–567 d.p.m. per ml. of serum per hour (the amount of tritium released from labelled collagen) and there was no evidence of any increase in activity in patients with diseases of the connective tissues. On the other hand, three patients with hepatomas all had extremely high serum proline hydroxylase activity. Stein *et al.* found that a substantial proportion of patients with hepatitis or cancers with liver metastases had significantly higher than normal serum proline hydroxylase activity. In liver disease there was a good correlation between increased alkaline phosphatase activity and increased proline hydroxylase.

Among patients with cancer, six out of fifteen with lymphoma had increased proline hydroxylase and, of these, four also had increased alkaline phosphatase; eleven out of forty-one patients with other cancers had increased proline hydroxylase, nine of them also having increased alkaline phosphatase.

Elevated serum alkaline phosphatase can also be a symptom of bone disease. It is therefore interesting that in patients with bone metastases, primary bone tumours and Paget's disease, many with great increases in serum alkaline phosphatase, only one patient had slightly more proline hydroxylase than normal.

To investigate whether wound healing would increase proline hydroxylase activity the enzyme was assayed in sixteen patients before and at daily intervals after major surgery. In six patients given nitrous oxide anaesthesia supplemented with muscle relaxants there was no change in proline hydroxylase activity. The other ten patients received either methoxyflurane or halothane as the primary anaesthetic agent, and in all of them concentrations of serum proline hydroxylase increased considerably a few days after the operation. The increase is therefore unlikely to be due to wound healing and may reflect an effect of the anaesthetic on liver metabolism. Both anaesthetics have been suspected of causing liver damage, but these patients showed no evidence of this by any of the conventional tests of liver function.

In view of the finding that serum proline hydroxylase seems to depend on liver activity and not, as might be expected, on the activity of connective tissue, it might prove to be a useful test of liver function; tests used at present are not entirely satisfactory.

STOMATA

Ion Pump at Work

from our Plant Physiology Correspondent

A technique which makes possible direct measurement of the concentration and nature of particular ions in small samples of tissue has provided strong support for the latest theory of stomatal movement. B. L. Sawnhey and I. Zelitch of Connecticut Agricultural Research Station have found that in conditions which favour stomatal opening, potassium ions accumulate in the guard cells. In contrast, an efflux of potassium from the guard cells occurs as the stomata close (*Plant Physiol.*, **44**, 1350; 1969).

Stomata are the minute pores which perforate the epidermal surfaces of leaves and so ventilate the leaf interior. The size of the pore aperture is related to the degree of turgor of the guard cells, two kidney shaped cells which line the mouth of the pore. When the turgor pressure of the guard cells equals that of the surrounding epidermal cells, the guard cells are pressed together, and the pore is closed. In conditions which favour opening, such as light or a decrease in carbon dioxide concentration, the guard cells take up water and become turgid. The peculiar structure of these cells forces them to separate when turgid, and so the pore opens. Stomata are the principal channels for the movement of water and gases between the external atmosphere and the interior of the leaf and, as such, play an important part in regulating the water economy of the whole plant and controlling the rate of photosynthesis.

Economically it would be of great value to be able to regulate either or both of these processes by modifying the stomatal aperture artificially, but more than 100 years of research have not revealed the underlying mechanism. Once it was established that stomatal movement depends on the osmotic movement of water, the problem seemed reduced to a search for a solute which when accumulated in the guard cells could cause an increase in the turgor pressure of these cells, and consequently opening of the pores. It now seems, in the light of recent findings, that the apparently significant presence of chloroplasts in the guard cells has been an appealing but unfortunate red herring. Guard cells are unique among epidermal cells in possessing chloroplasts, the minute green organelles which house the photosynthetic reaction centres. What more likely than that the active solute should be a soluble carbohydrate, produced photosynthetically in the light, but converted into insoluble starch in conditions which favour stomatal closure? Nevertheless, it has proved difficult to find experimental support for this view, and quite recently the work of M. Fujino (*Sci. Bull. Fac. Educ. Nagasaki Univ.*, **18**, 1; 1967), and R. A. Fischer (*Science*, **160**, 784; 1968), has indicated that the movement of potassium ions into and out of the guard cells represents a more likely mechanism.

Sawnhey and Zelitch have used the electron microprobe technique to build up a picture of the accumulation and loss of potassium ions from the guard cells as they open and close in light and darkness. The microprobe focused a stream of electrons onto prepared specimens of plant material causing ions in the material to emit their characteristic X-ray spectra. The spectra were then analysed both qualitatively and quantitatively, and a congruent image of the distribution of particular ions in the samples was obtained. In darkness the pores were closed and no clear pattern in the distribution of potassium in the tissues could be distinguished. After 1·5 hours in the light, however, the concentration of potassium in the guard cells increased from 0·21 M to 0·50 M, and the congruent image shows the open stomata clearly outlined by the accumulation of potassium in the guard cells.

The stomata closed when returned to darkness and the distribution of potassium again became random. The quantitative nature of the electron microprobe technique made it possible to demonstrate a linear relationship between the concentration of potassium ions in the guard cells and the stomatal aperture. Calcium and phosphorus ions were also measured, but these elements showed no regular pattern of movement as the stomata opened and closed.

These results strongly suggest that stomatal movement is controlled by an active transport of potassium ions through the cell membrane of the guard cells. Sawnhey and Zelitch believe that the problem is half solved; the next line of attack must be aimed at elucidating the nature of the ion pump itself.

GEOMAGNETIC FIELD

Spherical Harmonics Superseded?

from our Geomagnetism Correspondent

SPHERICAL harmonic analysis has done sterling service in the cause of geomagnetism during the past hundred years or so. Ever since Gauss carried out the first such analysis in 1839, this has been the standard mathematical technique for representing both the Earth's main field and its secular change. Although a description of the Earth's field in terms of spherical harmonics is a purely mathematical procedure and contains no implication that each term or group of terms corresponds to a different physical effect, other evidence suggests that this is the case. Thus Gauss himself was able to show that although the main field is of internal origin the Earth possesses a very small physically distinct external field. Furthermore, the internal field itself may be divided into dipole and non-dipole components. The different rates of movement of these components with respect to the Earth's surface during the past hundred years suggest that their origins are also different.

In spite of the success of spherical harmonic analysis it remains essentially a mathematical convenience, and so during the past few years attempts have been made to formulate models which are physically more meaningful. As a first step, Alldredge and Hurtwitz (*J. Geophys. Res.*, **69**, 2631; 1964) used a model with a central dipole and eight radial dipoles at a common distance of 0·26 Earth radius from the centre to simulate the field in 1955, and succeeded in reducing the r.m.s. residual of the best fitting field to about 700γ. To reduce this residual field even more, the original model has been extended by Alldredge and Stearns (*J. Geophys. Res.*, **74**, 6583; 1969) to include many more dipole sources. They show that the Finch–Leaton spherical harmonic field for 1955 may be simulated by a central dipole and twenty other dipoles at 0·21 Earth radius from the centre, with an r.m.s. residual of only 28γ. The International Geomagnetic Reference Field (IGRF) for 1965 may similarly be simulated by a central dipole plus thirty-four dipoles at 0·22 Earth radius from the centre, with an r.m.s residual of only 25γ.

Thus in order to represent the Earth's main field, a purely dipole model is as successful as the more familiar dipole–non-dipole spherical harmonic model. But does it describe the secular change field just as well? Apparently it does, for Alldredge and Stearns are able to show that by varying the dipole parameters it is possible to match the Z component of the Finch–Leaton 1955 field with an r.m.s. residual of only $2{\cdot}15\gamma$ per year, and the Z component of the 1965 IGRF with an r.m.s residual of only $1{\cdot}26\gamma$ per year.

So far the new model is as mathematical as spherical harmonic analysis; and there is little evidence that it is physically more realistic. In one sense it clearly is not, for the new models possess dipoles at a distance of about 0·2 Earth radius from the centre, whereas it is widely believed that magnetic changes so deep in the core would not be felt quickly enough at the Earth's surface to account for the observed secular change. Nevertheless, the purely dipole model describes the secular change field remarkably well. For the 1965 IGRF it leads to a westward drift of 0·2 degrees longitude a year, almost identical with the observed westward drift velocity.

The real field sources in the Earth's core are certainly very complex; and a complete understanding awaits a tractable hydrodynamic model. Meanwhile, Alldredge and Stearns hope that their kind of model will lead to a better understanding of the nature of the geomagnetic field and its secular variation without enquiring too deeply into the basic physics of the core motions.

UNUSUAL STARS

Infrared Object which Varies

by our Astronomy Correspondent

There seems to be no easy explanation for the odd behaviour of the infrared object which E. E. Becklin *et al.* of California Institute of Technology have found to be fluctuating in brightness by two magnitudes, equivalent to a factor of more than six (*Astrophys. J. Lett.*, **158**, L133; 1969). The favoured model for several infrared objects in the galaxy—that they are surrounded by a dust cloud which absorbs the energy from the central star and re-radiates it at infrared wavelengths—gives no hint as to why there should be such striking variability. But the magnitude and period of the fluctuation are like that of the Mira variables, and one possibility is that the central star is a Mira variable. The dust cloud would then have to be opaque enough to obscure certain strong absorption bands which are prominent features in the infrared spectra of long period variables but which are absent in the object IRC+10216 under study.

At 5 microns the object is the brightest thing outside the solar system, and clearly it is going to be an embarrassment to infrared astronomy if its nature cannot be cleared up fairly speedily, at least in outline. At visual wavelengths the object is insignificant—fainter than eighteenth magnitude—but on a plate taken by Arp at the 200-inch it has an elliptical non-stellar appearance. It has been observed at 2·2 microns since 1965 using a 62-inch infrared telescope, and these are data reported by Becklin *et al.* The time scale of the brightness variations seems to be 600 days or so.

The location of IRC+10216 is still a mystery. Absence of proper motion and of parallax sets a lower limit on distance of 100 parsec and this is, of course, still within the galaxy. Assuming a distance of 200 parsec—a typical distance for a nearby member of the galaxy—the luminosity comes out to be similar to that of the infrared source in the Orion Nebula and to long-period Mira variables. But Becklin *et al.* cannot rule out that the object is extragalactic, in which case its size as inferred from the 600 day fluctuation places it at 200 kiloparsec with a luminosity comparable with that of the galaxy.

Clearly Becklin *et al.* favour the view that IRC+10216 is in the galaxy, even though it refuses to fit any particular classification. Its similarity to the infrared point source in the Orion Nebula suggests that it may be a protostar, yet it is in an unlikely area where there is no gas or dust and no other young stars, and the brightness variations are awkward. One interesting speculation is that IRC+10216 may be a Mira variable evolving into a planetary nebula, and the clue here is the elliptical nebulosity which surrounds the object.

ELECTRON BEAMS

Electrons for Carrying Light

Two members of the Signals Research and Development Establishment at Christchurch have suggested an explanation for an apparently mystifying effect discovered in an electron diffraction experiment (Schwarz and Hora, *Appl. Phys. Lett.*, **15**, 11; 1969, reported in *Nature*, **225**, 15; 1970). The effect is that if a beam of electrons is diffracted by a thin crystal film of silicon or aluminium oxide through which a laser beam is shone at right angles to the electron beam, the diffraction pattern seen on a non-fluorescent screen of alumina contains spots of the same colour as the light in the laser beam. Drs R. L. Harris and R. F. Smith write:

It appeared to us, at first sight, not particularly surprising that the effect should have been observed. Indeed, analogous interactions between electromagnetic waves and electron beams have been used for some time in devices such as klystrons. Energy at the electromagnetic wave frequency is carried by the electron beam as velocity modulation, resulting in bunching which varies in intensity with distance along the beam.

But, on closer inspection, bunching as it is normally understood cannot directly cause the observed effect. This conclusion was reached by consideration of the electron density in the beam. Using the figures given by Hora and Schwarz, we calculated a value of 500 electrons per centimetre of the beam. A typical figure for the klystron would be between 10^4 and 10^6 times this value, and the modulating wavelength would be, say, 1 cm. In the case of light, the wavelength is only about 4×10^{-5} cm, and so the electrons are, on average, separated by many wavelengths. In spite of this, the effect may still be explained by velocity modulation if bunches of electrons occur separated in time by integral numbers of periods of the original optical wave. This requires the recovery process in the screen to be non-linear, thus producing harmonics of the electron bunching frequency.

If the effect is due to velocity modulation, then movement of the screen along the axis of the electron should result in a periodic variation in intensity of the optical emission from the screen. Because the distances between these peaks of intensity are dependent on the velocity of the electron beam, the reported reduction in intensity of the optical images with a decrease in electron velocity is possible for a particular position of the screen.

The reported disappearance of the effect on rotation of the plane of polarization of the incident light is to be expected if the velocity modulation explanation is true.

Fairly simple experiments can be devised to disprove the velocity modulation explanation, and this should be done before more erudite explanations involving "modulation" of individual electrons are proposed.

Extra-galactic Distance Scale

by

SIDNEY VAN DEN BERGH

David Dunlap Observatory,
University of Toronto,
Richmond Hill, Ontario, Canada

Nine different methods are used to determine the value of the Hubble constant. A mean value $H = 95^{+15}_{-12}$ km s^{-1} Mpc^{-1} is obtained from all data that are now available.

THE determination of the extra-galactic distance scale is essentially a two step process. First the distances to nearby galaxies which are members of the Local Group have to be determined. Next these nearby galaxies are compared with galaxies which are at such large distances that their systematic velocities of recession are large compared with their random motions.

Data on the distances to galaxies of the Local Group[1] are summarized in Table 1. The final distance moduli which are adopted for M 31 and the Magellanic Clouds depend rather critically on the relative weights that are given to different methods of distance determination. A weight of three has been assigned more or less arbitrarily to moduli derived from Cepheids, a weight of two has been given to moduli obtained from novae and RR Lyrae stars, and unit weight has been assigned to distance moduli determined from W Virginis stars, Population II giants, globular clusters, MK classifications and Hβ and Hγ photometry. It should perhaps be emphasized that the resulting distance moduli of the Local Group members depend rather critically on the assumption that the Cepheids in all galaxies obey a universal period-luminosity relation.

All determinations of distances beyond the Local Group are ultimately based on the assumption that recognizable types of distant objects are similar in nearby and in distant galaxies of the same type. This assumption may not be correct for any particular type of distance indicator because of possible differences in age, evolutionary history and abundance of the elements. Systematic errors due to such differences will be minimized if the largest possible number of methods of distance determination is used.

Nine distinct methods of distance determination beyond the Local Group are now available. The internal agreement between the distances obtained by these different methods provides some indication of the degree of confidence which may be placed in the resulting distance scale.

Diameters of HII Regions

Measurements of the diameters of HII regions on plates obtained with the 100 inch and 200 inch telescopes have been reported by Sérsic[2]. Unfortunately only blue plates, on which the contrast between nebulae and background is often quite small, were available in this study. A comparison of the mean diameters of the three largest HII regions in Local Group galaxies with the mean diameters of the three largest HII regions in thirteen distant galaxies of types Sc$^+$, dSc and Ir yields a value of $5 \log (H/100) = -0{\cdot}2 \pm 0{\cdot}4$, which corresponds to $H = 91^{+19}_{-15}$ km s^{-1} Mpc^{-1}. A more detailed study of giant emission regions in extra-galactic nebulae is currently in progress at the Mt Wilson and Palomar Observatories.

Luminosity Classification of Galaxies

Observation shows that the degree to which spiral structure is developed in galaxies correlates strongly with absolute luminosity. Intrinsically bright spirals are found to have long and well developed spiral arms whereas intrinsically faint spirals show only a rudimentary and ill defined spiral pattern. From a comparison of the luminosity classifications[3-5] of distant spiral nebulae with the luminosity classifications of the Local Group members M 31 and M 33, a value of $5 \log (H/100) = -0{\cdot}05 \pm 0{\cdot}4$, which corresponds to $H = 98^{+19}_{-17}$ km s^{-1} Mpc^{-1}, is obtained. The chief uncertainty of this result is due to the fact that only two Local Group galaxies are available for calibration.

Brightest Globular Clusters in Galaxies

Because of their high luminosities, globular clusters can be seen out to very large distances. This makes globulars potentially valuable as calibrators of the extra-galactic distance scale[6]. It should, however, be emphasized that the assumption that the brightest globular clusters in different galaxies have exactly the same luminosity is as yet unproven. The brightest galactic globular cluster is ω Centauri[7] for which $M_B = -9{\cdot}7$ if $\langle M_B \rangle = +0{\cdot}8$ is adopted for the RR Lyrae stars in this cluster. This is significantly fainter than the value $M_B = -10{\cdot}05$ obtained from recent 200-inch observations of the cluster Mayall II in M 31. (The clusters Nos. 39, 42, 93 and 327 in M 31 have luminosities which are comparable with that of MII. The data for M 31 and the galaxy yield a mean absolute magnitude $\langle M_B \rangle = -9{\cdot}88$, with an uncertainty of, say, 0·3 magnitudes, for the brightest globular clusters in supergiant spirals. This value may be compared to $B = 21{\cdot}2 \pm 0{\cdot}2$ which Racine[8] obtains for the brightest globular clusters in the supergiant elliptical galaxy M 87 in the Virgo cluster. If it is assumed that the brightest globulars in M 87, M 31 and the galaxy have the same absolute luminosity, then $(m-M)_{\text{pg}} \simeq (m-M)_0 = 31{\cdot}08 \pm 0{\cdot}36$ for the Virgo cluster. Combining this value with the mean observed[9]

Table 1. APPARENT VISUAL DISTANCE MODULI OF LOCAL GROUP GALAXIES*

Method	LMC	SMC	M 31	M 33	NGC 6822	IC 1613
Cepheids	19·0 ± 0·10	19·52 ± 0·10	24·66 ± 0·13	24·64 ± 0·14	24·78 ± 0·2	24·82
Novae	19·39 ± 0·4	19·42 ± 0·15	24·49 ± 0·12	—	—	—
RR Lyrae stars	18·65 ± 0·2	19·10 ± 0·2	—	—	—	—
W Virginis stars	—	19·31 ± 0·15	24·74 ± 0·10	—	—	—
Pop. II giants	18·37 ± 0·5	19·05 ± 0·5	24·40 ± 0·3	—	—	—
Globular clusters	—	—	23·9 ± 0·1	—	—	—
MK classification	18·70 ± 0·3	—	—	—	—	—
Hβ + Hγ	18·20 ± 0·4	18·49 ± 0·3	—	—	—	—
$\langle m-M \rangle_v$	18·84 ± 0·17	19·24 ± 0·14	24·50 ± 0·13	24·64	24·78	24·82
V	0·1	2·3	3·5	5·7	8·5	9·6
M_v	−18·7	−16·9	−21·0	−18·9	−16·3	−15·2
A_v (foreground)	0·18	0·06	0·33	0·09	0·57	0·09

* Errors quoted for each method are internal mean errors.

radial velocity of the Virgo cluster, $< V > = 1{,}175 \pm 64$ km s^{-1}, yields a value of 5 log $(H/100) = -0{\cdot}7 \pm 0{\cdot}4$. This corresponds to $H = 72^{+15}_{-12}$ km s^{-1} Mpc^{-1}. This value depends critically on the assumption that the brightest globular clusters in giant spiral and giant elliptical galaxies have exactly the same absolute luminosity.

Mass-to-light Ratios

Recently, Roberts[10] and Vorontsov-Velyaminov[11] have discovered that the mass-to-light ratios of spiral and irregular galaxies do not depend on their Hubble type. Furthermore, Roberts's data show no clear-cut correlation between the luminosity and the mass-to-light ratio of galaxies. This result implies that the extra-galactic distance scale may be obtained by comparing the mass-to-light ratios of distant galaxies with the presumably rather well known mass-to-light ratios of Local Group members. The mass of a galaxy, as determined from its rotation curve, is proportional to the assumed distance D whereas the absolute luminosity of a galaxy is proportional to D^2 so that $\mathcal{M}/L \sim D^{-1} \sim H$. Reducing Roberts's data to the distance scale in Table 1 yields $< \log(\mathcal{M}/L) > = 0{\cdot}63 \pm 0{\cdot}11$ for the spiral and irregular galaxies in the Local Group. Substitution of this value into $< \log(\mathcal{M}/L) > - \log(H/100) = 0{\cdot}61 \pm 0{\cdot}05$, which is obtained from Roberts's data for sixty galaxies with distances smaller than four Mpc, yields 5 log $(H/100) = +0{\cdot}1 \pm 0{\cdot}6$. This value corresponds to $H = 105^{+33}_{-26}$ km s^{-1} Mpc^{-1}.

Third Brightest Cluster Galaxy

A very rough estimate of the value of the Hubble constant can be obtained by comparing M 33, which is the third brightest member of the Local Group, with the third brightest galaxies in other small clusters of galaxies. From inspection of the Palomar Sky Survey it is found that the small clusters associated with NGC 3227, NGC 3368 NGC 3627 and NGC 4274 probably resemble the Local Group rather closely. Comparison of the third brightest members of these small clusters with M 33, for which $M_B = -18{\cdot}2 \pm 0{\cdot}5$, yields 5 log $(H/100) = +0{\cdot}5 \pm 0{\cdot}7$ which corresponds to $H = 126^{+48}_{-35}$ km s^{-1} Mpc^{-1}. It should be emphasized that this value is based on the unproven assumption that all small clusters of galaxies have similar luminosity functions. Furthermore, this determination of H involves a subjective choice of clusters which are believed to be similar to the Local Group.

Supernovae and the Extra-galactic Distance Scale

Distant supernovae can be compared directly with the supernovae in our own galaxy without using the members of the Local Group as intermediate stepping stones. Unfortunately, little is known about the intrinsic dispersion in the absolute magnitudes of different types of supernovae at maximum light. Furthermore, only fragmentary information is available on most of the supernovae that are known to have occurred in the galaxy during the last 1,000 years.

Table 2. COLOUR OBSERVATIONS OF TYCHO'S SUPERNOVA

Date	Colour	Adopted $B-V$	$(B-V)_0$	A_V
Nov. 1572	Like Venus ($B-V = 0{\cdot}83$) and Jupiter ($B-V = 0{\cdot}82$)	0·82	$0{\cdot}3 \pm 0{\cdot}1$	1·64
Jan. 1573	Similar to Mars ($B-V = 1{\cdot}36$) and Aldebaran ($B-V = 1{\cdot}52$)	1·44	$1{\cdot}0 \pm 0{\cdot}1$	1·48
Apr. 1573	Similar to Saturn ($B-V = 1{\cdot}04$)	1·04	$0{\cdot}5 \pm 0{\cdot}1$	1·75

Neither the maximum apparent magnitude nor the distance to the supernova of AD 1006 in Lupus is accurately known. According to Minkowski[12] the Crab supernova of 1054, for which both the distance and the apparent magnitude at maximum light are reasonably well known, seems to have been an unusual object for which no extra-galactic counterpart is known with certainty. In the case of Kepler's supernova of 1604, both the apparent magnitude at maximum light and the reddening are known but the distance is not. Finally, the supernova[13] of 1667, which produced the radiosource Cassiopeia A, was not seen by seventeenth century observers because of excessive interstellar absorption. Only Tycho's supernova of 1572 was sufficiently well observed to permit a rough estimate of the extra-galactic distance scale. From a definitive study Baade[14] obtained $V = -4{\cdot}0 \pm 0{\cdot}3$ for the maximum apparent magnitude of a Tycho's supernova. The reddening suffered by this supernova is surprisingly well determined from the colour estimates given by sixteenth century observers. A comparison of the observed colours with Mihalas's[15] intrinsic colours of type I supernovae is given in Table 2. The data in the table give $A_V = 1{\cdot}62$ with a formal mean error of about 0·1 magnitudes. Adopting this value for the absorption yields V_0 (max) $= -5{\cdot}62 \pm 0{\cdot}32$. From a comparison of observations of 21 cm absorption lines with rotation models of the galaxy (ref. 16 and unpublished work of Minkowski) the distance of the supernova is found to be greater than 3·0 kpc but probably less than 3·5 kpc, that is $(m-M)_0 = 12{\cdot}56 \pm 0{\cdot}16$. Combining this with the value with V_0 (max) $= -5{\cdot}62 \pm 0{\cdot}32$ yields M_V(max) $= -18{\cdot}18 \pm 0{\cdot}36$. Comparison of this value with the mean absolute magnitude of type I supernovae given by Kowal[17,28] yields 5 log $(H/100) = +0{\cdot}45 \pm 0{\cdot}7$, which corresponds to $H = 123^{+47}_{-34}$ km s^{-1} Mpc^{-1}. This estimate, which is based on ancient observations of a single supernova, is of course highly uncertain.

Surface Brightness and Diameter of Galaxies

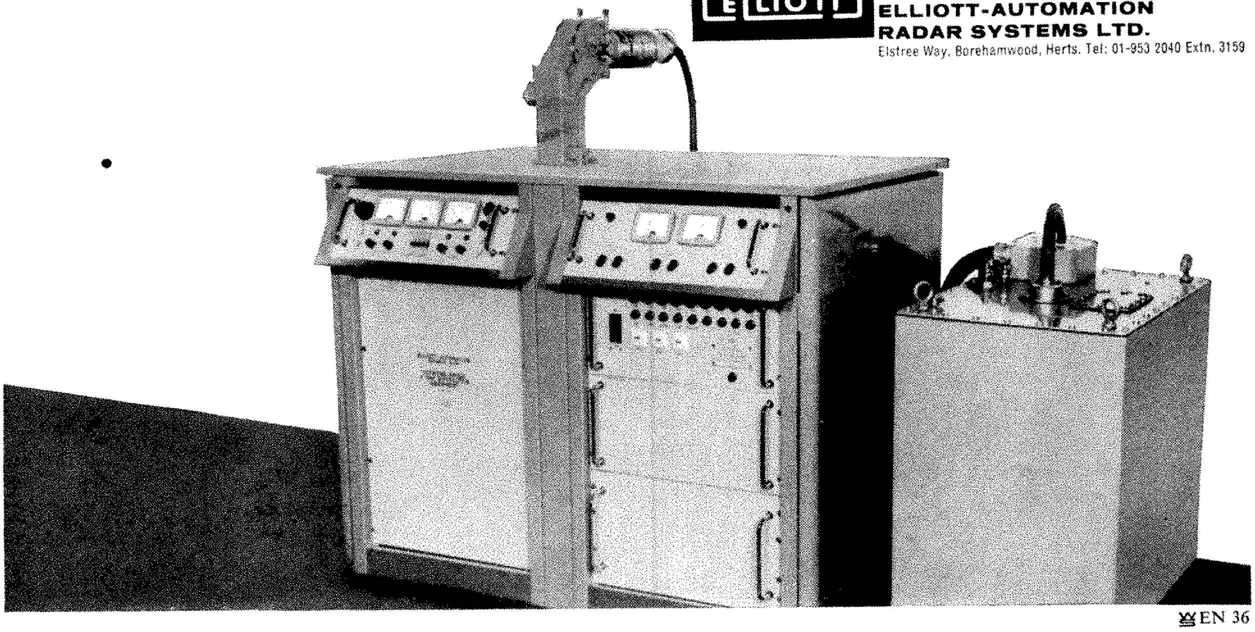
Holmberg[18] has shown that the surface brightness of spiral galaxies correlates with their intrinsic luminosity. In the mean, bright galaxies have a higher surface brightness than do faint galaxies. Intercomparison of the surface brightnesses of M 31 and M 33 (both corrected for galactic intersteller reddening) and the surface brightnesses of galaxies in the Virgo cluster (for which $< V > = 1{,}175 \pm 64$ is assumed) yields 5 log $(H/100) = -0{\cdot}15 \pm 0{\cdot}9$. This corresponds to $H = 93^{+48}_{-31}$ km s^{-1} Mpc^{-1}. The rather large mean error of this result is due to the large cosmic dispersion in the relation between the surface brightness and absolute magnitude of galaxies.

It is well known that intrinsically bright galaxies have larger diameters than do faint galaxies. Heidmann[19] has shown that the relation between the luminosity and diameter of galaxies may be used to estimate the Hubble constant. Comparison of Holmberg's[18] observations of galaxies in the Virgo cluster with the known absolute magnitudes and linear diameters of M 31, M 33, the Large Magellanic Cloud and Small Magellanic Cloud yields 5 log $(H/100) = -0{\cdot}25 \pm 0{\cdot}9$, which corresponds to $H = 89^{+46}_{-30}$ km s^{-1} Mpc^{-1}. The Local Group dwarf NGC 6822 has not been included in this comparison because extrapolation of its diameter to zero reddening is both large and uncertain. The dwarf galaxy IC 1613 was excluded because the validity of the relation between log diameter and m_{pv} in the Virgo cluster has not been established for such intrinsically faint galaxies.

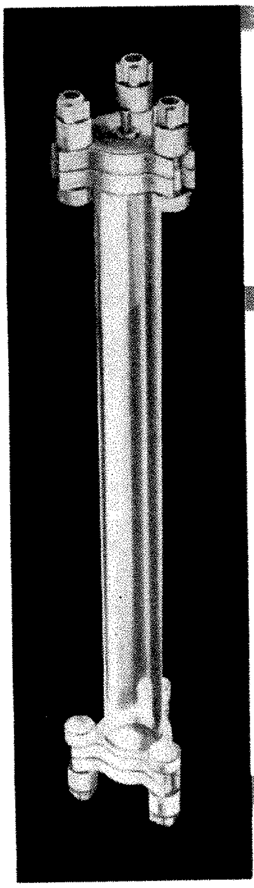
Elliott's GX3 rotating anode X-ray diffraction equipment includes an exclusive fine focus cathode system with a rotating anode of high power loading capability. 7 kW/mm^2 (actual area) is available with one thousand hours filament life. The plant includes the rotating anode tube mounted on a robust console which houses the vacuum system, cooling system and all controls. Tube current and voltage are stabilised within ±0·1%. Focal spot sizes are easily interchanged without disturbing the filament and advanced long-life seal design ensures trouble-free operation from the dynamically balanced anode.

ELLIOTT

Full literature available from:
The Neutron Division,
ELLIOTT-AUTOMATION
RADAR SYSTEMS LTD.
Elstree Way, Borehamwood, Herts. Tel: 01-953 2040 Extn. 3159



EN 36



wright

ACRYLIC chromatography columns

versatile inexpensive laboratory columns for chromatographic and gel filtration procedures employing aqueous solvents

Simple to use—unique patented design in acrylic and nylon with sintered plastics bed support.
Minimal dead space with low-volume connections. Diameters selected to produce cross-sectional area of 2, 4, 8, & 16 sq. cms.
Fixed or adjustable columns with or without jackets.
Standard lengths of 30, 60 and 90 cms.
Columns easily lengthened by joining.
Prompt delivery of standard columns from stock.
Preparative columns with bed volumes up to 50 litres are available to special order

Prices from **£5**

Phone or write for detailed catalogue to Dept. CC1/N

wright SCIENTIFIC LIMITED 3 Lower Road, Kenley, Surrey, CR2 5NH *Tel:* 01-668 4611

Cambridge Journals

Experimental Agriculture

Edited by Professor
J. P. HUDSON, *Wrington, Bristol*

This journal publishes the results of experimental work in animal and crop husbandry, with particular emphasis on problems in tropical countries. It includes accounts of new experimental techniques, and review articles on new developments in scientific agriculture.
Annual subscription (four issues) £5 ($16.50 in the USA). Single parts 35s ($5.00 in the USA).

Archaeometry

Edited by Mrs J. REID,
Richmond
Managing Editors:
Dr M. J. AITKEN and
Dr E. T. HALL, *Oxford*

Archaeometry is the only research journal devoted to applications of the natural sciences in archaeology. It includes work on dating methods, locating devices, archaeo-magnetic measurements, and analyses of coins and other artefacts.
Annual subscription (two issues) £3 ($9.50 in the USA). Single parts 40s ($6.00 in the USA).

The Journal of Hygiene

Edited by Dr R. M. FRY,
Department of Pathology, Cambridge

The Journal of Hygiene is concerned with bacterial and virus diseases of man and animals, and particularly with the identification of pathogens, epidemiology, and immunology.
Annual subscription (four issues) £7 ($23.00 in the USA). Single parts 40s ($6.50 in the USA).

The British Journal of Social and Clinical Psychology

Edited by Professor
THELMA VENESS
University of London Institute
and Dr J. G. INGHAM
Llandough Hospital, Penarth

Published for the British Psychological Society, the journal contains articles and book reviews on social psychology, criminology, psychotherapy, personality measurement and related topics.
Annual subscription (four issues) £6.10.0 ($22.00 in the USA). Single parts 45s ($7.50 in the USA).

Please write for further information and specimen copies of these, or any other journals published by the Press. Details of available back issues will also be sent on request.

CAMBRIDGE UNIVERSITY PRESS
P.O. Box 92, London, N.W.1
American Branch: 32 East 57th Street, New York, N.Y. 10022

Brightest Stars in Galaxies

For galaxies with redshifts smaller than about 1,500 km s^{-1} the brightest individual stars may be used as distance indicators. In 1953 Hubble and Sandage[20] suggested that the brightest blue variables in galaxies might prove to be useful in this way. Recent observations by Tammann and Sandage[21], however, indicate that these blue variables may have a considerable range in luminosity at maximum light. In fact it seems quite probable that the brightest blue irregular variables form a continuum which bridges the gap between the brightest stable main sequence stars and supernovae of type II.

Table 3. SUMMARY OF DATA ON THE HUBBLE CONSTANT

Method	5 log (H/100)
Diameters of HII regions	-0.2 ± 0.4
Luminosity classifications of galaxies	-0.05 ± 0.4
Brightest globular clusters in galaxies	-0.7 ± 0.4
Mass-to-light ratios of galaxies	$+0.1 \pm 0.6$
Third brightest member of small clusters	$+0.5 \pm 0.7$
Tycho's supernova	$+0.45 \pm 0.7$
Surface brightness of galaxies	-0.15 ± 0.9
Diameters of galaxies	-0.25 ± 0.9
Brightest stars in galaxies	$+0.1 \pm 1.0$
Adopted mean*	-0.1 ± 0.3

* Corresponds to $H = 95^{+15}_{-12}$ km s^{-1} Mpc^{-1}.

The brightest non-variable stars of Population I may be used as distance indicators after allowance has been made for the fact that the absolute magnitudes of these stars depend on both nebular type and on total Population I content. In practice additional difficulties arise because bright star clusters cannot be distinguished from individual stars of high luminosity. Furthermore, the bright end of the steller luminosity function is thinly populated with stars so that the statistical separation of truly extragalactic stars of high luminosity from foreground field stars is uncertain. Determinations of the absolute magnitudes of the brightest stars in the members of the Virgo cluster are at present in a thoroughly unsatisfactory state. Perhaps the best guess that can be made from a comparison of the brightest stars in the Virgo cluster with the brightest stars in M 31 and M 33 is that $(m-M)_{pg} = 30.25 \pm 1.0$ for the Virgo cluster. The assumption of zero reddening and a mean cluster velocity $<V> = 1,175 \pm 64$ km s^{-1} Mpc^{-1} yields $5 \log (H/100) = +0.1 \pm 1.0$. The corresponding value of the Hubble constant is $H = 105_{+51}$ km s^{-1} Mpc^{-1}. The uncertainty of this value could be significantly reduced by new photoelectrically calibrated measurements of the brightest stars in Virgo cluster galaxies.

Tammann and Sandage[21] have recently suggested that the brightest red stars of Population I might be useful distance indicators. The possibility that the absolute luminosity of such highly evolved stars might be very sensitive to the effects of chemical composition[22,23] should, however, be kept in mind.

The results of all nine methods of distance determination which are currently available have been summarized in Table 3. Assigning weights that are inversely proportional to the squares of the mean errors quoted for each method yields $5 \log (H/100) = -0.14 \pm 0.19$.

The nine methods of distance determination listed in Table 3 are not entirely independent. This is so because all methods, except that based on supernovae, depend on the distance scale within the Local Group. This distance scale is particularly sensitive to the zero point that is adopted for the Cepheid period-luminosity relation. For example, making all Cepheids brighter by 0·2 magnitudes would change H from 95 to 100 km s^{-1} Mpc^{-1}. A change of 10 per cent in the adopted distance to the Virgo cluster would change the Hubble constant by approximately 5 per cent. A final value of $5 \log (H/100) = -0.1 \pm 0.3$, which corresponds to $H = 95^{+15}_{-12}$, will be adopted. The quoted mean error makes allowance for the interdependence of the nine methods of distance determination listed in Table 3.

Regional Variations of the Hubble Constant

Available data on the distances and velocities of galaxies[24] suggest that individual galaxies have random velocities of the order of 200–300 km s^{-1}. The velocity of the galaxy relative to the absolute reference frame provided by the 3° K microwave radiation[25] is also of this order. The value of the Hubble constant determined here is particularly sensitive to possible random motions of clusters of galaxies in the vicinity of the Local Group, that is the locally determined value of H may not be representative of the universe as a whole.

Recently, Sandage[26] has shown that the absolute magnitude of the brightest member of rich clusters of galaxies exhibits a dispersion of only 0·3 magnitudes. A comparison of NGC 4472, which is the brightest galaxy in the Virgo cluster, with Sandage's data on the brightest galaxies in distant clusters yields $H(0)/H(\infty) = 1.04 \pm 0.15$ in which $H(0)$ is the local value of the Hubble constant and $H(\infty)$ is the Hubble constant beyond the Local Supercluster. Taken at face value this result indicates that there is no systematic difference between the Hubble flow inside and outside the Local Supercluster. Similar results, of rather lower accuracy, are obtained by Kowal[17,28] from a comparison of supernovae in the Virgo cluster with more distant supernovae. A rather different conclusion, however, is obtained by Abell and Eastmond[27], who compared the galaxian luminosity function of the Virgo cluster with the luminosity functions of the Coma and Corona Borealis clusters. Additional observations comparing the expansion of nearby space with the Hubble flow in more distant regions of the universe are clearly available.

I thank Dr Allan Sandage for several stimulating discussions on the time scale of creation.

[1] Bergh, S. van den, *Galaxies and the Universe* (edit. by Sandage, A. and M.) (Univ. Chicago Press, 1970).

[2] Sérsic, J. L., *Zs. f. Astrophys.*, **50**, 168 (1960).

[3] Bergh, S. van den, *Publ. David Dunlap Obs.*, **2**, 159 (1960).

[4] Bergh, S. van den, *Astrophys. J.*, **131**, 215 (1960).

[5] Bergh, S. van den, *Astrophys. J.*, **131**, 558 (1960).

[6] Sandage, A. R., *Astrophys. J. Lett.*, **152**, L149 (1968).

[7] Gascoigne, S. C. B., and Burr, E. J., *Mon. Not. Roy. Astron. Soc.*, **116**, 570 (1956).

[8] Racine, R., *J. Roy. Astron. Soc. Canada*, **62**, 367 (1968).

[9] Bergh, S. van den, *Mon. Not. Roy. Astron. Soc.*, **121**, 387 (1960).

[10] Roberts, M. S., *Astron. J.*, **74**, 859 (1969).

[11] Vorontsov-Velyaminov, *Astron. Circ. USSR*, No. 534 (1969).

[12] Minkowski, R., *Astron. J.*, **71**, 371 (1966).

[13] Bergh, S. van den, and Dodd, W. W., *Nature*, **223**, 814 (1969).

[14] Baade, W., *Astrophys. J.*, **102**, 309 (1945).

[15] Mihalas, D., *Pub. Astron. Soc. Pacific*, **75**, 256 (1963).

[16] Menon, T. K., and Williams, D. R. W., *Astron. J.*, **71**, 392 (1966).

[17] Kowal, C. T., *Astron. J.*, **73**, 1021 (1968).

[18] Holmberg, E., *Lund Obs. Medd., Ser. 2*, No. 136 (1958).

[19] Heidmann, J., *CR Acad. Sci.*, **268**B, 1782 (1969).

[20] Hubble, E. P., and Sandage, A. R., *Astrophys. J.*, **118**, 353 (1953).

[21] Tammann, G. A., and Sandage, A. R., *Astrophys. J.*, **151**, 825 (1968).

[22] Hofmeister, E., *Zs. f. Astrophys.*, **65**, 194 (1967).

[23] Schlesinger, B. M., *Astrophys. J.*, **157**, 533 (1969).

[24] Humason, M. L., Mayall, N. U., and Sandage, A. R., *Astron. J.*, **61**, 97 (1956).

[25] Partridge, R. B., *Amer. Scientist*, **57**, No. 1, 37 (1969).

[26] Sandage, A. R., *Observatory*, **88**, 91 (1968).

[27] Abell, G. O., and Eastmond, S., *Astron. J.*, **73**, S161 (1968).

[28] Kowal, C. T., *Pub. Astron. Soc. Pacific*, **81**, 608 (1969).

●

Career Choices by Science Studies Students

by
D. O. EDGE
Science Studies Unit,
University of Edinburgh,
34 Buccleuch Place,
Edinburgh EH8 9JT

Graduates who took science studies at Edinburgh are more likely to choose careers in industry and commerce than specialist science students. The decision to take a course of this type may reflect a prior career decision.

THE Faculty of Science in the University of Edinburgh initiated in October 1967 a one-year course in science studies as an option in the curriculum of science students. It is an introduction to some of the social and intellectual implications of the rise of modern science and technology; the material is selected from studies in history, philosophy, sociology and political science, and the topics are related so as to explore the social nature of science (the course is described in the Science Studies Unit's First Report, 1966–69). The course is usually taken in the student's second or third year (the honours year in Scotland is the fourth), and occupies half the time for that year. It is a "generalist" course, in the sense of the Swann Report when it mentions "interweaving several diverse disciplines to show their inter-relationships and relevance . . ."[1]. Some information about the career choices of those who take this course is now available.

Table 1. CAREER CHOICES OF GRADUATES

Group	Category *a*	 *b*	 *c*	 *d*
Science studies group	12	4*	4	4
Reference group	5	12	1	6†

* If PhD in clinical psychology included, add 1.

† Includes one graduate doing teaching temporarily, while trying to make a career in journalism.

Of the fifty-five students who registered for the initial course in 1967/68, twenty-three were in their third year at university and were intending to go on to honours in science (the honours schools involved were botany, chemistry, geography, geology, physics and psychology); a further two students were in the second year of an ordinary (3 year) degree course. Twenty-five students were therefore due to graduate in the summer of 1969. We have attempted to compare the career choices of these students with a reference group of science students who, in October 1967, had similar degree intentions but did not choose to take the science studies course nor any of the other "complementary" courses then available (history of science or economics). The reference group was matched to the science studies group within each honours school by number and sex, individuals being selected at random from the relevant pools of students not taking science studies.

Students in both groups were asked to state their plans for 1969/70 as soon as these were known. They were also asked about their long term career plans (if any), to find out what careers they had in mind, and how firmly they were committed to these plans. Members of the science studies group were also asked whether the science studies course had any influence on their career decisions; responses to this question were coded from *A* (crucially) to *E* (not at all).

From the results of the questionnaire, the "immediate destinations" of the graduates can be grouped into the following broad categories. (*a*) Those going directly into industrial or commercial employment, or undergoing short term training explicitly designed for such employment. Because the numbers involved are small, this includes those going into government employment (an administrative post with the Science Research Council, a scientific officer at the Ministry of Defence, an experimental officer with the Ministry of Overseas Development in Malawi, and a physicist in the British Antarctic Survey—two of these were from the science studies group, and two from the reference group). It also includes two graduates emigrating to Australia in search of professional scientific jobs: one a geologist (science studies), and one a chemist (reference); and a chemist (reference) who is abandoning chemistry to study accountancy and computer techniques for an industrial career. (*b*) Those going into postgraduate training which could lead to an academic career, whether or not that is their expressed long term preference. (*c*) Those preparing for clinical or social work. This category includes one graduate (science studies) who is studying for a PhD in clinical psychology and could be classified under (*b*) although his long term career preference is not academic. (*d*) Those going into school-teaching, or teacher training.

The destination of one science studies student is unknown. The matched figures are shown in Table 1.

Twenty-four students in the science studies group ventured subjective estimates of the influence of the course on their career choices. The distribution of these estimates is given in Table 2.

Table 1 shows that science students who choose to take, and who successfully complete, the science studies course are more likely to proceed directly into industrial and commercial employment and less likely to pursue an academic career than their strictly specialist counterparts. Table 2 suggests that this tendency might be, at least in part, a result of the course itself. These subjective estimates are, however, likely to over-stress the effect of the course: there is evidence that many respondents were "eager to please". Moreover, further elaboration of these figures exposes a more complex situation.

Graduates in category (*a*) can be subdivided into (i) those going to practise as scientists in manufacturing industry (including oil and chemical industries), (ii) those going to practise as scientists elsewhere (for example, in government service), (iii) those going into technical and production management, and (iv) those going into general administration, personnel management, marketing, cost accountancy and so on.

Table 2. SUBJECTIVE ESTIMATE OF INFLUENCE OF COURSE ON CAREER DECISION

	Class				
	A	*B*	*C*	*D*	*E*
No. of responses	4	4	4	6	6

Table 3 shows the results of this subdivision, together with the subjective estimates of course influence. It seems that, if the science studies course has any influence on career choice, it serves chiefly to deflect scientists, not into the practice of science elsewhere, but into the general field of management. Those embarking on a career as "applied scientists" tend to give low estimates of the course's influence. Their replies indicate that many of them had made their career decision before starting the course, and that they chose to take the course because of its likely relevance to that career.

Table 3. DESTINATION OF GRADUATES IN CATEGORY (*a*)

Destination subdivision	Reference group	Science studies group
(i)	1	4 (1*A* †, 2*D*, 1*E*)
(ii)	3*	3* (1*C*, 2*E*)
(iii)	0	1 (1*E*)
(iv)	1	4 (1*A*, 3*B*)

* Includes one emigrant to Australia.

† This graduate expressed a long term commitment to abandon science for personnel management.

Of the twelve graduates in the reference group in category (*b*), seven expressed a long term preference for an academic career, two for a non-academic career and three were undecided. Of the five science studies graduates who could be included in this category, two (one *D*, one *E*) had non-academic aims. Only one had chosen an academic career; he assessed himself to have been "considerably" influenced by the course, and it is known that he gave very long and careful thought to his decision.

The distribution of estimates of influence by the science studies students in the other two categories is given in Table 4.

Table 4. ESTIMATES OF GRADUATES IN CATEGORIES (*c*) AND (*d*)

	Estimate				
Category	*A*	*B*	*C*	*D*	*E*
Social and clinical	1	0	1	1	1
Teaching	0	0	2	1	1

Returning to a consideration of the whole sample, one important difference between the two groups might be found in their eventual academic attainments, as measured by their degrees and final honours classes. This may partly account for the differences expressed in Table 1: those who do not obtain good second class degrees are not offered the chance of postgraduate work, eventually leading to an academic career, and they must decide otherwise.

Of the twenty-three students in the science studies sample who originally intended to take honours degrees, eleven did not proceed to honours and graduated with an ordinary degree in the summer of 1968; the corresponding figure in the reference group is five. Failure to proceed to honours can result from academic failure, but it can also be a deliberate choice by the student. Of the eleven science studies ordinary graduates, it would seem that at least four (one *D*, three *E*) were taking a "short cut" to a career already decided on; one (*E*) was the result of serious illness; and not more than three (one *A*, two *B*) were deliberate choices to switch from honours science (to management, in all three cases).

(The notion of "deliberate choice" is, of course, problematical. It may arise from the genuine perception of a more desirable career; or it may be merely an honest, if perhaps unconscious, estimate of the likely honours class. The two are not unrelated. An able student may be influenced by devoting less time to his principal science subject; this will be reflected in his ongoing performance and so will alter his assessment of his likely degree class. This may lead to a decreasing interest in honours science, a resulting increasing interest in something else, and, finally, a "deliberate choice". Neither level of interest, nor expectation of degree class, is necessarily a measure of intrinsic ability.)

Twelve science studies students and eighteen students in the reference group proceeded to honours. Any significant difference between these groups could be detected only by dividing the second class honours degree, but this is not the practice at Edinburgh. Some information made available to the author suggests, however, that the science studies group may have contained a relatively smaller percentage of good second class degrees. The difference is not significant, but it is not inconsistent with the suggestion that career decisions and the related decision to take the science studies course may reflect the student's assessment of his likely degree class.

This small sample and informal survey therefore seems to provide some evidence both for the suggestion that the decision to take a generalist course of this type reflects a prior career decision, and that such a course helps to influence (perhaps to "crystallize") career decisions. More detailed research into career choice by science students is being conducted by Mr S. B. Barnes in the Science Studies Unit.

I thank Mr D. C. Bloor, Mr S. B. Barnes and Mr L. E. Schwartz for helping to create the science studies course, and the Wolfson Foundation, whose grant led to the foundation of the Science Studies Unit.

---

[1] *The Flow into Employment of Scientists, Engineers and Technologists*, 76 (Cmnd 3760, September 1968).

Complementary Oligonucleotide Binding to the Anticodon Loop of fMet-transfer RNA

by

OLKE C. UHLENBECK*
JULIE BALLER†
PAUL DOTY

Department of Biochemistry and
Molecular Biology,
Harvard University, Cambridge, Massachusetts

Exposed, unpaired areas of an RNA molecule will bind complementary tri and tetranucleotides. The binding can be measured by dialysis equilibrium methods and if the RNA sequence is known the conformation of the molecule can be mapped. The method is here applied to the anticodon loop of formylmethionine tRNA.

STUDIES of the stability of helical complexes formed from short self-complementary oligonucleotides (O. C. U., F. H. Martin and P. D., manuscript in preparation) suggest that oligonucleotides of only three or four units should interact with available complementary regions of an RNA molecule to such a degree that the association constant could be readily measured by conventional dialysis equilibrium. With the use of suitable controls and a variety of oligonucleotides it should therefore be possible to identify the open, unshielded and unpaired regions of an RNA molecule of known sequence. We report here the development of the methodology and its use in defining which bases are available in the anticodon loop of $\mathrm{tRNA}^{\mathrm{Met}}_{\mathrm{f}}$. The article which follows this demonstrates the extension of the method to the mapping of the whole length of 5S RNA[1]. It is apparent that this method cannot be used to detect available sequences shorter than its shortest probe, three nucleotides. In this way it complements chemical methods which generally recognize single unshielded bases. It must also be expected that sequences held in a rigid single-stranded conformation incompatible with bihelical conformations will not be identified. Thus the available sequences identified may be somewhat less than the total not in bihelical regions.

The anticodon loop of transfer RNAs of known sequence provides a useful prototype for evaluating the techniques of oligomer binding. The reactivity of this loop to reagents specific for single stranded residues[2,3] and ribonucleases[4] and the absence of complementary sequences prove that this loop is not involved in secondary or tertiary structure. Our concern is to explore the availability of the bases within the loop, and adjacent to it, for helix formation.

A study of the interaction of oligomers with tRNA can also be useful in determining whether the properties of the codon–anticodon interaction are the same as those observed for tRNAs and oligomers interacting in the presence of ribosomes. In particular, $\mathrm{tRNA}^{\mathrm{Met}}_{\mathrm{f}}$ has been found to bind to ribosomes in the presence of either AUG or GUG[5]. This ambiguity could be explained by hypothesizing a G·U base pair at the 5′ position of the codon rather than at the 3′ position[6]. Experiments in the absence of ribosomes would thus determine whether the observed relaxation of the specificity of base pairing is the result of the particular environment supplied by the ribosome or is an inherent property of the tRNA–codon interaction.

Preparation of Tri and Tetranucleotides and $\mathrm{tRNA}^{\mathrm{Met}}_{\mathrm{f}}$

Nucleoside diphosphates (NDP) of high specific activity were added to di and trinucleotides with primer dependent polynucleotide phosphorylase isolated from *Micrococcus luteus*[7]. Typical reaction conditions for the synthesis of a trimer XpYpN were: 0·1–1·0 mM ^{3}H-NDP (1–20 Ci/mmole, Schwartz), 6 mM XpY (Sigma), 10 mM $MgCl_2$, 0·4 M NaCl, 0·2 M glycine buffer (*p*H 9·3) and 0·3 mg/ml. polynucleotide phosphorylase. After 4 h at 34° C, the reaction was terminated by boiling and 0·1 mg/ml. alkaline phosphatase was added and reacted for an additional hour to degrade unreacted NDP. The products were separated from the reactants by descending paper chromatography as described by Thach[8]. Because the NDP concentration was much less than that of the primer, the major product was XpYpN, although smaller amounts of the tetramer, XpYpNpN, were also obtained. Usually 50 to 90 per cent of the NDP was incorporated into product molecules.

The synthesis of tetramers followed along similar lines: 0·1–0·3 mM NDP, 1 mM XpYpZ, 10 mM $MgCl_2$, 0·4 M NaCl, 0·2 M glycine buffer (*p*H 9·3) and 0·2 mg/ml. polynucleotide phosphorylase were reacted for 2 h to give 5 to 30 per cent of the NDP incorporated into XpYpZpN.

The $\mathrm{tRNA}^{\mathrm{Met}}_{\mathrm{f}}$ was provided by Oak Ridge National Laboratory, and has been assayed to have 100 per cent methionine and formyl accepting activity. The nucleotide sequence of *Escherichia coli* $\mathrm{tRNA}^{\mathrm{Met}}_{\mathrm{f}}$ has been recently elucidated[9].

Detection of Oligomer-tRNA Interaction

Dialysis was carried out in chambers made from a pair of 2·5 cm 'Plexiglas' acrylic plastic disks with a shallow cylindrical depression cut into each (1·8 × 12 mm). A membrane cut from dialysis tubing was placed between the disks to form two chambers each having a volume of about 200 µl. A narrow hole at the top of each disk allowed access to each side.

Experiments were carried out by loading a concentrated (80 µM) solution of tRNA on one side and 0·3–0·8 µCi of an oligomer on the other and allowing the system to come to equilibrium at 0° C. In 1·0 M NaCl, 10 mM $MgCl_2$ and 10 mM Na_2HPO_4 (*p*H 7·0), the buffer used in all these experiments, equilibration was reached in 24 h for trimers and 48 h for tetramers. After equilibrium, 3 µl. samples were withdrawn in triplicate from each side and counted.

If K is the association constant of an oligomer to a single complementary site on the tRNA molecule, and R is the ratio of oligomer counts in the chamber with the tRNA to the chamber without, then the relationship $R = 1 + K[\mathrm{RNA}]$ will hold as long as the molar RNA concentration greatly exceeds the molar oligomer concentration[10]. This requirement is met in these experiments because the RNA concentration is 40–80 µM and the oligomer concentration is 0·1–1·0 µM. (In these conditions the value of R is independent of the actual oligomer concentration, so that K values can be compared with oligomers of different specific activity.)

Interaction of Anticodon Loop with Oligomers

The association constants of the codon triplet, AUG, and a number of related oligomers with $\mathrm{tRNA}^{\mathrm{Met}}_{\mathrm{f}}$ at 0° C, are listed in Table 1. In interpreting these values it is important to note that association constants of less than about 250 correspond to R values, in the conditions of our experiments, of less than 1·02. Such values are taken to indicate no binding for the oligonucleotides used. It

---

* Present address: Department of Chemistry, University of California, Berkeley, California.

† Present address: Harvard Medical School, Boston, Massachusetts.

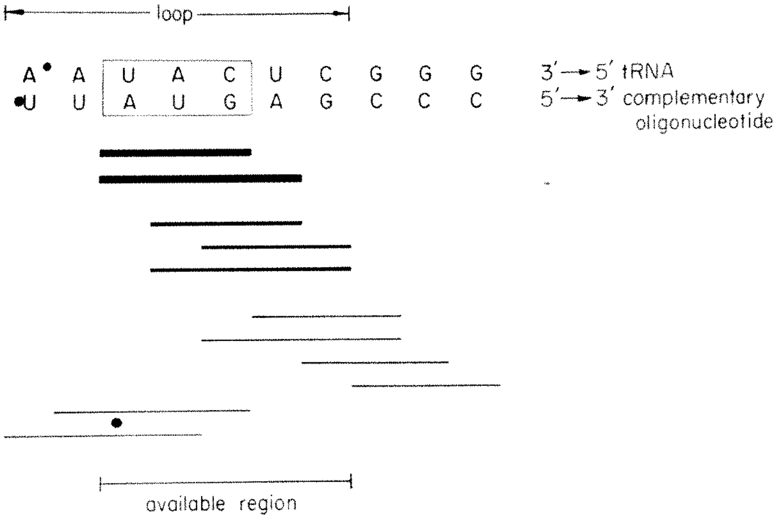


Fig. 1. The segment of *E. coli* fMet-tRNA investigated using complementary oligonucleotides. A heavy line indicates that the corresponding oligomer was bound; a thin line that it was not. A line of intermediate thickness indicates those oligomers whose binding constants were judged to be intermediate. The region of the anticodon loop found to be available for oligonucleotide binding is shown.

is likely that trinucleotides containing only A and U probably do have association constants in this range, but we have found it possible to avoid using this limited class.

Turning to Table 1, it is seen that the association constant for AUG is 1,200 l./mole. On the basis of association constants of other trimers[1] this value seems to be at the lower end of the range expected for trinucleotides containing only one G or C base. And because the complement, CAU, occurs only in the anticodon region of this RNA, it would appear that we are observing codon–anticodon association. Two sequence isomers were examined next: GAU and UAG. Neither was bound significantly. The complement to the first occurs once elsewhere but with one of its bases presumably in one of the helical regions. The complement of the other does not occur. Thus the lack of binding is understandable. The third sequence isomer, UGA, has an association constant of 400. This isomer has its complement in a triplet shifted one base from the anticodon; yet the *K* value is so low as to be ambiguous and is termed intermediate.

Table 1. MOLAR ASSOCIATION CONSTANTS OF OLIGOMERS WITH fMet-tRNA

Oligomer	*K*
AUG	1,200 ± 200
GAU	150
UAG	150
UGA	400
AUGA	13,500
AUGU	1,400
AUGC	900
AUGG	1,000

K was measured in 1·0 M NaCl, 10 mM $MgCl_2$, 10 mM sodium phosphate buffer (*p*H 7·0) at 0° C.

Table 2. MOLAR ASSOCIATION CONSTANTS OF OLIGOMERS WITH fMet-tRNA

Oligomer	*K*
UGA	400 ± 200
GAG	1,000
UGAG	2,600
AGC	150
GAGC	1,100
GCC	0
CCC	0
AUG	1,200
UAUG	1,100
UUAU	400

To examine whether or not the U on the 5′ side of the anticodon could participate in binding, the tetramer AUGA was synthesized and its binding measured. Similar tetramers with the other three bases at the 3′ end were also examined as controls. The results listed in Table 1 show clearly that this U in the anticodon loop can bind because the *K* value for AUGA is found to be quite high, 13,500. The other three tetramers display *K* values of about 1,000: this is similar to the association constant for AUG alone. Thus the specificity of the AUGA binding is emphasized by this order of magnitude increase in the value of *K* against no enhancement when the fourth base is non-complementary.

To complete the exploration of the availability of bases in the anticodon loop, a number of other complementary oligomers were made and their binding was measured. The results are listed in Table 2 and displayed in Fig. 1. It is seen that intermediate binding occurs at the second as well as the first base on the 5′ side of the anticodon; that is, GAG and UGAG bind with values that are significant but less than that expected when two G·C pairs are possible. But the following base, G, seems to be quite unavailable: GAGC binds about the same as GAG and AGC; GCC and CCC do not bind. Exploration of the availability of the bases on the 3′ side of the anticodon was limited to two oligomers, UAUG and UUAU. Because neither bound, it seems that both the first A and the second A to the 3′ side of the anticodon are not available for binding. It is most unlikely that they are paired with any other bases, so it follows that they must be held in a strained configuration incompatible with helix formation with a complementary oligomer.

The oligomer binding data summarized in Fig. 1 indicate that the sequence of five bases CUCAU in the anticodon loop are sufficiently single stranded to be able to bind complementary oligomers. The remainder of the loop, as well as the helical region proposed to close the loop, is not capable of binding oligomers. These data seem inconsistent with the model of the anticodon loop proposed by Fuller and Hodgson[11]. If the two A residues on the 3′ side of the anticodon are stacked with the anticodon along the same helix axis as the helix closing the loop, it would be expected that these A residues would be available for base pairing. Similarly, if the U and C residues on the 5′ side of the anticodon were strained, it would be expected that they would not be able to bind oligomers. Because we find that the A residues are not available to bind oligomers and the U and C residues are available but perhaps only after overcoming a minor restraint, it seems that some other conformation of the anticodon loop is correct.

The Wobble Interaction of GUG with $\text{tRNA}^{\text{Met}}_{\text{f}}$

In order to examine the possibility of a wobble interaction at the 3′ end of the anticodon, GUG and a number of related oligomers were presented to $\text{tRNA}^{\text{Met}}_{\text{f}}$ and their association constants were determined (Table 3). The association constant of GUG with $\text{tRNA}^{\text{Met}}_{\text{f}}$ was found to be the same as that of AUG. Further, GUGA has a much higher *K* than GUGU, suggesting that GUG is binding to the anticodon. This can be confirmed by performing competition experiments. If a large amount of non-radioactive AUG is included in a dialysis experiment, a sufficient number of anticodons are covered and the observed value of *K* for the radioactive triplet is

Table 3. MOLAR ASSOCIATION CONSTANTS OF OLIGOMERS WITH fMet-tRNA

Oligomer	*K*
GUG	1,200 ± 200
GUGU	1,000
GUGA	9,800
UUGU	400
AUG	1,200
AUG + 1·1 mM AUG*	500
GUG + 1·1 mM AUG*	600
GUG + 1·1 mM AAG*	1,100

* Non-radioactive oligomer.

decreased for that site. The results (Table 3) show that in conditions where the binding constant for labelled AUG is halved by unlabelled AUG, the observed value of K for GUG is similarly decreased. The presence of a similar amount of non-radioactive AAG does not, however, affect the value of K for GUG. We can therefore conclude that AUG and GUG bind to the same site on $\text{tRNA}^{\text{Met}}_{\text{f}}$.

Because the G·U bond at the 5′ position of the codon is only observed with $\text{tRNA}^{\text{Met}}_{\text{f}}$, it has been suggested that this is a reflexion of the special interaction of the initiator tRNA with the ribosome[12]. This possibility does not seem to be the case, for GUG will bind to the anticodon in the absence of ribosomes. The fact that no G·U bond was observed with the other U in the anticodon loop implies that the ability of the tRNA to form a particular wobble bond with the messenger RNA is a consequence of the structure of the tRNA loop and not of the environment supplied by the ribosome.

The technique of oligomer binding has proved useful in examining the detailed structure of a fairly well defined RNA loop. It should also be useful in exploring the conformation of the rest of the $\text{tRNA}^{\text{Met}}_{\text{f}}$ molecule as well as that of other RNA molecules of known sequence.

We appreciate helpful discussions with J. B. Lewis. This work was supported by a US National Science Foundation grant and the award of a National Institutes of Health predoctoral fellowship to O. C. U.

Received December 12, 1969.

[1] Lewis, J. B., and Doty, P., *Nature*, **225**, 510 (1970) (following article).

[2] Brostoff, S. W., and Ingram, V. M., *Science*, **158**, 666 (1967).

[3] Cramer, F., Doepner, H., van de Haar, F., Schlimme, E., and Seidel, H., *Proc. US Nat. Acad. Sci.*, **61**, 1348 (1968).

[4] Nelson, J. A., Ristow, S. C., and Holley, R. W., *Biochim. Biophys. Acta*, **149**, 590 (1967).

[5] Clark, B. F. C., and Marcker, K. A., *J. Mol. Biol.*, **17**, 394 (1966).

[6] Crick, F. H. C., *J. Mol. Biol.*, **19**, 548 (1966).

[7] Thach, R. E., and Doty, P., *Science*, **148**, 632 (1965).

[8] Thach, R. E., *Procedures in Nucleic Acid Research* (edit. by Cantoni, G. L., and Davies, P. R.), 520 (Harper and Row, New York, 1966).

[9] Dube, S. K., Marcker, K. A., Clark, B. F. C., and Cory, S., *Nature*, **218**, 232 (1968).

[10] Edsall, J. T., and Wyman, J., *Biophysical Chemistry*, chap. 11 (Academic Press, New York, 1958).

[11] Fuller, W., and Hodgson, A., *Nature*, **215**, 817 (1967).

[12] Bretscher, M. S., *Nature*, **218**, 675 (1968).

Derivation of the Secondary Structure of 5S RNA from its Binding of Complementary Oligonucleotides

by
JAMES B. LEWIS
PAUL DOTY
Department of Chemistry,
Harvard University,
Cambridge, Massachusetts

Using the oligonucleotide binding procedure outlined in the previous article, the secondary structure of 5S RNA has been explored. Most of the unpaired sequences have been identified, although the findings are not compatible with any of the published models of 5S RNA.

INCREASING knowledge of the sequences of various small RNA molecules has prompted much recent work on the secondary and tertiary structure of RNA. For the transfer RNAs it has been possible to arrive at plausible secondary structures by inspecting their sequences and pairing the Watson–Crick complementary regions into helices and thereby forming the familiar cloverleaf configuration[1,2]. Various physical and chemical studies support this result. But for 5S RNA, although sequences are known for that from *Escherichia coli*[3] and that from KB cells[4], no such uniquely plausible secondary structure is evident. A greater interest has therefore developed in the various physical and chemical techniques which may indicate the extent of base pairing in 5S RNA, although so far the clues provided have not been sufficient to provide a unique model of the secondary structure of 5S RNA. (As used here, a model of the secondary structure means a specification of which regions of the molecule are base paired in a double stranded fashion. The further folding of the molecule to give a three dimensional structure will be referred to as tertiary structure.)

Hypochromicity measurements of *E. coli* 5S RNA indicate that 60 per cent of the residues are base paired[5]. Ultraviolet difference spectra and optical rotatory dispersion suggest that the molecule is 67 to 82 per cent base paired, 60 to 70 per cent of these base pairs being G·C (ref. 6).

Other data suggest more specifically which regions are base paired. Certain regions of the 5S RNA molecule were found to be resistant to ribonuclease, suggesting that they were double stranded[3]. Oxidation with monoperphthalic acid indicated that only ten A residues are unpaired[7]. Most of the bases are inaccessible to carbodiimide, but the U residues in two particular sequences are more exposed and available for reaction[8].

Several of the secondary structures that have been proposed for *E. coli* 5S RNA (refs. 1, 3, 5, 9, 10) meet the approximate requirements imposed by the spectral studies, but they agree less well with the chemical and enzymatic analysis of secondary structure. Moreover, by contrast with tRNAs, the number of *a priori* plausible secondary structures having a proper percentage of bases paired is large[11] so that it seems impossible to propose a secondary structure of the molecule without additional knowledge of the specific sequences which are unpaired.

We wish to report the identification of most of the unpaired sequences in 5S RNA by determining whether or not complementary oligonucleotides bind to 5S RNA. The RNA molecule should only bind well those oligonucleotides whose Watson–Crick antisequences are unpaired in the molecule; it should show little affinity for those whose antisequences are base paired or otherwise inaccessible. By the use of many oligonucleotides, the entire length of the molecule can be mapped, overlapping oligonucleotides permitting an accurate assessment of the limits of the unpaired sequences. The technique employed is that used by Uhlenbeck, Baller and P. D.[12] to explore the structure of the anticodon loop in $\text{tRNA}^{\text{Met}}_{\text{f}}$.

On the basis of earlier work on the dependence of oligonucleotide interactions on base composition (O. C. Uhlenbeck, F. Martin and P. D., to be published) and the self consistency of the present results, one can readily decide if the observed value of the association constant K lies within the range expected if all bases are interacting with their complementary bases in the RNA; it is sufficient to restrict the oligonucleotides to trimers and tetramers. Within this narrow size range the value of K for binding to unpaired complementary regions at 0° C ranges from 400 to 400,000. Below 400 the binding is too small to be accurately measured. The variations within this thousand-fold range of *K* values are to be attributed to (*a*) the fraction of base pairs that are G·C, (*b*) the specific sequence and (*c*) the oligonucleotide length—three or four. Further, the conformation of the single stranded regions in the RNA may also influence the value of *K*. For example, an oligonucleotide may exhibit a different *K* value if it binds to the ends rather than to the middle of a looped out region. Additional problems may arise from the existence of denatured conformations of 5S RNA (ref. 13) inasmuch as the presence of small amounts of the denatured form could cause significant binding of an oligonucleotide the antisequence of which is not available in the native form. Our experience indicates, however, that all these effects are small compared with the three items listed above.

Of forty-one trinucleotides tested, twelve (including three controls the antisequences of which are not present in 5S RNA) did not bind ($K < 400$) and fifteen were judged to bind in the range expected. The remaining fourteen displayed some binding, but the *K* values were low and probably represented binding to a distorted region, to a small amount of denatured 5S RNA or to involve only a part of the sequence. The criterion for the binding of tetranucleotides was the finding of a value of *K* much larger (five to ten times) than the sum of the *K*'s of its two constituent trinucleotides. Of fifty-seven tetranucleotides tested, thirty-eight (including six controls the antisequences of which are not present in 5S RNA) did not bind and seven did; nineteen displayed intermediate values of *K*.

In these experiments, a *K* value of less than 400 is not experimentally different from no binding. Approximately half of the *K* values determined were done in duplicate. The duplicates displayed an average deviation from the mean of ±10 per cent for oligonucleotides with *K* values larger than 5,000, and ±25 per cent for oligonucleotides with smaller *K* values. For those trinucleotides capable of forming only one G·C bond, *K* values of 800 to 1,200 were observed. Those with more GC content had values between 2,000 and 5,000, except for those containing a GG sequence, in which case the constants were near 10,000 if the GG was at the 3′ end of the trinucleotide, and 20,000 or more if at the 5′ end. All the tetranucleotides observed to bind were capable of forming at least two G·C bonds, and had *K* values of greater than 30,000. If GG sequences were present, the *K* value was greater than 100,000. The association constants of a number of oligomers that illustrate these generalizations are displayed in Table 1. Examination of these results shows that the observed distribution of *K* cannot be explained as an artefact resulting from differences in composition and sequence of the oligonucleotides, but instead arises from the difference in accessibility of the antisequence on the RNA. Despite similar composition, three of the seven tetranucleotides have *K* values which clearly indicate binding as tetranucleotides; the other four can be explained by the binding only of their constituent trinucleotides.

Table 1. EFFECT OF BASE COMPOSITION ON *K*

Oligonucleotide	*K*	Classification
GUU	$0{\cdot}2 \times 10^3$	—
AUG	0·5	Intermediate
CAG	0·9	Intermediate
CUG	1·4	Intermediate
UGG	9·2	+
CGG	9·7	+
AGG	10·2	+
GGU	18·0	+
* GGC	61	+
AUGG	4·0	—
CUGG	12·2	—
CAGG	15·0	—
† GGUU	24	—
* AGGC	224	+
AGGU	139	+
* CGGC	423	+

* There seem to be two *E. coli* 5S RNA sequences, present in roughly equal amounts, differing in position 13 (ref. 2). One would expect a binding of 0·5 mole of AGGC and 1·5 moles of CGGC per mole of 5S RNA. Also, GGC probably binds at both positions 10–12 and 61–63. True molar *K*'s will accordingly differ from the observed *K*'s.

† Control whose antisequence is not present in 5S RNA.

To construct a map of secondary structure from these data, all of the antisequences of the oligonucleotides that did not bind well were underlined. Underlining a region once means only that at least one position is unavailable. If overlapping oligonucleotides are found not to bind, a region may be underlined several times, suggesting that most of the region is unavailable. Placing the oligonucleotides that did bind is less simple. The antisequences of some of the binding oligonucleotides are only contained once in the 5S RNA sequence. As much as possible, the other binding oligonucleotides are grouped around the six unique sequences to give the maximum overlap. The final result is summarized in Fig. 1, which shows four accessible regions ranging in size from four to eight nucleotides.

As an illustration of the method, consider the unpaired region nearest the 3′ end. The relevant binding data are listed in Table 2. It can be seen that the trinucleotides complementary to CAU and AUG bind very little, if at all, while the complement of UGC shows a small degree of binding. The complement to GCG binds, but the complement of CGA binds very weakly, and may be binding to positions 43–45. The complements of GAG and AGA do not bind. Further, the complement of CCAU does not bind, while the complements of CAUG and AUGC show

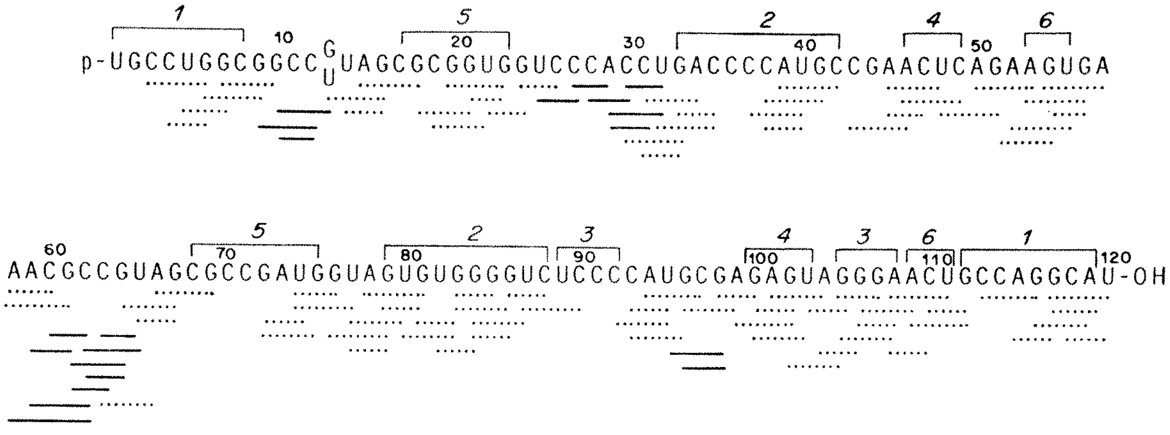

1
10
5
20
30
2
40
4
50
6
p-UGCCUGGCGGCC G/U UAGCGCGGUGGUCCCACCUGACCCCAUGCCGAACUCAGAAGUGA
60
5
70
80
2
90
3
4
100
3
6
110
1
120
AACGCCGUAGCGCCGAUGGUAGUGUGGGGUCUCCCCAUGCGAGAGUAGGGAACUGCCAGGCAU-OH

Fig. 1. Oligonucleotide binding to 5S RNA. All binding experiments were performed according to Uhlenbeck *et al.*[12]. The sequences underlined with dotted lines are those whose antisequences did not bind or bound very weakly. The sequences underlined with solid lines are the most probable sites for those oligonucleotides that did bind. The numbered brackets indicate those regions that may base pair. The 5S RNA used in these experiments was prepared by the method of Comb and Zehavi-Willner[14]. It was free of large rRNA and tRNA after chromatography on 'Sephadex *G*-100', using a 2·5 × 100 cm column with a flow rate of 2 ml./h/cm². The elution buffer was 0·1 M NaCl and 0·01 M potassium acetate (*p*H 7·0). 5S RNA exhibits several conformations, chromatographically separable on 'Sephadex *G*-100' (ref. 13). The material used here was not exposed to any conditions known to denature 5S RNA and eluted as one symmetrical peak from the column.

Table 2. MAPPING OF THE FOURTH LOOPED OUT REGION (... ^{93}CAUGCGAGA^{101} ...)

Oligonucleotide	5S RNA antisequence	K	K_{tri}*	Classification
AUG	CAU	0.5×10^3		Intermediate
CAU	AUG	0.3		−
GCA	UGC	1.0		Intermediate
CGC	GCG	2.6		+
UCG	CGA	1.4		Intermediate
CUC	GAG	0		−
UCU	AGA	0		−
AUGG	CCAU	4.0	9.7×10^3	−
CAUG	CAUG	4.4	0.8	Intermediate
GCAU	AUGC	9.0	1.3	Intermediate
CGCA	UGCG	34	3.6	+
UCGC	GCGA	4.8	4.0	−

* The sum of the K's of the two trinucleotides constituting the tetranucleotide.

intermediate binding. The complement of UGCG, which occurs only at this position in the 5S RNA sequence, definitely binds. The complement of GCGA does not. We conclude that the looped out region extends from position 93 to 98 or 99, although positions 93 and 94 are too distorted to bind oligonucleotides well.

An analysis of the base pairing possibilities of *E. coli* 5S RNA suggests that it may not have a unique secondary structure[11]. Our results indicate that there is at least one structure or perhaps several very similar structures that predominate. The four available, unpaired sequences that this work suggests are incompatible with all the published secondary structures for 5S RNA. Positions 9–11, 13, 27–31, 61–65 and 95 in Cantor's model; 9–13, 25, 28–32, 61–65 and 95–97 in Madison's; 9–13, 25 and 29–31 in Boedtker's model are said to be base paired, while these results show them to bind oligonucleotides. In addition, various oligonucleotides that would be expected to bind do not. One could say that a region predicted to be single stranded might be involved in further folding and thus not bind oligonucleotides, but it is difficult to visualize how a region predicted to be double stranded could bind oligonucleotides.

A possible base pairing scheme consistent with our results is presented in Fig. 1 by designating the most likely base paired regions. The two largest are both common to several of the secondary structures mentioned here[5,9]. The model can exist only in three dimensions, unlike the tRNA cloverleaf. There are no unpaired regions longer than three bases which were not found to bind oligonucleotides. Many of the oligonucleotides classified as intermediate can be considered to be binding at the distorted ends of the looped out regions. For example, GCC ($K = 1.4 \times 10^3$) and GAC ($K = 0.7 \times 10^3$) could be binding at the 5′ ends of the first and second looped out regions, respectively. UACG ($K = 15 \times 10^3$) could be binding at the 3′ ends of both first and third looped out regions.

In accordance with the spectral data[5,6], the model is 60 per cent base paired (counting four G·U bonds) and 64 per cent of the base pairs are G·C. One helix is interrupted by a looped out A residue. Of the twenty-four A residues, fourteen are unpaired, although not all of these fourteen are necessarily available for chemical reaction. The simple looped out A residues at positions 73 and 104 may not react to a reagent, such as monoperphthalic acid[7], in the same way as would an A residue in the middle of a larger unpaired region. The interpretation of the carbodiimide experiments[8] in terms of this model is less clear. The looped out regions proposed here provide several sites for the more reactive of the two reactive U bases. The less reactive U bases could only come from the melting of helical region 2 during the course of the reaction. Moreover, the model does not explain why several U residues available for oligonucleotide binding did not react with carbodiimide. Because the pairing pattern shown here requires the folding of the sequence in three dimensions, the tertiary structure thus imposed may sterically restrict the number of bases available for reaction with a given reagent.

This work was supported by a grant from the US National Institutes of Health, a US Public Health Service traineeship and a US National Science Foundation graduate fellowship. We appreciate useful suggestions and help from Dr Olke Uhlenbeck.

Received December 12, 1969.

[1] Madison, J. T., *Ann. Rev. Biochem.*, **37**, 131 (1968).

[2] Philipps, G. R., *Nature*, **223**, 374 (1969).

[3] Brownlee, G. G., Sanger, F., and Barrell, B. G., *Nature*, **215**, 735 (1967).

[4] Forget, B. G., and Weissman, S. M., *Science*, **158**, 1695 (1967).

[5] Boedtker, H., and Kelling, D. G., *Biochem. Biophys. Res. Commun.*, **29**, 758 (1967).

[6] Cantor, C. R., *Proc. US Nat. Acad. Sci.*, **59**, 478 (1968).

[7] Cramer, F., and Erdmann, V. A., *Nature*, **218**, 92 (1968).

[8] Lee, J. C., and Ingram, V. M., *J. Mol. Biol.*, **41**, 431 (1969).

[9] Cantor, C. R., *Nature*, **216**, 513 (1967).

[10] Raacke, I. D., *Biochem. Biophys. Res. Commun.*, **31**, 528 (1968).

[11] Richards, E. G., *Europ. J. Biochem.*, **10**, 36 (1969).

[12] Uhlenbeck, O. C., Baller, J., and Doty, P., *Nature*, **225**, 508 (1970) (preceding paper).

[13] Aubert, M., Scott, J. F., Reynier, M., and Monier, R., *Proc. US Nat. Acad. Sci.*, **61**, 292 (1968).

[14] Comb, D. G., and Zehavi-Willner, T., *J. Mol. Biol.*, **23**, 441 (1967).

New Approach to Cancer

by

P. R. J. BURCH
MRC Environmental Radiation Unit,
Department of Medical Physics,
University of Leeds,
General Infirmary, Leeds

A unified theory of growth, cytodifferentiation and disease can explain many features of natural cancers which are incompatible with purely cytological explanations.

IN 1962, Smithers[1] published a vigorous challenge, "Cancer, an Attack on Cytologism". The following quotation conveys the essence of his thesis: "Cancer is a disease of organization, not a disease of cells; its field of study is the science of organismal organization; it cannot be encompassed simply by cytology"[1]. Smithers attacked the idea that a normal cell can be converted by a single, all or none, and irreversible process into "the cancer cell". In particular, he listed the following incompatibilities with cytologism. "(1) The multicentric origin of neoplasia. (2) The long prediagnostic natural history and the many predisposing factors in the development of tumours. (3) Age-incidence and geographical variations. (4) Progression and regression in tumour behaviour. (5) The conditional persistence of some tumours. (6) The hormone dependence of others"[1].

My collaborators and I have recently put forward a unified theory[2-7] of growth, cytodifferentiation and disease, and in this article I wish to show that it can account for those several features of neoplasia that, in Smithers' view[1], cannot be explained by "cytologism".

Outline of Unified Theory

We believe that, beyond a certain stage of embryogenesis, a central mesenchymal system regulates normal growth through a homoeostatic or feedback mechanism. In our view, many age-dependent diseases, including, perhaps, all natural malignancies, arise from a breakdown in the central part of the control system. The central control has two principal sections, corresponding to the anatomical division of target tissues between those on the blood side, and those behind, blood-tissue barriers. In the first section, one class of small lymphocyte (the kind involved in homograft rejection and delayed hypersensitivity) acts as the effector of symmetrical mitosis[2-4]. Humoral factors necessarily control symmetrical mitosis when the target tissue lies behind a blood-tissue barrier. Usually, these factors seem to migrate on electrophoresis with the α_2-globulin fraction: basophilic granulocytes and mast cells might be connected with their synthesis[3,5,6].

Effectors of symmetrical mitosis, which we call mitotic control proteins (MCPs), have to locate the correct target tissue, the cells of which are identified by tissue coding factors (TCFs). Tissue coding factors comprise histocompatibility antigens, tissue-specific antigens and subtler determinants that usually remain undetected in transplantation studies. Target cells secrete humoral TCFs which act as affector signals, and their rate of flow is proportional to the size of the target tissue. These affectors have to locate their cognate control element in the central system and inhibit the release of effector MCPs. Recognition, therefore, is a two-way problem.

We infer[2,3] that it can have only one genetic basis. The genes that code for some or all of the polypeptide components of a given MCP are identical with those that code for the corresponding polypeptide components of the cognate TCF[2,3]. When effector MCPs and affector TCFs are both humoral, they have to be distinguished: the difference resides in the non-protein components of the molecules, MCPs being complexed with carbohydrates, and TCFs chiefly with lipids[3].

A homoeostatic system of growth-control must contain a "comparator" to determine whether the target tissue has attained the correct size. We deduce that each growth-control element has its own comparator, which, from about the time of birth and onwards, consists of a fixed number of stem cells[2-7]. These cells are located principally or wholly in the bone marrow[3].

I have argued[3] that, in addition to their recognition function, TCFs also determine cytodifferentiation by derepressing, directly or indirectly, a particular set of genes. Specific cellular adhesiveness and the inhibition of mitosis produced by contact between similar neighbouring cells depend, we suggest, on the specific contact and self-recognition interactions between the TCFs of similar, adjacent cells[2,3].

Neoplastic Transformation in Autoaggressive Disease

Spontaneous autoaggressive disease of a tissue bearing a particular TCF is initiated by random gene mutations in a comparator stem cell of its central growth-control element[2-7]. Following Burnet[8,9], we believe that the mutant mesenchymal stem cell propagates a "forbidden clone" of similarly mutant descendant cells. "Mutant" MCPs attack the target tissue, the growth and size of which they usually regulate in their non-mutant form[2-7]. This attack results from a changed type of interaction. Whereas the protein components of the normal MCP bear an identity relationship to those of the TCF, the "mutant" MCP is complementary to the target TCF.

In the simplest form of carcinogenesis, "mutant" MCPs probably interact directly with target cells to transform them eventually into the neoplastic state[10]. Transformation probably entails a change in the structure of the TCF brought about by an induced change in gene expression. Invasiveness, and the destruction of normal by malignant tissue, result from strong interactions between the altered (mutant) TCFs in cancer cells and complementary TCFs in the cells of normal tissue.

Two kinds of experimental evidence support the idea that mutant MCPs change the structure of TCFs through an inductive mechanism. First, the influence of normal mesoderm on the differentiation of epithelia during embryogenesis is well established[11]. Only recently, however, has it been shown[12] that for some anatomical sites (ear, sole of foot, trunk) the normal dermis of the adult guinea-pig or hamster can determine the type of the overlying epidermis in recombinant grafts. Second, the lability of certain antigens on the surface of mammalian cells is revealed in the phenomenon of antigenic modulation[13]. The phenotype TL+, associated with certain mouse thymocytes, can be converted to TL− by the action of anti-TL antibody[13]. That the character of TCFs in target epithelial cells could be changed inductively by appropriately mutant humoral MCPs—which are somewhat analogous to classical antibodies—has therefore good precedents.

Evidence which I shall refer to later suggests that a target cell attacked by mutant MCPs might have to undergo one or more mitoses before neoplastic transformation is fixed. Even then, mitosis of the transformed cells is probably stimulated by, and during the first (pre-terminal) stage of cancer *in vivo* depends on, mutant MCPs.

In various non-carcinomatous malignancies, including Burkitt's lymphoma, Hodgkin's disease and certain leukaemias, proliferative change probably results from an indirect mechanism[3]. Here, the primary targets of the autoaggressive attack are likely to be processing cells that usually regulate the proliferation of lymphocytes or granulocytes[3,10]. The Reed-Sternberg cell is probably the altered target cell in Hodgkin's disease[3].

In certain types of leukaemia proliferating leucocytes probably come directly from a mutant growth-control stem cell[3].

Defence Mechanisms

Many chronic autoaggressive conditions can be exacerbated by specific infective agents[3,4]. Classical infectious diseases such as poliomyelitis develop only when the host is invaded by a particular type of microorganism. Nevertheless, the polio virus is not a sufficient cause. In an epidemic, a high proportion of the population produces anti-polio antibodies, but less than one per cent develops degenerative changes in the central nervous system.

I find that in populations which have not received prophylactic immunization, the sex distribution and age distributions of mumps, poliomyelitis and infectious hepatitis obey the same stochastic rules as those followed by idiopathic disorders[3]. This implies that the characteristic changes associated with these diseases develop in a person only if a forbidden clone has been recently initiated[3]. The average interval or latent period between the completion of initiation and the onset of symptoms is very short for these infectious diseases—some 0 to 6 months[3]. A combination of invading microorganisms and a recently formed forbidden-clone in the non-immunized person is essential to the expression of these infectious diseases.

Infective agents, of the appropriate specificity, probably precipitate pathological change by competing for immunoglobulin antibodies that would usually suppress the growth of a forbidden clone[3]. In chronic non-infectious diseases such competition increases the activity of the forbidden clone: the classical infectious diseases manifest only when microorganisms compete specifically for particular defence resources.

If the pathogenesis of cancers closely resembles that of non-neoplastic autoaggressive conditions, specific infective agents might sometimes play similar quantitative or qualitative parts in their development.

Relation of Unified Theory to Smithers's Critique

A unified theory of growth and age-dependent disease can call on a vast range of evidence for its support or refutation. For example, numerous observations relating to the multicentric origin of a non-malignant disease, clinical dental caries[3,7,15], become relevant to the understanding of the distribution of cancer foci. Indeed, two of Smithers's "incompatibilities with cytologism"—the multicentric origin and age-incidence—form the chief quantitative foundations of our theory[3–7,10].

Many stochastic models can account for the broad features of the age-patterns of cancer during adult life[3]. Sex distributions and age distributions for the range 0 to 20 years of age provide more exacting tests. When cohort changes or diagnostic error do not obtrude, the age patterns of numerous malignant and non-malignant diseases fit a simple biological model[3–7,10].

Our model postulates first that a specific autoaggressive disease can only arise in people genetically predisposed to it. Sometimes, as with osteoarthrosis, an entire community can be predisposed, but for other diseases such as Huntington's chorea, only a small fraction is genetically susceptible[3].

Second, a spontaneous autoaggressive disease is initiated in a predisposed person by a specific set of random gene mutations in growth-control stem cells. One or more mutations may occur in a single stem cell, or in multiple-stem-cells. The average rate of occurrence of each specific initiating mutation stays constant from about the time of birth to the onset of disease: it is unaffected by ordinary environments[3–7,10]. This constancy of rates during growth virtually eliminates biological models that would locate the initiating mutations in target cells. Most target tissues increase greatly in size between birth and maturity: the average rate of a particular somatic mutation in such tissues would increase with the growth in the number of cells. By locating the initiating mutations in the growth-control comparator, with its fixed number of cells, we can explain the constancy of mutation rates during growth.

According to our theory, the siting of neoplastic or degenerative foci depends on the anatomical distribution of target cells the TCFs of which are complementary to the mutant MCPs of the forbidden clone[3,7,15]. The simultaneous bilateral presentation of various tumours[16,17] can be understood readily in these terms.

I turn now to Smithers's second point concerning predisposing factors and prediagnostic signs. In our theory, the gene complement at fertilization determines predisposition. Inheritance is seldom simple autosomal dominant[3,18].

The progression from dysplasia, through carcinoma *in situ*, to invasive carcinoma of the cervix provides an outstanding example of prediagnostic signs[19]. Two distinctive groups of women (at least) are genetically predisposed to cervical cancer[10]. For the early-onset group with initiation-mode at about 45 yr (and, perhaps, for the late-onset group with initiation-mode at about 76 yr), four forbidden clones initiate the disease, and each stem cell requires three somatic mutations[10]. Probably one and/or two clones produce the early dysplasia, two and/or three induce carcinoma *in situ*, and four forbidden clones result in invasive carcinoma.

Hyperplasia precedes cancer of the breast which often presents unilaterally or bilaterally at many sites in the breast ducts[16]. Two groups of women (at least) are genetically predisposed to breast cancer and, in the early-onset group, three forbidden clones initiate the disease[10]. Probably, one and/or two of these clones promote hyperplasia.

Smithers's third point includes geographical variations of incidence. For spontaneous cancers, the principal factor in geographical variation is probably gene frequency, which determines the proportion of the community that is predisposed[3,10,20]. Changes of incidence with time also occur, and they often differ from one region to another. With the recent, and largely successful, conquest of the fatal infectious diseases, some of which are likely to be genetically associated (positively or negatively) with malignant diseases[3], we must now be living through a period of pronounced transition in gene frequencies. Cohort changes in the age-incidence of some cancers may well be the result of changing gene frequencies, produced by altered selection pressures. Should viruses prove to play an essential part in the pathogenesis of any cancer in man they could, of course, give rise to marked geographical and temporal variations of incidence.

The phenomena of progression, regression, conditional persistence and hormonal dependence—Smithers's points (4) to (6)—are commonly exhibited by many non-malignant autoaggressive diseases. In general, the course of a chronic disease will be determined by the balance or imbalance between the proliferative tendency of the forbidden clone and the restraining action of the endogenous defence mechanism[3,6,14]. Total remission, however, may follow from an appropriate mutation in the stem cell propagating a forbidden clone.

I suggest that the growth of many tumours before the terminal phase depends, at least in part, on mitotic stimuli produced by the interaction of mutant MCPs with their altered target cells. Such tumours will respond to fluctuations in the activity of forbidden clones. Steroids and adrenocorticotrophic hormone affect the balance between the defence and the level of mutant MCPs: they constitute the basis of many therapeutic measures. These and other hormones may also exert direct effects on the growth of certain tumours.

Laird[21,22] has found that in experimental animals, the growth of many primary and transplanted tumours follows a Gompertzian equation, to reach a limiting size. She concludes that a tumour grows . . . "as though it were a single organism, rather than a population of dissociated individual cells, each the progenitor of an independent line of tumour cells"[22]. These observations[21,22] suggest that the host controls the growth of a tumour, and of normal tissues, by similar mechanisms. The tumour grows disproportionately because the normal relation between the comparator and the target tissue has been altered.

It is commonly supposed that the persistent growth and invasiveness of a tumour and its metastases cause death. This view cannot easily be reconciled with the mathematical form of the survival curve, which can often be represented by a single negative exponential function ($S_\tau = \exp - \lambda\tau$), or by the sum of two such functions. S_τ is the fraction of patients surviving (corrected for mortality from other diseases) at time τ after diagnosis: the constant λ is characteristic of the type of tumour. This form of survival curve suggests that death might be precipitated by a single random event occurring during the post-diagnostic phase. From evidence that is too extensive to review here I believe that the "single random terminal event" may prove to be a somatic mutation in a cell of the forbidden clone that causes the cancer. This additionally mutant cell then propagates a new clone, which induces a fatal progression.

Chemical Carcinogenesis

The idea that random somatic mutations in stem cells of the central system of growth-control initiate spontaneous cancers seems to be contradicted by evidence for the local induction of tumours by carcinogens applied locally. When a small area of skin is treated with a chemical carcinogen such as 20-methylcholanthrene, skin tumours appear in the treated area. Such treatments cannot directly affect relevant growth-control stem cells in the bone marrow, and at first sight epidermal tumours might seem to result from the direct action of the chemical carcinogen on epidermal cells. Transplantation experiments[23,24] undermine this simple view. When pure epidermal- or thin Thiersch-grafts of skin treated with carcinogen are autotransplanted to untreated sites, no tumours develop in the graft skin[23,24]. A substantial yield of tumours can be obtained, however, when untreated epidermis is transplanted to a denuded area which had previously been treated with 20-methylcholanthrene. Thick Thiersch grafts and whole thickness grafts of treated skin yield a few tumours after autotransplantation. On the other hand, when whole thickness grafts of skin treated with carcinogen are re-implanted into the beds from which they have been cut, a larger yield of tumours arises more quickly than in unoperated controls[23,24].

These and other experiments suggest that direct action of the carcinogen on superficial epidermal cells *in vivo* cannot be responsible for their conversion to the cancerous state[23-25]. In our theory, a series of feedback loops connects the growth-control stem cells in the bone marrow to the final (peripheral) executive cells[2,3]. When the target tissue, such as the epidermis, lies behind a blood-tissue barrier, circumstantial evidence indicates that mast cells might in some cases act as the local executive cells of the central system of growth control[2,3].

The findings of Billingham *et al.*[23] described above agree with the idea that the carcinogen first induces mutations in growth-control cells in the dermis directly underlying the treated epidermis. (Recent observations[26,27] indicate, contrary to earlier suspicions, that hair follicles as such play no direct part in the production of tumours from the superficial epidermis.) Probably, the mutant peripheral growth-control cells then secrete mutant humoral MCPs which interact with overlying epithelial cells to produce carcinomatous transformation. To judge from the experiments with full thickness grafts, the effectiveness of this process depends, to some extent, on the proximity of the peripheral growth-control cells to the previous stage of the central system of growth-control—possibly neighbouring lymph nodes. When wounds—such as the removal and then restoration of a whole thickness graft—activate the growth-control mechanism locally to stimulate the secretion of mutant MCPs, the rate of tumour development is accelerated.

From electron microscope studies of the sequence of changes during the development of spontaneous and induced murine mammary tumours, Tarin[28] has argued that epithelial tumours may result from disturbed interactions between the epithelium and underlying connective tissue.

Radiation Carcinogenesis

Ionizing radiation delivered locally induces tumours in the irradiated area[29]. Nevertheless, dose-response relations[30-32] for the induction of certain dermal and epidermal tumours suggest[10] that the cells in which the initiating mutations are induced do not usually have to undergo cell division to produce a tumour. Again, mast cells in the dermis are possible candidates for the cells at direct mutational risk, and their secreted mutant MCPs might induce a neoplastic transformation in overlying viable epidermal cells.

This interpretation receives further support from experiments[33-35] designed to assess the depth beneath the surface of those cells that are at risk with respect to the induction of epithelial skin tumours. Glucksmann[33] reported that 0·3 MeV electrons failed to induce skin tumours in the mouse whereas 0·7 MeV electrons were effective. Albert *et al.*[34] found that protons penetrating 0·17 mm into the skin of rats did not induce tumours. From experiments with beams of electrons, these workers estimated that the cells at risk lie about 0·3 mm below the surface[34]. Although these workers suggest[34,35] that epithelial cells in hair follicles are the cells at carcinogenic risk, this attribution seems improbable in the light of other evidence[26,27]. Mast cells reside specifically in the radio-sensitive zone and their numbers show changes related to the course of chemical co-carcinogenesis and anti-carcinogenesis[36]. Riley[37] has already proposed that mast cells are involved in the "promotion" phase of chemical carcinogenesis.

Plastic Film Carcinogenesis

Many experiments have shown that target cells in tissue culture can be transformed into the malignant state by physical and chemical carcinogens and by oncogenic viruses. Cell replication is required to fix the transformed state of cells *in vitro* after injection of simian virus 40 (ref. 38). When cells are irradiated *in vitro*, two cell generations have to elapse before the transformed state becomes established[39]. Studies of the age-patterns of disease in man, on the other hand, offer no support for the idea that viruses contribute to the "initiation" phase of cancer or of non-malignant diseases[3,10].

All these observations can be reconciled if MCPs, normal or mutant, exert a controlling influence over the type of TCF that is synthesized by their cognate target cells. I propose that spontaneous mutations of TCF genes occur in target cells (probably "strand-switching" mutations[3]), but in ordinary circumstances do not lead to cancer, because certain of these mutations are reversed *in vivo* by an inductive interaction between the normal MCP and the mutant target cell.

An interpretation of plastic film carcinogenesis can now be offered. The presence of the physical barrier between cells reduces the area of cell to cell contact, diminishes the contact inhibition of cell division, and thereby promotes hyperplasia. This eliminates the need for cell division promoted by MCPs, and hence the monitoring role of the latter is also lost. Spontaneous strand-switching mutations of genes coding for TCFs in target cells become fixed through repeated cell division. Cells which have acquired the necessary complement of mutations propagate a cancer.

Value of Unified Theory

By locating initiating somatic mutations in central (mesenchymal) growth-control stem cells—instead of in cells of the target tissue that subsequently becomes neoplastic—we can readily explain those many awkward features of natural cancers in man that, in Smithers's view[1], dispose of earlier aetiological theories. Another advantage of our theory over earlier ideas is that it represents but one facet of a unified and consistent scheme of growth, cytodifferentiation and age-dependent diseases, both neoplastic and non-neoplastic. The theory incorporates the phenomenon of cancer into the general biology of multi-tissue organisms.

Should our synthesis prove to be substantially correct, new approaches to the problems of prophylaxis and therapy might be advantageous[3].

Received October 20; revised December 8, 1969.

[1] Smithers, D. W., *Lancet*, i, **443** (1962).

[2] Burch, P. R. J., and Burwell, R. G., *Quart. Rev. Biol.*, **40**, 252 (1965).

[3] Burch, P. R. J., *An Inquiry Concerning Growth, Disease and Ageing* (Oliver and Boyd, Edinburgh, 1968; University of Toronto Press, Buffalo, New York, 1969).

[4] Burch, P. R. J., and Rowell, N. R., *Acta Derm.-venereol. Stock.*, **45**, 366 (1965); **48**, 33 (1968).

[5] Burch, P. R. J., and Milunsky, A., *Lancet*, i, 554 (1969).

[6] Burch, P. R. J., de Dombal, F. T., and Watkinson, G., *Gut*, **10**, 277 (1969).

[7] Jackson, D., and Burch, P. R. J., *Gerontologia*, **15**, 203 (1969).

[8] Burnet, F. M., *The Clonal Selection Theory of Acquired Immunity* (Cambridge University Press, London, 1959).

[9] Burnet, F. M., *Brit. Med. J.*, **1**, 338 (1965); *Lancet*, i, 1383 (1968); Burnet, F. M., and Holmes, M. C., *Nature*, **207**, 368 (1965).

[10] Burch, P. R. J., in *Radiation-Induced Cancer*, 29 (Proceedings Series, International Atomic Energy Agency, Vienna, 1969).

[11] Billingham, R. E., and Silvers, W. K., *New Engl. J. Med.*, **268**, 477 (1963).

[12] Billingham, R. E., and Silvers, W. K., *J. Exp. Med.*, **125**, 429 (1967).

[13] Boyse, E. A., Stockert, E., and Old, L. J., *Proc. US Nat. Acad. Sci.*, **58**, 954 (1967).
[14] de Dombal, F. T., *Gut*, **9**, 144 (1968).
[15] Jackson, D., *Proc. Roy. Soc. Med.*, **61**, 265 (1968).
[16] Smithers, D. W., *A Clinical Prospect of the Cancer Problem* (Livingstone, Edinburgh and London, 1960).
[17] Willis, R. S., *Pathology of Tumours*, third ed. (Butterworth, London, 1960).
[18] Lynch, H. T., *Hereditary Factors in Carcinomas* (Springer-Verlag, Berlin, Heidelberg, New York, 1967).
[19] Johnson, L. D., Nickerson, R. J., Easterday, C. L., Stuart, R. S., and Hertig, A. T., *Cancer*, **22**, 901 (1968).
[20] Ashley, D. J. B., and Davies, H. D., *Gut*, **7**, 542 (1966).
[21] Laird, A. K., *Brit. J. Cancer*, **18**, 490 (1964).
[22] Laird, A. K., *Brit. J. Cancer*, **19**, 278 (1965).
[23] Billingham, R. E., Orr, J. W., and Woodhouse, D. L., *Brit. J. Cancer*, **5**, 417 (1951).
[24] Marchant, J., and Orr, J. W., *Brit. J. Cancer*, **7**, 329 (1953).
[25] Orr, J. W., *Brit. Med. Bull.*, **14**, 99 (1968).
[26] Bond, B., and Orr, J. W., *Brit. J. Cancer*, **23**, 188 (1969).
[27] Arundell, F. D., Karasek, M. A., and Gates, A. H., *J. Invest. Dermatol.*, **52**, 119 (1969).
[28] Tarin, D., *Brit. J. Cancer*, **23**, 417 (1969).
[29] Bond, V. P., Shellabarger, C. J., Cronkite, E. P., and Fliedner, T. M., *Radiat. Res.*, **13**, 318 (1960).
[30] Burns, F. J., Albert, R. E., and Heimbach, R. D., *Radiat. Res.*, **36**, 225 (1968).
[31] Hulse, E. V., Mole, R. H., and Papworth, D. G., *Intern. J. Radiat. Biol.*, **14**, 437 (1968).
[32] Barendsen, G. W., in *Radiation-Induced Cancer*, 413 (Proceedings Series, International Atomic Energy Agency, Vienna, 1969).
[33] Glucksmann, A., in *Cellular Basis and Aetiology of Late Somatic Effects of Ionizing Radiation* (edit. by Harris, R. J. C.), 121 (Academic Press, London, 1963).
[34] Albert, R. E., Burns, F. J., and Heimbach, R. D., *Radiat. Res.*, **30**, 515 (1967).
[35] Heimbach, R. D., Burns, F. J., and Albert, R. E., *Radiat. Res.*, **39**, 332 (1969).
[36] Riley, J. F., *Experientia*, **24**, 1237 (1968).
[37] Riley, J. F., *Lancet*, ii, 1457 (1966).
[38] Todaro, G. J., and Green, H., *Proc. US Nat. Acad. Sci.*, **55**, 302 (1966).
[39] Borek, C., and Sachs, L., *Proc. US Nat. Acad. Sci.*, **59**, 83 (1968).

Gigantopithecus and the Origins of Hominidae

by
DAVID PILBEAM
Anthropology Department,
Yale University,
New Haven, Connecticut 06520

A re-examination of the available *Gigantopithecus* material has revealed that most of the supposed "man-like" characteristics of this fossil primate are not, in fact, hominid at all.

Gigantopithecus is an extinct man-like primate which has long been a puzzle to anthropologists and palaeontologists. The first species recorded, *G. blacki*, was discovered more than 30 years ago in Pleistocene deposits in China; recently, an older species of the same genus, *G. bilaspurensis*, has been located in Indian beds of middle Pliocene age. The exact taxonomic position of both species is controversial, and they have been variously interpreted as man-like apes, aberrant hominids or direct early human ancestors. An attempt is made in this article to outline the course of later Cenozoic hominoid evolution which culminated in the Chinese *Gigantopithecus*, to interpret the functional significance of the morphological trends in this lineage, and to review possible selective factors which might have produced these changes. This re-examination of the material reveals that most, if not all, the supposed "man-like" characters are not in fact resemblances to hominids at all, and that *Gigantopithecus* should not therefore be regarded as a hominid.

Records of *Gigantopithecus*

The first *Gigantopithecus* specimen was purchased in 1935 by Professor Ralph von Koenigswald in a Hong Kong drugstore[1]. This specimen, a right M_3, was chosen as the type of a new genus and species, *Gigantopithecus blacki*. Between 1935 and 1939, von Koenigswald purchased a further seven teeth of the same species. All eight teeth seemed to have come from Pleistocene deposits in southern China. Three of these were described by Weidenreich[2] in 1945. He concluded that *Gigantopithecus* was a gigantic ancestor of the middle Pleistocene hominid, *Homo erectus*. Von Koenigswald[3] described the eight teeth in 1952. He pointed out that the most reasonable age for the materials was middle Pleistocene and that they were therefore contemporaries of *Homo erectus*. He also concluded that, although *G. blacki* was a hominid, it was not ancestral to any later forms, being merely an extinct side-branch of Hominidae.

In late 1955 and early 1956 a survey team from the Academia Sinica located fifty more *Gigantopithecus* teeth in collections from caves in Kwangsi Province, southern China. In the summer of 1956 a mandible with most of the teeth preserved was discovered by a peasant in a cave in the same province, and in 1956 and 1958 two more mandibles, with much of their dentitions intact, came from the same cave. Between 1956 and 1960 more than a thousand isolated teeth were found in that cave and in others in Kwangsi Province.

After the discovery of this first jaw in 1956, Pei[4] published a short descriptive note in which he described *G. blacki* as an "ape", but from his discussion it is clear that he believed *Gigantopithecus* to be what would now be described as an aberrant hominid. In 1958, von Koenigswald[5] discussed all the material from China, which at that time consisted of two lower jaws and numerous isolated teeth. He concluded that *G. blacki* should be grouped with *Australopithecus* in a "pre-hominid" group, distinct from living and fossil pongids. The most complete account of *G. blacki*, including a description of all material known at the time, was published by Woo[6] in 1962. Like von Koenigswald, Woo concluded that *G. blacki* was a primitive hominid. In contrast, several other authors, including Simons and myself[7], and Remane[8], have treated *Gigantopithecus* as a rather peculiar, though nevertheless definite pongid.

The geological age of *G. blacki* is known only from a study of the associated faunal remains (there being no associated rocks suitable for radiometric dating) and is probably middle Pleistocene, perhaps around 0·5 to 0·75 million years old. That is, the samples are younger than *Australopithecus* in Africa, and of roughly the same age as the earliest *Homo erectus* finds in Asia and Africa. The associated faunas seem to include animals adapted to a number of different environments, including forest and woodland savannah[9].

The ancestry of *G. blacki* was conjectural until the recent description by Simons and Chopra[10] of a new species of the genus, *G. bilaspurensis*. This find, a mandible with almost all of the teeth still preserved, comes from beds of middle Pliocene age in Northwest India for which, unfortunately, there are no absolute dates. From studies of the associated faunas, the fossil can be given a probable age of between 5 and 9 million years[11]. These faunas also suggest that at this time north-west India was covered with grassland and relatively open woodland. Simons and Chopra[10] consider it likely that the "man-like" resemblances of the mandible are parallelisms, and that the *Gigantopithecus* finds are best considered as representing an independent line of apes. The basic morphological features of *G. bilaspurensis* are similar to those of *G. blacki*, although the earlier species was probably smaller, with lower-crowned and less cuspidate cheek teeth, and less markedly bicuspid front lower premolars.

Ancestry

The ancestry of *G. blacki* has been uncertain until now, although several authors have discussed possible ties with earlier forms. In 1952, von Koenigswald[3] very tentatively suggested a relationship with a pongid species that he had newly described as *Indopithecus giganteus* from late Miocene or early Pliocene beds in India and Pakistan. Simons[12] pointed to a possible link with the aberrant late Miocene hominoid *Oreopithecus*. With the recovery of *G. bilaspurensis*, some light can now be thrown on the ancestry of the Chinese form, because the Indian species is fairly clearly ancestral to, or close to the ancestry of, *G. blacki*. Because *G. bilaspurensis* is more primitive in a number of features, it is now much easier to look for possible ancestors among earlier Tertiary hominoids. These ancestors are probably to be located among Asian dryopithecines (primitive apes) of the Miocene and Pliocene.

There is one rather enigmatic specimen—a lower second or third molar—which may represent an early stage in the ancestry of *Gigantopithecus*. This tooth (Geological Survey of India specimen *D*175) comes from beds in West Pakistan; its probable age is early Pliocene, perhaps between 10 and 12 million years old, and it was associated with animals typical of forested environments. The specimen was first described in 1915 by Pilgrim[13] as *Dryopithecus giganteus*. Von Koenigswald later placed it in a new species and genus *Indopithecus giganteus*[14] (a conclusion with which Hooijer[15] disagreed). In 1965, Simons and myself[7] transferred this tooth to *Dryopithecus indicus*. It is a most interesting specimen, for although it is similar to *D. indicus* it is larger and narrower than other second or third lower molars of that species. In outline and in the general size, relative proportions, and disposition of the cusps, this tooth is very similar to *G. bilaspurensis*. Indeed, it is quite possible that *D*175 is sampled from a species ancestral to the entire *Gigantopithecus* lineage. The cusp morphology of *D*175 is, however, more primitive and resembles closely that of *Dryopithecus*. For this reason, it may be advisable to restore this tooth to Pilgrim's original species, *D. giganteus* (bearing in mind that it is generally poor taxonomic procedure to base new taxa on single teeth).

D. giganteus is similar enough to *D. indicus*[7] for it to be probable that the lineage leading to *G. blacki* evolved originally in Asia from a dryopithecine similar to (perhaps even identical with) *D. indicus*. *D. giganteus* is from the same horizons as *D. indicus*, although the latter occurs in earlier beds too. (A relatively complete lower dentition of *D. indicus* from early Pliocene beds in India will shortly be described by Simons and myself.) The lower molars and posterior premolar, in spite of having more sharply defined cusps, are very like *G. bilaspurensis* in outline, relative size of cusps, and so forth. The anterior lower premolar, although of fairly typical pongid sectorial form, nevertheless shows some features which foreshadow the premolars of *G. bilaspurensis*. I therefore propose the following as a tentative phylogenetic sequence: *Dryopithecus indicus* (late Miocene)→*D. giganteus* (early Pliocene)→*G. bilaspurensis* (middle Pliocene)→*G. blacki* (middle Pleistocene). This becomes, in my opinion, one of the best documented fossil ape lineages, extending through 10 or more million years. It is one, moreover, which shows a significant amount of morphological change.

Morphological Trends

The morphology of the molars in *D. giganteus* is of typical *Dryopithecus* form; the occlusal surface is rather restricted, and the cusps are conical. In *G. bilaspurensis*, cheek tooth cusps show much less relief and are flattened and plate-like rather than conical; occlusal surfaces are relatively broad and flat. *G. blacki* shows still further interesting changes; cheek teeth are higher crowned and also much more cuspidate.

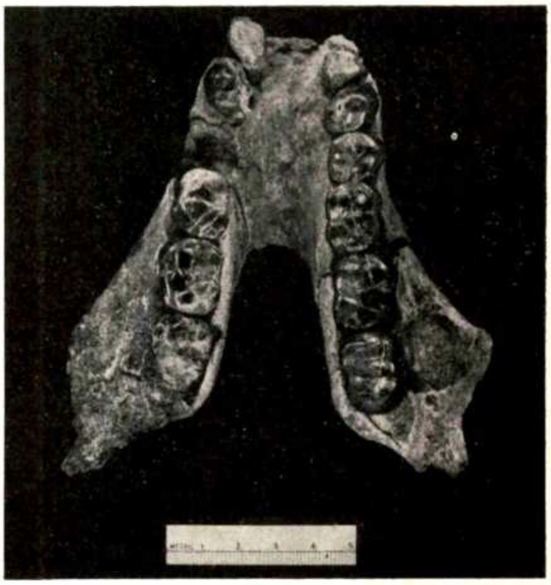


Fig. 1. *Gigantopithecus bilaspurensis* mandible from middle Pliocene beds in India.

The anterior lower premolar of *D. indicus* is an elongated, sectorial tooth. The principal external cusp is prominent, and although an internal cusp is usually present it is poorly developed. By the middle Pliocene the internal cusp was bigger, and the tooth was arranged in the mandible so that the long axis was oriented at less than 45 degrees to that of the other cheek teeth. In *G. blacki* the front premolar was even more clearly bicuspid; however, the trigonid was still quite well developed, suggesting that the upper canines sheared against the anterior face of the lower premolars. The cheek teeth of *Gigantopithecus* were packed closely together and showed considerable interstitial wear.

Canines were already relatively small in *G. bilaspurensis*. Even presumed males of *G. blacki* had canines which projected little beyond the plane of the cheek teeth. In both "males" and "females", the canines wore down rapidly on the tips, even at an early dental age. Both the upper and the lower canines were stout, broad teeth. Incisors were relatively small and closely packed between the canines. Relative to cheek tooth size, the incisors were smaller even than in the primitive dryopithecines of the Miocene and Pliocene. Between the Miocene and the Pleistocene there were trends therefore toward increased cheek tooth size, more molarized premolars, higher crowned and more cuspidate molars and premolars, smaller incisors, and reduced canines with occlusal surfaces more or less contiguous with those of the other cheek teeth.

Mandibular changes were equally marked. The mandibular body became very deep and robust in *Gigantopithecus*; the largest mandible of *G. blacki* is twice as deep as that of some male gorillas. The symphysis was strongly buttressed by superior and inferior transverse tori. The ascending rami originate opposite M_3 or the rear part of M_2, suggesting that the face was probably short; the gonial angles were thick and everted. Mandibular rami and tooth rows of *Gigantopithecus* diverged posteriorly, presumably because incisors and canines were small while the face was short relative to basicranial breadth.

Functional Interpretation of Morphology

How can these morphological trends and changes best be interpreted in functional terms ? One of the most distinctive and noticeable characteristics of both *Gigantopithecus* species is their large jaw size. Presumably these species had adult body sizes as large as or larger than modern gorillas: *G. blacki* was probably, on average, larger even than the mountain gorilla. This would suggest that they were creatures at least as terrestrial as the living African ape. Although ancestral *Dryopithecus* species seem to have been forest dwellers, *G. bilaspurensis* is found associated with an open country fauna[11], and one can probably infer a similar non-forested habitat for *G. blacki*[9]. It is likely therefore that *Gigantopithecus* species were adapted to foraging in relatively open country. None of the extant apes are so adapted ecologically, and for analogies among living primates it is necessary to turn to the ground-living Old World monkeys.

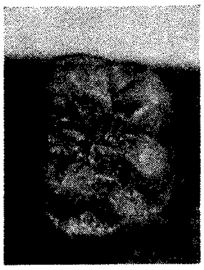


Fig. 2. Molar of a possible ancestor of *Gigantopithecus* from early Pliocene beds in Pakistan (*GSI D*175). Max. length: 18·3 mm.

Recently, Dr Clifford Jolly has shown[16] (and personal communication) that the dental, mandibular and facial adaptations of gelada baboons (*Theropithecus*) differ from those of other baboons (*Papio*) in features which can be correlated with differences in diet and manner of feeding. *Theropithecus* species, both living and extinct, have large cheek teeth coupled with small incisors, contrasting with the relatively larger front teeth of species of *Papio*[17]. Because of heavy use, gelada molars and premolars become packed close together and are heavily worn. Jolly relates the "*Theropithecus* complex", as he has called it, to the fact that these monkeys feed on small items—seeds, grass corms, and so forth—in large quantities. Such a diet requires large grinding cheek teeth and powerful masticatory muscles, while incisors are relatively unimportant. These features, and others, geladas share with the early hominids *Ramapithecus* and *Australopithecus*. Many components of the "*Theropithecus* complex" are also to be found in *Gigantopithecus*.

The small incisors of *Gigantopithecus*, fitting tightly between the canines, contrast markedly with the large cheek teeth. The molars and premolars were often closely packed and heavily worn. Premolars were molarized, so much so that the anterior lower premolar was fully bicuspid in *G. blacki*, thus increasing available chewing area. This was supplemented by what amounts to the functional incorporation of the canines into the posterior chewing battery. The canines of *G. blacki*, in particular, were small, stubby and broad. They became worn down rapidly from their tips, presumably as they erupted, so that they probably never actually projected beyond the other teeth. Although wear facets from upper canines can be observed on the P_3s of *Gigantopithecus*, these facets are only poorly developed. The canines of *Gigantopithecus* then became essentially grinding teeth additional to the premolars and molars. Thus it is important to note that although *Gigantopithecus* canines were reduced like those of Hominidae (and *Oreopithecus*), they were not incisiform slicing teeth like those of hominids. Functionally they were totally different, having become crushing rather than cutting teeth.

Numerous features of the mandible also point to powerful mastication. The symphysis was long, deep, and powerfully buttressed by large tori. These probably reduced anterior torsion during chewing, and Scott[18] has recently suggested a relationship between degree of development of such tori and the size of the masticatory muscles and cheek teeth. The horizontal mandibular rami were thick, deep and massively built; Vogel[19] believes that mandibles of this sort evolve in forms that are powerful masticators. The ascending rami turn upward quite sharply opposite M_2, and were probably set relatively vertically to the horizontal ramus. This would indicate that the face was also deep. Short deep faces—like those of *Theropithecus*, *Ramapithecus*, and *Australopithecus*—are adapted to powerful mastication. Chewing stresses can more readily be dissipated in a deep skull[20]. Masseters originating more anteriorly can exert greater power because their moment arm around the mandibular condyle is increased. That the masseters were powerful is also confirmed by the everted and buttressed mandibular gonial angles.

In conclusion, it is interesting to note the range of opinions concerning the status of *Gigantopithecus*, from the view that it is a true human ancestor to the idea that it was merely a man-like ape. I hope to have shown that, in phylogenetic terms, it was a pongid—although a peculiar one. If "traits" are simply listed, then "man-like" features are certainly to be found in *Gigantopithecus*: small incisors and canines, crowded and heavily worn cheek teeth, bicuspid front lower premolars, anteriorly convergent tooth rows, massive mandibles, and so forth. Viewed in functional perspective, however, it can be seen that these so-called "man-like" morphologies need not necessarily imply any relationship at all to Hominidae. Although the canines of *Gigantopithecus* were small like those Hominidae, morphologically and functionally they were very different. *Gigantopithecus* canines were large grinding teeth, not the chisel-like slicers of Hominidae. While it is true that bicuspid P_3s are typical of hominids, their presence in *Gigantopithecus* was probably the result of two factors. First, the reduction of the upper canines removed the necessity for an elongated P_3 to provide a blade-like shearing surface; second, the premolars became molarized because of selection pressures favouring more efficient mastication through increased tooth area. These two trends inevitably produced a bicuspid, barely sectorial P_3. The mere listing of such a trait has, in the case of *Gigantopithecus*, helped obscure the true relationships of that genus.

I thank Professor E. L. Simons, P. C. Ettel, Ian Tattersall and M. F. Gibbons, jun., for help during the preparation of this manuscript.

Received August 11, 1969.

[1] Von Koenigswald, G. H. R., *Proc. Kon. Akad.* v. *Wet.*, **38**, 872 (1935).

[2] Weidenreich, F., *Amer. Mus. Nat. Hist. Anthrop. Papers*, **40**, **5** (1945).

[3] Von Koenigswald, G. H. R., *Amer. Mus. Nat. Hist. Anthrop. Papers*, **43**, 295 (1952).

[4] Pei, W-C., *Amer. Anthrop.*, **59**, 834 (1957).

[5] Von Koenigswald, G. H. R., *The Leech*, **28**, 101 (1958).

[6] Woo, J-K., *Acad. Sinica*, No. 146 (1962).

[7] Simons, E. L., and Pilbeam, D. R., *Folia Primat.*, **3**, 81 (1965).

[8] Remane, A., in *Menschliche Abstammungslehre*, 249 (Gustav Fischer, 1965).

[9] Kahlke, H. D., in *Evolution und Hominization*, 91 (Gustav Fischer, 1968).

[10] Simons, E. L., and Chopra, S. R. K., *Postilla, Yale Peabody Mus.*, No. 137 (1969).
[11] Tattersall, I., *Nature*, **221**, 451 (1969).
[12] Simons, E. L., *Amer. Sci.*, **48**, 179 (1960).
[13] Pilgrim, G. E., *Geol. Surv. India Recs.*, **45**, 1 (1915).
[14] Von Koenigswald, G. H. R., *Ecl. Geol. Helv.*, **42**, 515 (1950).
[15] Hooijer, D. A., *Amer. J. Phys. Anthrop.*, **9**, 79 (1951).
[16] Jolly, C. J., *The Baboon in Medical Research*, **11**, 23 (1966).
[17] Leakey, L. S. B., and Whitworth, T., *Coryndon Mus. Occ. Papers*, No. 6 (1958).
[18] Scott, J., *Symp. Zool. Soc. Lond.*, No. 10, 127 (1963).
[19] Vogel, C., in *Taxonomy and Phylogeny of Old World Primates with References to the Origin of Man*, 21 (Rosenberg Sellier, 1968).
[20] Davis, D. D. *Fieldiana, Zool. Mems.*, No. 3 (1964).

Teichoic Acids and Membrane Function in Bacteria

by
S. HEPTINSTALL
A. R. ARCHIBALD
J. BADDILEY
Microbiological Chemistry Research Laboratory,
School of Chemistry,
University of Newcastle upon Tyne, NE1 7RU

Selective destruction of teichoic acid reduces the ability of bacterial cell walls to bind Mg^{2+} ions.

ALTHOUGH studies relevant to the function of teichoic acids in bacteria are few, the widespread occurrence of these polyol phosphate polymers is evidence of their importance for the maintenance of cellular activity. Wall teichoic acids, including the structurally related polymers containing sugar 1-phosphate residues, are common among the Gram-positive bacteria[1] but are nevertheless absent from a number of species. In many of the species lacking wall teichoic acids, acidic polysaccharides possessing uronic acid residues are present in the walls, and it is likely that these polymers serve the same purpose. On the other hand, as far as reasonably extensive studies have shown, appreciable amounts of membrane teichoic acids are always present in Gram-positive bacteria. They occupy an ill-defined region[2] between the wall and the underlying cytoplasmic membrane; after mechanical disruption of cells of most organisms they sediment together with fragmented membrane[3], although they are sometimes absent from membranes prepared from protoplasts[2,4], thereby suggesting that the association with the cell membrane is loose or perhaps mesosomal. The walls and membranes of Gram-negative bacteria do not normally contain teichoic acids. They do, however, contain lipopolysaccharides which, like the teichoic acids, possess sugar residues, phosphate groups and basic residues, and which may serve a purpose similar to that of the teichoic acids.

The importance of these acidic macromolecular components in the outer regions of bacteria has been demonstrated experimentally[5] with *Bacillus subtilis*, where it has been shown that in continuous culture with phosphate limitation of growth, teichoic acid is no longer present in the walls, but in its place is found a polysaccharide containing uronic acid residues. The organism can presumably dispense with its wall teichoic acid provided that it can substitute another acidic polymer. It is especially noteworthy that even in the conditions of phosphate limitation of growth a membrane teichoic acid was found in the cells[6], suggesting a vital and indispensable role for this component.

It had been noted some time ago that, with the exception of the true halophiles, bacteria that are able to tolerate moderate concentrations of sodium chloride and other salts in the growth medium are relatively rich in wall teichoic acid, and the original suggestion[7] that these polymers might participate in ion-exchange and the control of access of ions to the inner regions of the cell has been strengthened and further developed by recent experiments. In particular it is likely that the ability[8] of isolated bacterial walls to bind cations, especially bivalent cations, has some bearing on the function of teichoic acids. It has been suggested[9] that this binding is the result of the presence of phosphate groups in the wall, and this work confirms that it is the phosphate groups of teichoic acids that are responsible for this effect; it is also shown that the amino groups in the alanine ester residues on the teichoic acid have a profound effect on cation binding, and this effect may be important in the proposed function of these polymers discussed here.

We have examined the walls of *Staphylococcus aureus* H and *Micrococcus* sp. 24 with respect to their ability to bind Mg^{2+}, and have studied the effect of removal of the alanine ester residues and of the teichoic acid. *S. aureus* H was grown in nutrient broth as previously described[10] and also in a medium of the same composition but containing 7·5 per cent NaCl; *Micrococcus* sp. 24 was grown in nutrient broth. Cells were disrupted with No. 11 'Ballotini' beads in a Braun cell disintegrator, and the walls were recovered by centrifugation and washed with 0·9 per cent sodium chloride and then water. The walls were heated at 100° C for 10 min in order to destroy autolytic enzymes, and then washed twice with a solution of 0·1 M EDTA at *p*H 6·8 and extensively with glass-distilled water and then freeze-dried. Atomic absorption spectroscopy after wet digestion[11] of the walls showed that the preparations did not contain detectable amounts of Mg^{2+}. Samples (25 mg) were suspended at 25° C in 50 mM sodium acetate buffer (10 ml.) containing 0·25 mmoles of $MgCl_2$ at *p*H 5·0, and portions were removed at intervals by centrifugation at 17,000*g*, washed four times with glass-distilled water to remove unbound cations and analysed for phosphate and Mg^{2+}. Alanine ester residues were removed from the teichoic acid in the walls by suspending a sample in 0·4 M hydroxylamine (salt-free) at *p*H 7·2 for 1 h at 45° C; earlier work has shown that in comparable conditions all alanine residues are converted to a mixture of alanine and its hydroxamate, and no detectable hydrolysis of phosphodiester linkages occurs[12]. Uptake of cations by many of the wall samples was complete after 30 min, but values quoted in Table 1 were determined after incubation for 16 h, when uptake was complete with all samples. During this time no alanine or organic phosphates were detected in the supernatant solutions.

Binding of Mg^{2+} Ions by Degraded Cell Walls

Walls prepared from *S. aureus* grown in standard conditions contained 0·59 moles of alanine ester for each phosphate. These walls bound 0·23 equivalent of magnesium for each phosphate (phosphodiester) group in the wall and after treatment with hydroxylamine this value rose to 0·56 equivalent. Thus removal of alanine results in a substantial increase in the ability of the wall to bind magnesium. Walls prepared from *S. aureus* grown in 7·5 per cent NaCl contained fewer alanine ester residues (0·20 mole/mole of phosphate) and bound a substantially greater amount of magnesium (Table 1). Removal of alanine from these walls also increased their magnesium binding capacity although to a lesser extent than was observed with walls that originally contained more alanine. Similar results were obtained (Table 1) for walls of the *Micrococcus* which contained 0·33 mole of alanine ester for each phosphate. These results show that the presence of alanine ester residues substantially affects the capacity of walls to bind magnesium, and it is possible that the alanine ester residues of the teichoic acid function in regulating surface charge and salt uptake.

Table 1. MAGNESIUM BINDING CAPACITY OF WALLS AND CHEMICALLY MODIFIED WALLS OF *Staphylococcus aureus* H AND *Micrococcus* SPECIES 24

	Alanine ester content of untreated walls (moles of ester alanine : mole of phosphate)	Magnesium content of wall samples (equivalents of magnesium : mole of phosphate)				
		Untreated walls	Walls after treatment with hydroxylamine	Walls after treatment with hydroxylamine and trypsin	Walls after treatment with 0·1 M sodium hydroxide	Walls after treatment with hydroxylamine and sodium periodate
Staphylococcus aureus H (grown in nutrient broth)	0·6	0·23	0·56	0·64	0·69	0·45
Staphylococcus aureus H (grown in nutrient broth containing 7·5 per cent NaCl)	0·2	0·51	0·79	0·86	0·82	0·65
Micrococcus sp. 24	0·33	0·55	0·79	0·87	0·84	0·86

Walls were incubated with magnesium chloride as described in the text. The resulting content of magnesium was determined by atomic absorption spectroscopy.

The wall samples from which alanine ester residues had been removed differed from each other in their binding capacity, suggesting that factors additional to the presence of alanine ester residues may moderate cation binding to some extent. Although this difference is not yet understood in detail, among the moderating factors appears to be the presence of protein. Thus after removal of alanine by suspension of walls in 0·1 M NaOH at 22° C for 60 min, the binding capacities (Table 1) were greater than those of walls that had been treated with hydroxylamine. Similar increases were observed after hydroxylamine treated walls had been incubated with trypsin (Table 1), and as it has been found that small amounts of protein are extracted from these walls by dilute alkali, the increased binding capacities are probably due to the removal of protein. Nevertheless, the binding capacities of walls from different organisms differed even after digestion with trypsin, so that further factors, possibly steric in nature, also influence the capacity of the phosphate groups to bind magnesium.

A direct demonstration that the phosphate groups are responsible for the cation binding was obtained by selective chemical removal of these groups from the wall. The phosphorus-containing component of the walls of *S. aureus* H is a ribitol teichoic acid possessing N-acetylglycosaminyl and alanine ester substituents[10]. Because the ribitol phosphate residues comprising the polymer chain are readily oxidized by periodate, this treatment will remove from the wall nearly all of the phosphate of the teichoic acid, leaving a small amount of phosphate associated with linkage between teichoic acid and peptidoglycan. On the other hand, the teichoic acid in the wall of the *Micrococcus*[13] possesses glycerol phosphate and N-acetylglucosamine 1-phosphate residues in its chain and is stable towards periodate. Because the peptidoglycan in both cases is stable towards periodate, the *Staphylococcus* can be used to study the effect on magnesium binding of selective removal of teichoic acid, and the *Micrococcus* represents an excellent control.

Periodate oxidation of walls was carried out by suspending them in 0·1 M sodium metaperiodate solution for 24 h at 21° C. Such oxidation of walls of *S. aureus* that had been grown in nutrient broth resulted in the removal of 75 per cent of the phosphate and a decrease of 80 per cent in the binding capacity of the residual wall material. Oxidation of walls prepared from cells grown in 7·5 per cent NaCl resulted in a loss of 92 per cent of the phosphate and 96 per cent of the binding capacity.

In contrast to the large loss in phosphorus and in magnesium binding capacity on oxidative removal of teichoic acid from the staphylococcal walls, the micrococcal wall was almost unaffected by periodate. Periodate treatment of untreated walls and of those that had had alanine ester residues removed yielded no soluble phosphates. The magnesium binding efficiency before removal of alanine ester was almost unchanged after the treatment with periodate.

The above results establish that the capacity of bacterial walls to bind bivalent cations is due to the presence in them of teichoic acids. Moreover, the binding capacity is greatly increased after removal of the alanine ester residues. It is proposed that a major function of both wall and membrane teichoic acids is to maintain a high concentration of bivalent cations in the region of the membrane. This is consistent with the cation binding properties of these polymers and its partial control through the amino groups of the alanine ester residues. It explains the observed structural diversity of teichoic acids and related polymers, for many structural varieties of polymers containing phosphate, amino and hydroxyl groups could satisfy the requirements for controlled cation binding. It also explains the vital requirement of cells for membrane teichoic acid; wall teichoic acids would be functionally less important and, although useful, could be substituted by other acidic polymers under unfavourable growth conditions. The superior importance of membrane teichoic acid is reflected in their much narrower structural diversity; thus all are glycerol phosphate polymers[1,14] and all possess D-alanine ester residues, the differences between them being confined to the type and number of glycosyl substituents.

In normal conditions of growth the most abundant bivalent cation would be Mg^{2+}, and the proposal that the major function of teichoic acids is to maintain a high bivalent cation concentration in the region of the membrane is supported by the observation[5] that organisms grown in a chemostat under conditions of Mg^{2+} limitation of growth possess exceptionally large amounts of wall teichoic acid. Presumably the teichoic acid content of the walls is raised in order to scavenge the limited supply of bivalent cations.

There are several reasons why it is important that the cell should be able to maintain a high concentration of bivalent cations in the region of its membrane. Many enzymes are bound to the membrane[15], including those required for the synthesis of membrane, wall and capsular components, for example, peptidoglycans, teichoic acids, capsular and wall polysaccharides, lipopolysaccharides, glycolipids and so on, as well as those required for electron transport and oxidative phosphorylation. The cation requirements of these enzymes have not been determined in all cases, but it is known that many require bivalent cations for optimal activity, and in some cases, for example, those for the synthesis of peptidoglycans[16,17] and teichoic acids[18,19], exceptionally high concentrations (20 mM or higher) of Mg^{2+} are required. The physical integrity of the membrane requires Mg^{2+}, as is shown in studies on the survival of protoplasts[20]. The association of ribosomes with the membrane during protein synthesis[21] also requires Mg^{2+}, as does the association of mesosomes with membrane[22].

Other Functions for Techoic Acids

Although we believe that the main function of teichoic acids is to provide the correct ionic environment for the membrane, this does not exclude the possibility that additional functions may exist in specific cases. Indeed, at least two cases of possible secondary functions are already known. In the *Pneumococcus* the dietary requirement for choline arises[23] through the presence of choline phosphate residues in the ribitol teichoic acid (C-substance)[24] in its wall. Ethanolamine can substitute for choline in the medium, and in these conditions ethanolamine residues are incorporated into the wall polymer. The effect of this structural change is dramatic[25]; reproduction is substantially unimpaired, but cell separation is inhibited and long chains of many hundreds of unseparated cells are formed. Moreover, the highly characteristic autolytic behaviour of old colonies of the organism is not shown by cultures grown with ethanolamine rather than choline. Although the mechanism of these changes has not yet been established, it appears that the effects are related to changes in the behaviour of lytic enzymes required for the process of separation of parent and daughter cells in cell division. In this connexion, the lytic properties of *Streptococcus zymogenes* are of interest[26]. This organism excretes enzymes into the medium which are powerfully lytic towards the walls of a number of other bacteria. Nevertheless, the walls of the *Streptococcus* itself are immune to attack. These walls possess a ribitol teichoic acid and, if the alanine ester residues are removed chemically, then the walls become sensitive towards the lytic enzymes. Again, the detailed mechanism of these effects is not yet established, but in both cases it seems that relatively small changes to the structure of wall teichoic acids, especially in the basic components, can have a large effect on the behaviour of lytic enzymes. As the cation requirement of these lytic enzymes has not been examined, it is not easy to decide whether these effects, caused by small changes in teichoic acid structure, are related to an independent secondary function of teichoic acids in controlling the activity of important lytic processes related to cell division, or whether the effects are again consequences of alteration in the cation balance which may cause changes in the activity of enzymes.

A further function of wall teichoic acids is associated with their charge. The demonstrable surface charge of bacteria is doubtless to be attributed in many cases to teichoic acid, and the maintenance of repulsive charges may be a desirable property enabling cell populations to disperse in the medium and thereby utilize nutrients efficiently. The effectiveness of this surface charge can be demonstrated with wall suspensions in buffers with constant ionic concentration but different *p*H values. At values below *p*H 7 the suspensions show a marked tendency to sediment. Moreover, walls that have had their teichoic acid removed by oxidation with periodate settle rapidly as a flocculent mass when attempts are made to suspend them in buffers over a wide range of *p*H.

We thank Miss Veronica Reid for assistance with atomic absorption spectroscopy. S. H. thanks the Science Research Council for a research studentship.

Received November 21, 1969.

[1] Archibald, A. R., and Baddiley, J., *Advances in Carbohydrate Chem.*, **21**, 323 (1966).

[2] Hay, J. B., Wicken, A. J., and Baddiley, J., *Biochim. Biophys. Acta*, **71**, 188 (1963).

[3] Critchley, P., Archibald, A. R., and Baddiley, J., *Biochem. J.*, **85**, 420 (1962).

[4] Shockman, G. D., and Slade, H. D., *J. Gen. Microbiol.*, **37**, 297 (1964).

[5] Tempest, D. W., Dicks, J. W., and Ellwood, D. C., *Biochem. J.*, **106**, 237 (1968).

[6] Ellwood, D. C., and Tempest, D. W., *Biochem. J.*, **108**, 40P (1968).

[7] Archibald, A. R., Armstrong, J. J., Baddiley, J., and Hay, J. B., *Nature*, **191**, 570 (1961).

[8] Cutinelli, C., and Galdiero, F., *J. Bact.*, **93**, 2022 (1967).

[9] Meers, J. L., and Tempest, D. W., *Soc. Gen. Microbiol. Proceedings*, **10**, Sept. (1968).

[10] Baddiley, J., Buchanan, J. G., RajBhandary, U. L., and Sanderson, A. R., *Biochem. J.*, **82**, 439 (1962).

[11] Eagon, R. G., *Canad. J. Microbiol.*, **15**, 235 (1969).

[12] Kelemen, M. V., and Baddiley, J., *Biochem. J.*, **80**, 246 (1961).

[13] Archibald, A. R., Baddiley, J., Button, D., Heptinstall, S., and Stafford, G. H., *Nature*, **219**, 855 (1968).

[14] Archibald, A. R., Baddiley, J., and Button, D., *Biochem. J.*, **110**, 559 (1968).

[15] Rogers, H. J., and Perkins, H. R., *Cell Walls and Membranes* (Spon, London, 1968).

[16] Chatterjee, A. N., and Park, J. T., *Proc. US Nat. Acad. Sci.*, **51**, 9 (1964).

[17] Meadow, P. M., Anderson, J. S., and Strominger, J. L., *Biochem. Biophys. Res. Commun.*, **14**, 382 (1964).

[18] Chin, T., Burger, M., and Glaser, L., *Arch. Biochem. Biophys.*, **116**, 358 (1966).

[19] Brooks, D., and Baddiley, J., *Biochem. J.*, **113**, 635 (1969).

[20] Weibull, C., *Exp. Cell Res.*, **10**, 214 (1956).

[21] Coleman, G., *Biochem. J.*, **112**, 533 (1969).

[22] Reaveley, D. A., and Rogers, H. J., *Biochem. J.*, **113**, 67 (1969).

[23] Tomasz, A., *Science*, **157**, 694 (1967).

[24] Brundish, D. E., and Baddiley, J., *Biochem. J.*, **110**, 573 (1968).

[25] Tomasz, A., *Proc. US Nat. Acad. Sci.*, **59**, 86 (1968).

[26] Davie, J. M., and Brock, T. D., *J. Bact.*, **92**, 1623 (1966).

Plate Tectonics, Orogeny and Continental Growth

by

J. F. DEWEY

Department of Geology,
University of Cambridge

BRENDA HORSFIELD

Further Education Television Department,
BBC, London

Ocean-driven plate mechanisms, the authors conclude, have been responsible for the growth and evolution of continents for at least 3×10^9 years. Some of the implications of this idea are discussed.

A GLOBAL tectonic mechanism in which essentially rigid lithosphere plates[1–4] are created at oceanic ridges, and consumed in oceanic trenches, provides a powerful framework for evaluating the development of orogenic belts and continents. Although continents are superficial passengers on moving plates, they place significant restraints[4] on plate evolution in that their buoyancy prevents their destruction by plate consumption. A variety of mountain, and continent, building mechanisms operate on leading plate margins as a result of the consumption of oceanic lithosphere. The plates of the present oceans probably contain no oceanic crust older than late Palaeozoic or early Mesozoic (personal communications from W. C. Pitman). It is therefore probable that the oceans suffer a complete turnover by plate accretion and consumption in about 250 m.y. The history of oceans before about 250 m.y. ago can only be elucidated by analysis of orogenic belts, in island arcs and continents, in which slices of oceanic crust (ophiolite suite) are preserved and mark the sites of former oceans. We take the view in this article that no kind of plate margin necessarily maintains a fixed position, that rates of plate production and consumption sum to approximately zero[5] on an Earth of essentially constant radius, and that lithosphere consumption only occurs on one side of a plate boundary. We consider below that the relationship of continents, island arcs and oceans has been determined by an ocean-based plate mechanism for at least 3×10^9 yr.

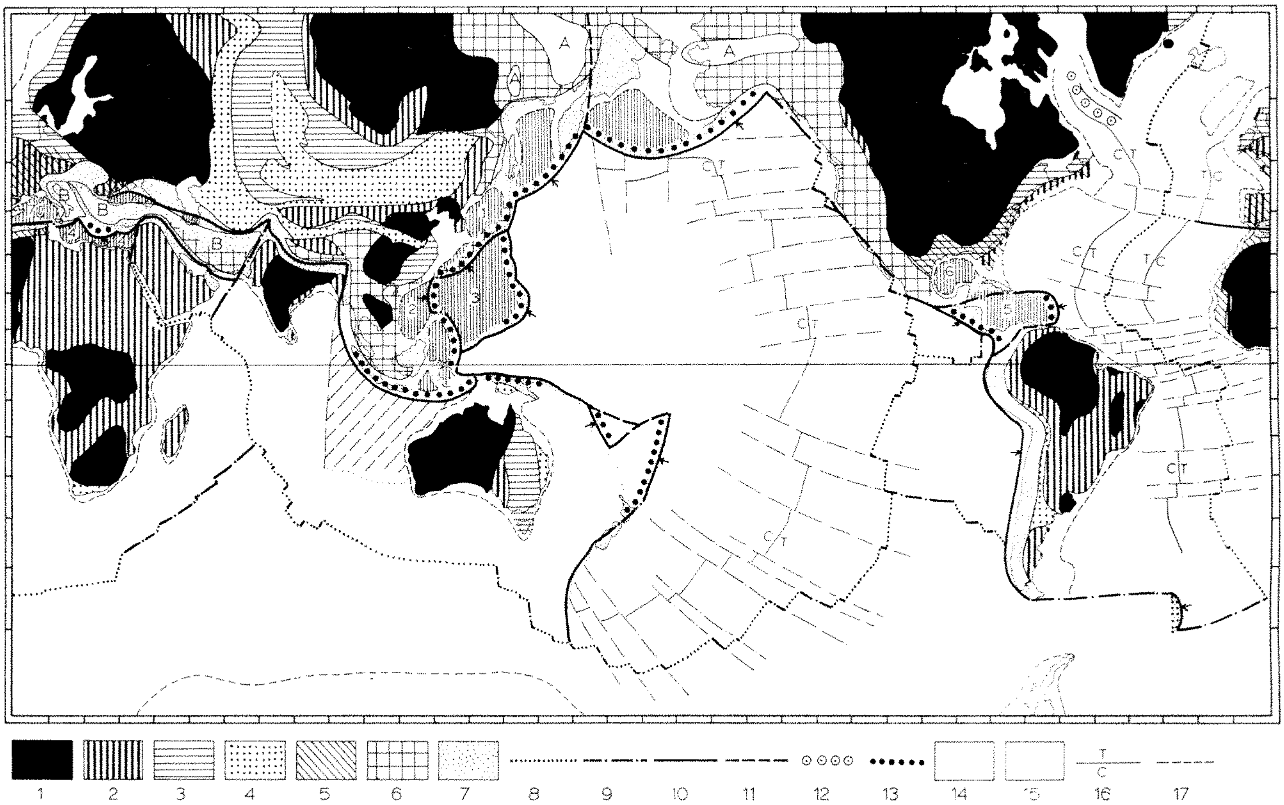


Fig. 1. Boundaries of present lithosphere plates and distribution of orogenic belts younger than 10^9 yr. Key: 1—Cratonic nuclei consolidated since 10^9 yr ago; 2—Late Pre-Cambrian (Baikalian) orogenic belts; 3—Lower Palaeozoic (Caledonian) orogenic belts; 4—Upper Palaeozoic (Uralian) orogenic belts; 5—Regions of Upper Palaeozoic regeneration (Alleghenian-Variscan); 6—Mesozoic (Cimmerian) orogens; 7—Tertiary-Quaternary (Alpine) orogens; 8—Accreting plate margins; 9—Transform plate margins; 10—Consuming plate margins (consuming side shown by small arrow); 11—Plate margins of indeterminate nature; 12—Extinct accreting plate margins; 13—Calc-alkaline vulcanicity; 14—Small ocean basins; 15—Remnants of Tethys; 16—Boundary of Cretaceous/Tertiary ocean floor; 17—Continental margins; A—Pre-Cambrian cratons of uncertain age; B—Internal microcontinents of the Alpine system of Europe and the Middle East. Latitude and longitude shown in 10° intervals.

Present Plate System

The Atlantic Ocean (Fig. 1) has been expanding in a varied and complex way since late Triassic times. The volume of sediments forming the continental rises depends on the rate of sediment discharge from adjacent continents from the time of continental separation. These continental margin sediments are largely non-volcanic and are thickest on the eastern margin of North America[6], reflecting accumulation since late Triassic times when Africa separated from North America. Elsewhere sediment volumes are smaller, reflecting separation of South America from Africa[7], Greenland and Europe from North America in Cretaceous times and Europe from Greenland in Palaeocene times. The result of the complex opening history of the Atlantic has been an exceedingly complex history of closure of Tethys now represented by remnant patches of ocean such as the Black, Caspian and Eastern Mediterranean Seas associated with the Alpine fold belt of Europe and the Middle East (unpublished results of J. Bonnin, J. F. Dewey, W. C. Pitman and W. B. F. Ryan). East of the Mediterranean further complexity arises from the growth of the Indian Ocean, whereby fragments of Gondwanaland such as India and Australia moved northwards to collide with Asia. The Indian Ocean thus consists of ocean developed from Cretaceous times with a much older Tethyan portion east of the Ninety East Ridge and west of Australia (Fig. 1).

In the Pacific, although lithosphere is being created at the East Pacific Rise and subsidiary ridges, it is being consumed in trenches at or near the Pacific Ocean margins. In western North America, the Mesozoic Cordilleran Orogen is bounded to the west by a ridge transform system argued[8] to be the result of driving the East Pacific Rise into a trench complex. In western South America, the Peru–Chile Trench precisely delineates the edge of the Mesozoic–Tertiary Andean Orogen on the continental margin. In the Western Pacific, however, plate consumption is occurring in trenches in front of island arc volcanic/orogenic belts within the ocean (Fig. 1). The simplest situation holds where, as in Japan, a small ocean basin lies between continental margin and island arc and the trench consumes the Pacific Plate. Further south there are complications. The Philippine Sea is a small contracting plate bounded by island arcs, pushing a finger westwards into, and therefore consuming part of, the South China Sea. The extending Fiji transform fault joins the New Hebrides Trench (consuming the northern Tasman Sea) to the Tonga Kermadec Trench (consuming the Pacific Plate). A range of present plate/ocean/continent combinations is shown in Fig. 2.

Evolution of Oceans

Lithosphere is being created by plate accretion along oceanic ridges in all the major oceans. In the Atlantic lithosphere consumption occurs in only two small regions (the Lesser Antilles and the Scotia Arc) and the continental margins are not plate margins. Because the Atlantic Ocean is therefore expanding, it is axiomatic that another major ocean is contracting. This is probably the Pacific, which has therefore to consume lithosphere at a rate that is the sum of its own rate of production and the rate of expanding oceans. The marginal Pacific trenches must therefore retreat into the ocean in front of continental margins and island arcs.

Although a pattern of linear oceanic expansion and contraction is an over-simplification, the effect of continental disruption, by plate growth, and convergence, by plate consumption, is that any continental margin will be the site of thick extensive sedimentation during continental separation and subsequently the site of orogeny during continental convergence[9]. This is the basis for our model of continental growth discussed below. In a sense this is an oceanic cycle but does not imply that the Atlantic, Pacific, Indian and Tethyan Oceans have all suffered alternate phases of expansion and contraction. There is evidence[10–12], however, that the Appalachian/Caledonian Orogen developed by contraction of a Proto-Atlantic Ocean roughly along the axis of subsequent opening of the central and northern Atlantic. Plate growth may begin along old lines of weakness and the effect of this will be a close association of orogenic belts of widely varying ages[13,14].

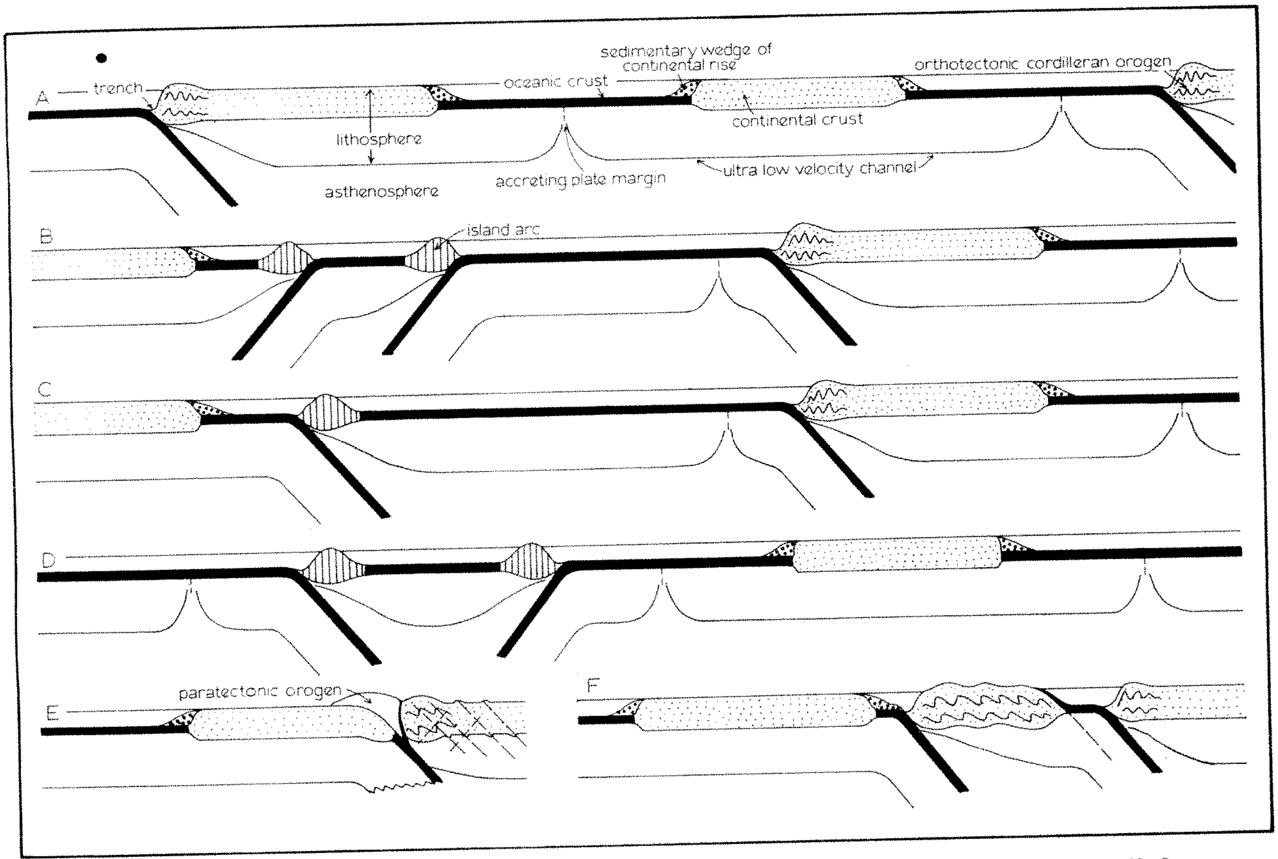


Fig. 2. Schematic relationships between lithosphere plates, continents, oceans, island arcs and orogenic belts. (A) Section from the Pacific Ocean, across South America, the Atlantic Ocean, Africa, and the Indian Ocean, to the Java Trench. (B) Section from South-East Asia, across the South China Sea, the Philippines, the Philippine Sea, the Marianas Arc, the Pacific Ocean, and South America, to the Atlantic Ocean. (C) Section from Australia, across the Tasman Sea, the New Hebrides Arc, the Pacific Ocean, and South America, to the Atlantic Ocean. (D) Section from the Pacific Ocean, across the Caribbean Sea, the Atlantic Ocean, and Africa, to the Indian Ocean. (E) Section from the Indian Ocean, across the Himalayas (site of lost Tethyan Ocean) to Tibet. (F) Section from the Atlantic Ocean, across Africa, the Eastern Mediterranean, Turkey, and the Black Sea, to the Caucasus.

A simplified view[9] of the present oceans is thus that they are either expanding (Atlantic) or contracting (Tethys). This is a grossly oversimplified way of analysing the behaviour of oceans because, although the Pacific is contracting, the oceans form a continuous system and cannot be considered separately. It is more accurate and instructive to consider the evolution of plates which may or may not carry continents as passengers. This emphasizes that the oceans and therefore plates are mutually interdependent and that continents may be disrupted and swept apart by plate motion, and may collide with island arcs or other continents. The great possible latitude and longitude changes involving continental separation and conjunction have fundamental implications for the distribution of animals and plants, particularly land-dwelling types. Faunal and floral province studies will contribute a great deal to our understanding of the relative positions of continents in the past.

Orogeny and Continental Accretion

The close spatial relationships of consuming plate margins, island arcs and the younger orogenic belts indicate with certainty that the energy source for mountain building is associated with plate consumption. Orogenic belts seem to be associated with both lithosphere consumption at continental margins (Andes) or in island arcs (Japan), and with continental collision (Himalayas). The conditions that determine the site and timing of plate consumption are so far obscure. It has been suggested[9,15] that an Atlantic-type continental margin with its thick pile of sediments on the margin of a wide old ocean, where the oceanic lithosphere is oldest and thickest, is eventually likely to become the site of lithosphere consumption and orogeny. Island arcs may be the result of the nucleation of trenches at microcontinents near continental margins. The onset of trench development may be related to the distance of a section of lithosphere from a ridge. If the lithosphere is regarded as a boundary conduction layer, steadily cooling and thickening away from the ridge, there may be a critical distance/thickness function at which time the plate sinks to form a trench. An alternative, or concurrent, possibility is that a major continental collision, after the closing of an ocean, resulting in cessation of plate consumption, leads to a situation where other active trenches cannot cope with lithosphere disposal and new trenches are forced to open, the likeliest positions being those already discussed.

There are at least four kinds of orogenic belt (Fig. 3) and the effectiveness of each type in accreting continents is discussed below.

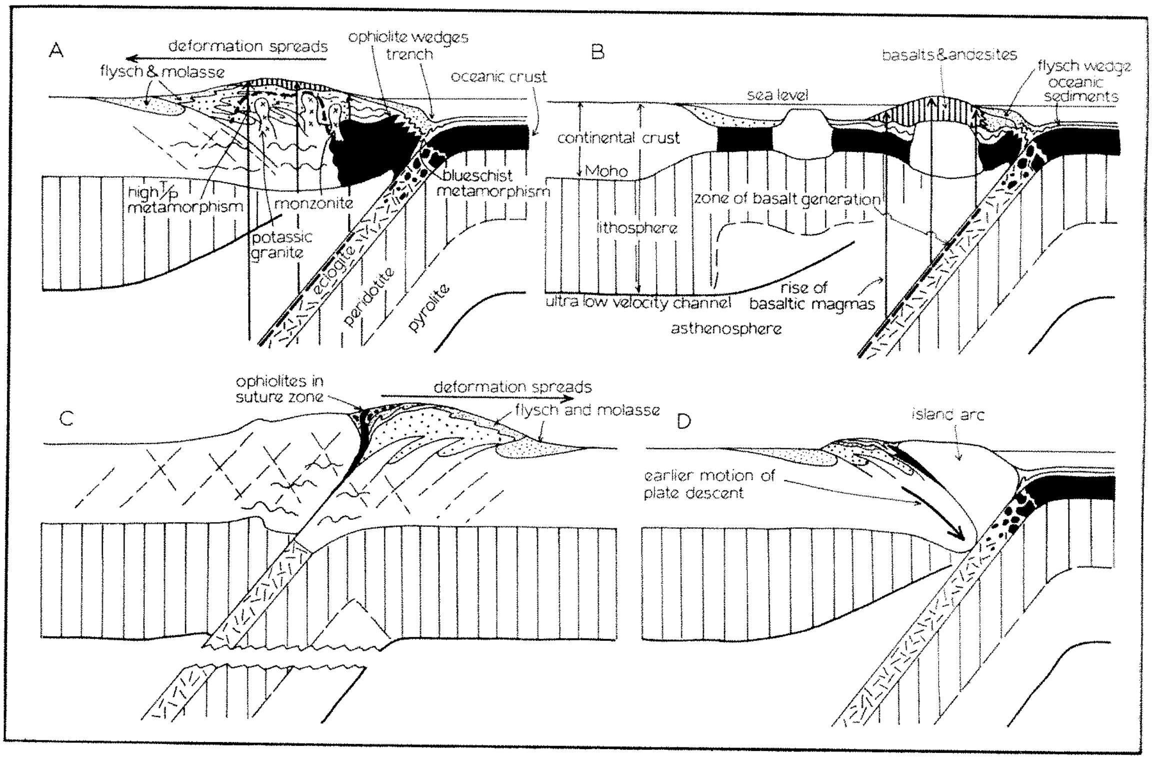


Fig. 3. Models for four basic types of orogenic belts developed at consuming plate margins. (*A*) Cordilleran-type orthotectonic orogen developed at the continental margin of a leading plate edge. The Mesozoic cordilleran orogen of the western United States is taken as the type example. (*B*) Island arc orthotectonic orogen developed on leading plate edge, perhaps nucleated on a microcontinent, within the ocean. (*C*) Himalayan-type paratectonic orogen developed by continental collision. (*D*) Paratectonic orogen developed by collision of a continent and island arc, followed by the development of a new consuming plate margin on the oceanic side of the arc. The Tertiary fold belt of New Guinea seems to have developed in this way. Southern New Guinea was part of the Australian continent and, after the contraction of an ocean (part of Tethys) between southern and northern New Guinea (Bismark island arc), collided with the Bismarck Island arc in Miocene times. A new trench then formed north of New Guinea to consume the Pacific Plate.

(1) *Cordilleran-type.* These develop at the continental margin of a leading plate edge when lithosphere consumption begins near the foot of a continental rise[9,13]. They are characterized by a deformation pattern (migrating towards the continent) developed by the contraction of the leading plate edge (Fig. 3*A*) weakened by the high heat-flow associated with the region behind the trench. On the inner wall of the trench, wedges of oceanic crust (ophiolite suite consisting of ultrabasic rocks overlain by basic intrusives and extrusives) are thrust up. These and the mélange of sediment scraped from the descending plate are deformed and metamorphosed in the high pressure, low temperature, regime of the blueschist facies. As the deformation spreads away from the trench, the sediments of the old continental rise are thrust, above basement wedges, on to the continent and affected by a high temperature metamorphism. Volcanics of the convergent tholeite-alkali basalt and calc-alkalic andesite suite are erupted and thus an orogen characterized by a paired metamorphic zonation is developed. The Jurassic/Cretaceous (Nevadan to Laramide) cordilleran orogen is believed to have developed in this way. The amount of lateral continental accretion achieved by a cordilleran orogeny may be quite small because, although continent-derived sediments are spread on to oceanic crust, the strip of oceanic crust trapped against the continent by the trench may be narrow and furthermore both this strip and the continental basement behind it probably suffer considerable contractions. The total width of an orogen such as the Cordilleras, the Andes, or the early Caledonides[11] cannot be considered to be a prism accreted during the orogeny. Only those parts underlain by ophiolite complexes may be taken as the accreted portion and the paucity of exposed ophiolites militates against an estimate.

(2) *Island arcs.* We suggest that island arcs are built when lithosphere consumption begins at some distance from the continental margin (Fig. 3*B*). Upthrusting of ophiolite wedges and the development of paired metamorphic belts occur[17] as in cordilleran orogeny. The crust of island arcs, perhaps nucleated on microcontinents, is built by the scraping up of oceanic sediments (of which chert is a significant component) and the accumulation of a thick pile of basalts and andesites and their acid derivatives[18]. Island arc development is an extremely effective mode for continental accretion. Not only is it a direct mechanism of building continental crust within an ocean, but the small ocean basins behind arcs are major sediment traps. The accumulation of a thick sedimentary sequence may convert a small ocean basin into continental crust, particularly as the high geothermal gradients[16] are likely to result in metamorphism.

(3) *Himalayan-type*. These are the result of the collision of an aseismic continental margin carrying a sedimentary wedge, against an opposing continental margin in the final stages of oceanic contraction. They are characteristically asymmetric and result from the squeezing of sediments which are heaped onto the continent of the underthrust plate (Fig. 3*C*). Upthrust wedges of oceanic crust (ophiolites) occurring in suture zones (for example, Indus Suture of the high Himalayas; Ivrea Zone of the Alps) are of great significance because these ophiolite belts represent the join-lines of collided continents. Underthrusting of a continent cannot proceed to any great degree because of buoyancy considerations and crushing and shortening begins to occur over a wide zone possibly following the detachment and sinking of the descending plate[4]. Himalayan-type orogeny works against continental accretion in that a small amount of continent may be underthrust.

(4) *New Guinea-type*. This type (Fig. 3*D*) results from the consumption of oceanic lithosphere on the continental side of an island arc, for example the consumption of the Tasman Sea on the west side of the New Hebrides arc. Eventually the continent carrying a marginal sedimentary wedge collides with, and partially underthrusts, the arc. Continued underthrusting is prevented by the buoyancy of the continent[4] and at this stage the descending slab is detached and a new trench is formed to consume lithosphere from the oceanic side. An island arc is thus accreted on to a continent. This notion of flip-over[4] in the direction of lithosphere underthrusting may be a partial explanation of why trenches are commonly associated with orogenic belts on continental margins. The trenches and island arcs develop within the ocean perhaps to be subsequently driven towards, and accumulate at, the continental margin.

Pre-Mesozoic Oceans

Plate tectonics will probably be used as a rigorous geometrical tool in working out the relationship between continents, orogenic belts and oceans well back into Mesozoic times. It will, however, only be valuable as a qualitative notion in understanding the relationships between older orogenic belts and long-vanished pre-Mesozoic oceans, unless large segments of transform faults like the San Andreas Fault are preserved and if palaeomagnetic isoclines are systematically deduced. If pre-Mesozoic orogenic belts were related to the evolution of the oceans, and the view that geosynclines are simply oceans and continental margins is accepted, detailed synthesis of sedimentary, igneous and metamorphic facies-assemblages in the older orogenic belts should yield valuable information for the evolution of the early oceans. This has been attempted for the Appalachian/Caledonian Orogen[10–12] where the geological history is consistent with this approach. The precise site of lithosphere consumption within the older orogens is likely to be represented by blueschist belts and ophiolite complexes. The ophiolites of the median suture of the Urals are associated with blueschist metamorphism and almost certainly lie on the site of a contracted ocean. The late pre-Cambrian (Mozambique–Damaran belts of Africa, Baikalian belt of Siberia, Fig. 1) orogens apparently present an obstacle to an oceanic origin in that large areas of these belts consist of regenerated earlier continental crust. The existence of earlier basement rocks beneath an orogenic belt, however, as argued above, does not imply a totally intra-continental origin, for during continental collision the ocean may be reduced to a narrow ophiolite bearing suture. A section across the Himalayas at a depth of 30 km might reveal a wide "regeneration" zone. Ophiolites occur along the edge of the Dahomey Belt in Ghana[19] but are rare in other African belts.

Within the oldest cratonic nuclei of Africa, such as the Rhodesian Craton, the mafic volcanic rocks and serpentine forming the lower parts (Sebakwian and Bulawayan) of the Schist Belt sequences[20] are overlain by more andesitic volcanics and coarse clastic sequences. It may be that the andesites and coarse clastic sediments represent island arcs built on a Sebakwian/Bulawayan ocean floor older than 3×10^9 yr. In the Yellowknife region of the Great Slave province of Canada tholeitic and calc-alkalic volcanics about $2{\cdot}6 \times 10^9$ yr old have been suggested to belong to an old island arc complex[21].

We submit, then, that ocean-driven plate mechanisms have been responsible for the growth and evolution of continents since at least 3×10^9 yr ago. During the early Pre-Cambrian, oceanic lithosphere plates perhaps considerably thinner and more numerous than at present controlled the growth of continental crust by the eruption of basalts and andesites and their acidic derivatives in island arc complexes. These were amalgamated by the moving plates until at some stage they formed small continental nuclei. We believe this process to be happening now, in a small scale way in the south-west Pacific, where the New Hebrides/Fiji/Tonga arc-transform system is building continental crust. Since the nuclei were formed, they were added to by the processes of accretion described here. During the early stages of continental growth island arc mechanisms must have been dominant, but as the nuclei enlarged they contributed increasing volumes of sediments to be spread on to the ocean floor as continental rises. Cordilleran accretion mechanisms have thus probably become progressively more important. This model of continental growth involving accretion on earlier nuclei does not imply a concentric accretion. The continuous evolution of oceans by plate growth and consumption leads to complex alternate phases of accretion on different margins of continents. As the continents grow and the oceans diminish in size, oceanic expansion–contraction cycles are likely to become more important. The cyclic opening and closing of oceans are inherent in the concept of plate tectonics because, if this were not the case, a single large continent would have been developed long ago. Continental accretion, however, may be exceedingly irregular, involving a continuous sequence of separation and amalgamation of continents, sometimes along old lines of earlier orogenic belts and sometimes across them to open new continent/ocean interfaces. Such terms as Gondwana, Laurasia and Pangea can therefore only properly be used to describe temporary conjunctions of continents.

We thank Professor Sir Edward Bullard for his encouragement and for critically reading the manuscript. J. F. D. acknowledges discussions with Professor C. L. Drake and Dr D. P. McKenzie.

Received December 10, 1969.

[1] McKenzie, D. P., and Parker, R. L., *Nature*, **216**, 1276 (1967).
[2] Morgan, W. J., *J. Geophys. Res.*, **73**, 1959 (1968).
[3] Isacks, B. L., Oliver, J., and Sykes, L. R., *J. Geophys. Res.*, **73**, 5885 (1968).
[4] McKenzie, D. P., *Geophys. J. Roy. Astron. Soc.*, **18**, 1 (1969).
[5] Le Pichon, X., *J. Geophys. Res.*, **73**, 3661 (1968).
[6] Drake, C. L., Ewing, M., and Sutton, G. H., in *Physics and Chemistry of the Earth*, **3**, 110 (Pergamon, Oxford, 1959).
[7] Dickson, G. O., Pitman, W. C., and Heirtzler, J. R., *J. Geophys. Res.*, **73**, 2087 (1968).
[8] McKenzie, D. P., and Morgan, W. J., *Nature*, **224**, 125 (1969).
[9] Dewey, J. F., *Earth Plan. Sci. Lett.*, **6**, 189 (1969).
[10] Wilson, J. T., *Nature*, **211**, 676 (1966).
[11] Dewey, J. F., *Nature*, **222**, 124 (1969).
[12] Bird, J. M., and Dewey, J. F., *Geol. Soc. Amer. Bull.* (in the press).
[13] Sutton, J., *Proc. Geol. Assoc.*, **79**, 275 (1968).
[14] Dearnley, R., *Nature*, **206**, 1083 (1965).
[15] Dietz, R. S., and Holden, J. C., *J. Geol.*, **65**, 566 (1967).
[16] McKenzie, D. P., and Sclater, J. G., *J. Geophys. Res.*, **73**, 3173 (1968).
[17] Miyashiro, A., *Medd. Dansk Geol. Foren.*, **17**, 390 (1967).
[18] Sugimura, A., Matsuda, T., Chinzei, K., and Nakamura, K., *Bull. Volc.*, **26**, 125 (1963).
[19] Grant, N. K., *Geol. Soc. Amer. Bull.*, **80**, 45 (1969).
[20] Amm, F. L., *Rhodesia Geol. Surv. Bull.*, **35**, 307 (1940).
[21] Folinsbee, R. E., Baadsgaard, H., Cumming, G. L., and Green, D. C., *Amer. Geophys. Union Geophys. Mem.*, **13**, 441 (1968).

●

LETTERS TO THE EDITOR

PHYSICAL SCIENCES

Polarization of the Radio Pulses from the Crab Nebula Pulsar

We have undertaken a systematic investigation of the polarization of radio pulses from a number of pulsars, using a polarimeter feed in the Mark I radio telescope at 408 MHz. The results for the Crab Nebula pulsar, NP 0532, are of special interest, because they may be compared with the optical polarization recently measured by Wampler *et al.*[1]. We therefore now present a preliminary analysis for the main radio pulse, both in the form of an integration over many pulses and for individual pulses recorded photographically.

Our apparatus records independently the four Stokes parameters, presenting them either on a 4-channel oscilloscope, which displays individual pulses, or as integrated pulse profiles, giving the average over some thousands of pulses received over a period of several minutes. The pulsar NP 0532 is notoriously variable in that it occasionally produces an individual pulse very much larger than the average, so that about 1 in 10^4 pulses is photographable on the oscilloscope. We therefore arranged to trigger the oscilloscope only for large pulses; these were detected in a separate radio receiver tuned 2 MHz higher in frequency, so that the pulse arrived 12 ms earlier in the triggering channel, due to the interstellar dispersion in pulse arrival time.

Tracings of some of these photographs are shown in Fig. 1. All were recorded in the space of 10 min. The receiver bandwidth was 330 kHz (to 3 dB points), and the time constant of the detector was 0·5 ms. The pulses with apparent half-widths about 3·5 ms are therefore somewhat lengthened by dispersion in the receiver bandwidth, amounting to 2 ms. The polarization is predominantly linear, and totals about 70 per cent. It may be seen that the position angle of the plane of polarization of the largest pulse differs by more than 60° from those of the smaller pulses. With the present time resolution there is no conclusive evidence of fine structure in these pulses.

The shape of the integrated pulse profiles is very similar to that of the individual pulses, and also shows high polarization. At the peak of the pulse the ratio

$$\frac{(Q^2+U^2)^{\frac{1}{2}}}{I}=0{\cdot}65\pm0{\cdot}15$$

The plane of polarization does not rotate by more than 20° during the central 3 ms of the pulse, and does not change between successive 4-min integrations. The interpulse is less strongly polarized.

The high degree of polarization of the main pulse shows that the plane polarization does not contain much fine structure with time scale less than the present resolution, and further that the position angle variation from pulse to pulse is usually small. Other measurements made at 240 MHz with a time resolution of 3 ms show the same high degree of linear polarization.

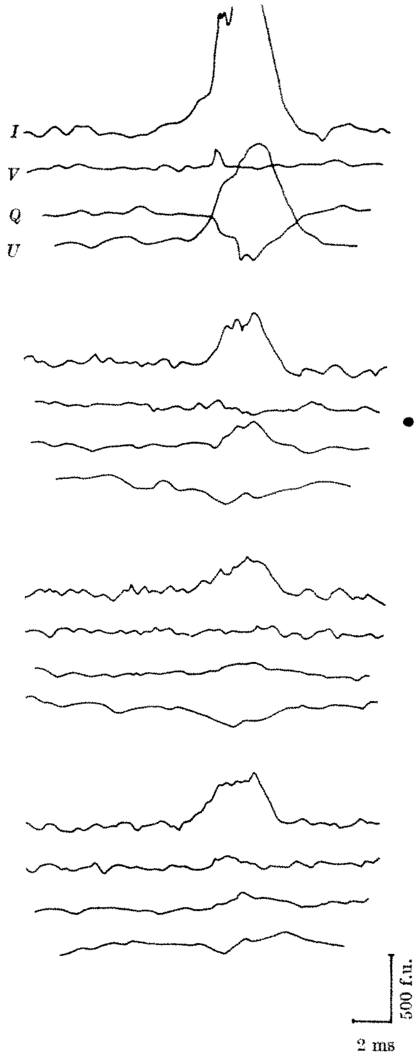


Fig. 1. Stokes parameters I, V, Q and U of four individual pulses from NP 0532.

These results are in sharp contrast to the optical measurements, which show only 10–15 per cent polarization, with a plane swinging through 120° during the centre 2 ms of the pulse. Wampler *et al.*[1] show that the optical results fit well with a simple geometrical model of the pulsar, in which the plane of polarization is determined by the orientation of two axes, a rotation axis and another related to the magnetic field. If the main radio and optical pulses are emitted simultaneously, then the model must evidently be revised. Observations at Arecibo, however (F. Drake, personal communication), have shown that the integrated profile of radio pulses observed at 408 MHz and lower frequencies shows a broad precursor pulse a few milliseconds before the sharp feature corresponding to the optical pulse. If our recordings refer to the precursor, it should be possible to describe the polarization angle as a continuous function through a considerable part of the pulsar cycle. We would then surmise that the sharp pulse and the precursor originate in different but adjacent regions.

D. A. Graham
A. G. Lyne
F. G. Smith

University of Manchester,
Nuffield Radio Astronomy Laboratories,
Jodrell Bank.

Received January 13, 1970.

[1] Wampler, E. J., Scargle, J. D., and Miller, J. S., *Astrophys. J. Lett.*, 157, L1 (1969).

Pulsar NP 0532: Average Polarization and Daily Variability at 430 MHz

MEASUREMENT of optical polarization has of course been limited to NP 0532. The optical radiation in both the main pulse and the interpulse, averaged over many pulses, is weakly linearly polarized (~15 per cent) with a position angle which rotates in a manner akin to that in PSR 0833–45 (refs. 1 and 2). The polarization properties, as well as the total intensity and pulse shape, seem to be time-dependent, however[3]. Circular polarization is absent.

With the single exception of PSR 0833–45, in an average over many pulses the polarized Stokes parameters tend to be reduced by the variability of pulsar polarization. It was therefore with some degree of temerity that we embarked on the measurement of the average Stokes parameters of NP 0532 at 430 MHz. Just to detect the pulsar in total intensity requires averaging over at least 10^4 pulses, and there are large fluctuations in the total intensity of individual pulses[4].

The observations were made with the 1,000-foot spherical reflector and 430 MHz line feed of the Arecibo Observatory. This feed provides two orthogonal senses of linear polarization, which are immediately combined to yield the two senses of circular polarization. These are then amplified, mixed to 30 MHz and transferred to the control building through coaxial cables.

Each sense of polarization was converted into two channels by mixing to baseband with a 30 MHz oscillator. The phase of the oscillator differed by $\pi/2$ for the two channels, so that the resulting voltage of the two channels at baseband differed in phase by $\pi/2$. Thus the four channels available at baseband consisted of the components $E_{\text{RH}}e^{i\omega t}$, $E_{\text{RH}}e^{i(\omega t+\pi/2)}$, $E_{\text{LH}}e^{i\omega t}$, $E_{\text{LH}}e^{i(\omega t+\pi/2)}$ where E_{RH} and E_{LH} denote the voltages available from the amplifiers on the two senses of right and left hand circular polarization. These four channels were passed through four identical filters which defined both the bandwidth and time constant; the bandwidth to 3-dB points was 8·2 kHz. The channels were simultaneously digitally sampled, recorded and later multiplied together[5] to provide the four Stokes parameters. For a total duration of 10 ms within each pulse period, samples were obtained at 5 μs intervals in windows centred on each pulse component. Samples were taken synchronously with the pulse period. The continuum emission from the Crab nebula itself was used as a calibration and to eliminate instrumental polarization; the source was assumed to be unpolarized.

Rotation of the feed during tracking was accounted for. The Faraday rotation due to the ionosphere was measured approximately using the critical frequency from the ionosonde at the Arecibo Observatory in conjunction with approximate profiles of ionospheric density kindly supplied by G. Nelson. The critical frequency was measured continually during our observations, and the Faraday rotation, which varied from about 13° to 26°, was accounted for.

Observations were made for two or three hours on October 26 and 27 and November 1 and 3, 1969. Unfortunately, the lengths of cables in the systems were changed before we were able to fix the directions to which the linearly polarized Stokes parameter refer. We cannot therefore specify the position angle of linearly polarized radiation.

Fig. 1 shows the four Stokes parameters, averaged over 757,600 pulses. The individual data points, whose separation is 50 μs and which are nearly independent, have been smoothed by 200 μs to increase signal/noise—no significant short time scale structure was present in any Stokes parameter before smoothing. It is immediately evident that there is a high degree of linear polarization whose position angle must be independent of time because one of the linear Stokes parameters is zero. This point was also clear on the original data, and therefore the direction of the reference system was rotated so that all of the polarized power appeared in one Stokes parameter, for the sole purpose of clarification in presentation.

Fig. 1 also shows the fractional linear polarization. This is normally defined as $(Q^2+U^2)^{1/2}/I$, but because all of the polarized power appears in only one Stokes parameter, the fractional polarization given in Fig. 1 was computed simply as Q/I.

We examined the results averaged over each day of observation. Although the small signal to noise ratio prohibited seeing variation of the polarized Stokes parameters (Q, U and V) from day to day, it was clear that the total intensity, I, was variable from day to day in some of the pulse components. We emphasize that our measurements of the Stokes parameters are in terms of the intensity of the Crab Nebula, which was responsible for 89 per cent of the system noise, and that our detector has a perfect square law because detection was done digitally. The flux, S, in each of the three components was obtained for each of the four days by integrating over the appropriate phase intervals within the pulse period (see Fig. 1) and are given in Fig. 2.

Fig. 1 confirms the existence of three components in the pulse period (unpublished work of Rankin *et al.*). From Fig. 1 we infer (errors quoted are maximum errors derived from the peak to peak noise level in the baselines of Fig. 1): (*a*) The precursor is essentially 100 per cent linearly polarized over most of its width and the variations are consistent with the noise levels in Q and I. The main pulse is about 18 per cent linearly polarized during its rise and at its peak and more highly polarized during its decay. The polarization of the interpulse is very weak indeed, less than 11 per cent at its peak. The fractional polarization increases near the final stages of both the main pulse and the interpulse, but this could easily result from the noise levels in Q and I. (*b*) The position angles of the precursor and the main pulse are the same, and the angles do not rotate within either pulse. Noise fluctuations in Q and U allow random variations of about ±20°. Systematic variation of the position angle within each pulse is much less. (*c*) The fractional circular polarization, V/I, is less than about 25, 8 and 10 per cent for the precursor, main pulse and interpulse respectively.

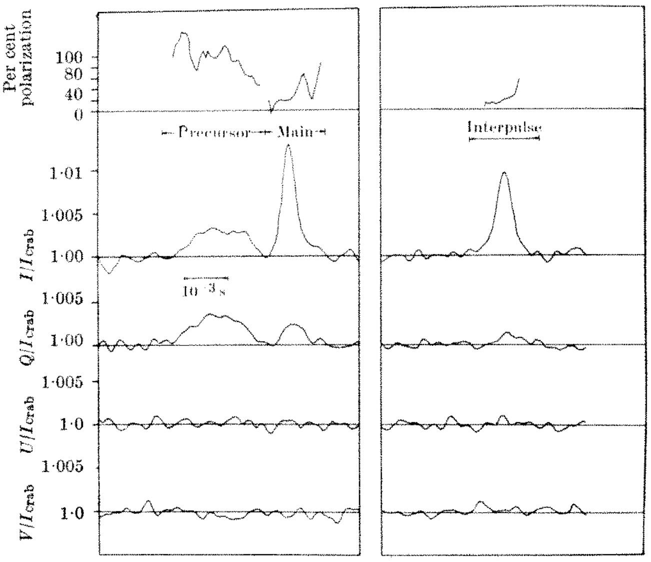


Fig. 1. From the top down: fractional linear polarization Q/I, in per cent; the total intensity Stokes parameter, I; a linearly polarized Stokes parameter, Q; the other linearly polarized Stokes parameter, U; the circularly polarized Stokes parameter, V. All Stokes parameters are in terms of the total intensity. Stokes parameter of the Crab Nebula radio source, I_{crab}.

Of course the high fractional linear polarization of the precursor itself implies little fractional circular polarization. (*d*) The polarization properties of the precursor must be constant from pulse to pulse, because the average polarized power in one of the linear Stokes parameters is essentially equal to the total intensity.

Our results for the main pulse and for the interpulse are somewhat affected by the measurement process. Our bandwidth of 8·2 kHz yields a time resolution of halfwidth about 120 μs because of interstellar dispersion and filter rise time. The halfwidth of the main pulse without the 200 μs smoothing in Fig. 1, not shown here, is about 250 μs. Thus instrumental broadening is not completely negligible, and variations of the Stokes parameters within the pulses are integrated over to some extent. If the position angle rotates by about 90°, as it does for PSR 0833–45, the average value of the Stokes parameter, *U*, across the pulse in Fig. 1 would be nearly zero; instrumental broadening would make it difficult to detect.

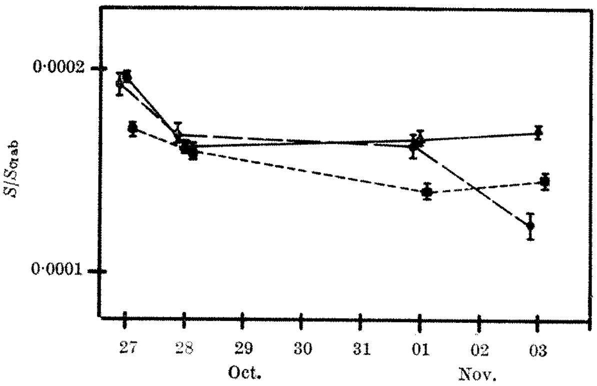


Fig. 2. Flux of the three pulse components averaged over the observing period of each day, in units of the flux of the Crab Nebula radio source, S_{Crab}. Errors shown are probable errors. ● – – – ●, Precursor; ▲—▲, main pulse; ■ - - - ■, interpulse.

The lack of rotation of the angle of rotation in the precursor, however, must be genuine, because the precursor does not suffer from inadequate time resolution. This constancy is in marked contrast to previous results and to the currently accepted view that the rotation is a general phenomenon. Given the picture of Radhakrishnan and Cooke[6] that the neutron star emits only in a cone centred on the magnetic axis, the lack of rotation of the polarization angle implies that the magnetic axis and line of sight to the Earth are instantaneously very closely aligned as the neutron star rotates. This implies that the precursor–mainpulse combination is associated with one magnetic pole and the interpulse with the other, and therefore that the magnetic axis is close to perpendicular to the rotation axis. The low probability of such a favourable alignment in the most prominent supernovae remnant perhaps casts some doubt on this theory.

The linear polarization of the precursor must be responsible, in large part, for the variation of its intensity found by Rankin *et al.*, who observed only one sense of linear polarization. They used a telescope with an alt-az tracking system and observed during the daytime when ionospheric Faraday rotation is large and variable. These effects would cause the essentially completely linearly polarized precursor both to vary its apparent intensity and to appear weaker, on the average, than it really is. Not all of its observed variation can be ascribed to polarization, however.

The total flux, *S*, of each pulse component was calculated by integrating the Stokes parameter, *I*, over time (Fig. 2). Of course, polarization cannot affect these results. The variations of the precursor and main pulse are much larger than the errors, while the flux of the interpulse varies somewhat less. There is no obvious correlation.

The physical cause for the variations must lie in the pulsar itself rather than in interstellar scintillation, because scintillation would be expected to affect all three components equally. Unfortunately, the Crab Nebula generates too much noise to examine the flux behaviour in our data on a time scale less than one day. But the large fluctuations in pulse intensity, which facilitated the discovery of NP 0532, show that variations exist on shorter time scales too—time scales which may include the range usually associated with interstellar scintillation. Variations on such time scales are found at optical wavelengths[3], but we in no sense imply any association whatsoever between the emission processes in the radio and optical, in view of the differences in pulse structure and polarization for the two wavelength regions. Other pulsars may have similar intrinsic variations; it is the multiple pulse structure of NP 0532 which allows us to conclude that scintillation does not play a part.

We thank D. W. Richards for discussions and J. Comella for assistance. The Arecibo Observatory is operated by Cornell University under contract to the US National Science Foundation and with partial support from the Advanced Research Projects Agency. This work was supported in part by the US Air Force Office of Scientific Research. One of us (J. M. R.) acknowledges the support of a NASA traineeship during the early part of this work and present partial support from the Van Allen–Link Fellowship Foundation.

D. B. CAMPBELL
C. HEILES*

Cornell–Sydney Astronomy Center,
Arecibo Observatory,
Arecibo, Puerto Rico.

J. M. RANKIN

University of Iowa,
Iowa City,
Iowa.

Received January 14, 1970.

* On leave from the University of California, Berkeley.

[1] Wampler, E. J., Scargle, J. D., and Miller, J. S., *Astrophys. J. Lett.*, **157**, L1 (1969).
[2] Warner, B., Nather, R. E., and MacFarlane, M., *Nature*, **222**, 233 (1969).
[3] Cocke, W. J., Disney, M. J., and Gehrels, T., *Nature*, **223**, 576 (1969).
[4] Staelin, D. H., and Reifenstein, E. C. III, *Science*, **162**, 1481 (1968).
[5] Cohen, M., *Proc. IRE*, **46**, 183 (1958).
[6] Radhakrishnan, V., and Cooke, D. J., *Astrophys. Lett.*, **3**, 225 (1969).

Size and Motion of the Interstellar Scintillation Pattern from Observations of CP 1133

THE time variations of the intensity of pulsar radiation at radio frequencies may be described by the superposition of short and long term fluctuations. Scheuer[1] has shown that the short term fluctuations are most probably intrinsic to the source, and both Scheuer and Salpeter[2] have suggested that the long term fluctuations may be scintillation caused by irregularities in the interstellar medium. B. J. R. (ref. 3 and unpublished work) provided some evidence for the interstellar scintillation hypothesis when he observed a correlation between dispersion measure and the characteristic bandwidth over which the intensity variations are correlated. Additional evidence has been provided by Lang[4], who has shown that the long term variations of four pulsars have power spectra, modulation indices, frequency distributions and decorrelation frequencies which are consistent with the predictions of scintillation theory.

The simplest theory of the scintillations relies on the assumption that the motion of the pulsar and/or the interstellar medium with respect to the Earth causes a stable diffraction pattern to be swept past a single antenna. We have observed the fluctuations of pulsar intensity with spaced antennae in an attempt to measure the scale size and motion of this diffraction pattern. In general, the fluctuations may be both partially decorrelated and also show a time shift between the two sites. The maximum cross-correlation coefficient and its associated time delay then give some information about the apparent scale size and velocity of the pattern, respectively.

The pulsar CP 1133 was observed for simultaneous three hour periods at Arecibo and Jodrell Bank on June 20 and 22, 1969. Observations were made with right hand circularly polarized antennae connected to radiometers centred at 406·0 MHz with bandwidths of 500 kHz and time constants of 10 ms.

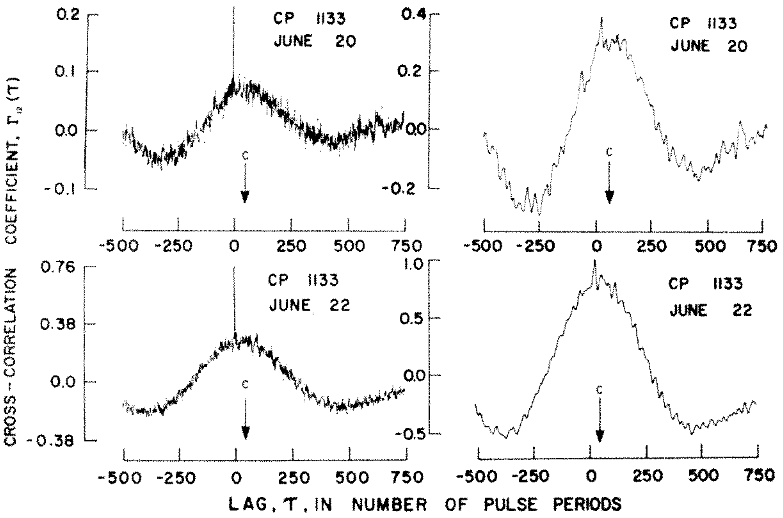


Fig. 2. Cross-correlations of pulse intensities detected at Arecibo and Jodrell Bank (left side). The right side shows cross-correlations between pulse intensities smoothed by taking running means over ten pulses. The centroid of the cross-correlation function is denoted by the letter C.

Pulse intensities (ON intensities) were calculated by integrating the power within windows of data which were 100 ms wide and which were centred in each pulse. Intensities due to system noise (OFF intensities) were similarly calculated for windows centred midway between pulses. The ON intensities were smoother by running means over ten data points, and the autocorrelation function, $\Gamma_{11}(\tau)$, was formed from the smoother intensities of each observation (Fig. 1). The correlation function $\Gamma_{12}(\tau)$ of the intensities $I_1(t)$ and $I_2(t)$ recorded at time t by radiometers 1 and 2 is defined as

$$\Gamma_{12}(\tau) = <[I_1(t) - <I_1(t)>][I_2(t+\tau) - <I_2(t+\tau)>]>/\sigma_1\sigma_2 \qquad (1)$$

where < > denotes a time average and σ_1^2 and σ_2^2 are the mean square deviations of $I_1(t)$ and $I_2(t)$ respectively. Also shown in Fig. 1 are the modulation indices, M, defined by

$$M = \{[(\sigma_{\mathrm{ON}})^2 - (\sigma_{\mathrm{OFF}})^2]/[<I_{\mathrm{ON}}(t)> - <I_{\mathrm{OFF}}(t)>]^2\}^{1/2} \qquad (2)$$

The autocorrelation functions and the modulation indices observed at the two observatories are nearly identical when the effects of noise are taken into account.

The cross-correlation function, $\Gamma_{12}(\tau)$, was formed from the ON intensities recorded at the two observatories (Fig. 2). These ON intensities were then smoothed by running means over ten data points, and the cross-correlation of the smoothed data is also shown in Fig. 2. The abscissae are defined so that a positive lag corresponds to a pulse group arriving at Arecibo after it arrives at Jodrell Bank. The observed decorrelation is thought to be the result of noise. The narrow spike at zero lag is a result of the short term fluctuations in the radiation intensity. This spike is an additional indication that short term fluctuations are intrinsic to the pulsars.

The peak value of the smoothed cross-correlation functions, $\Gamma_{12}(\tau)$, when corrected for the noise contribution to

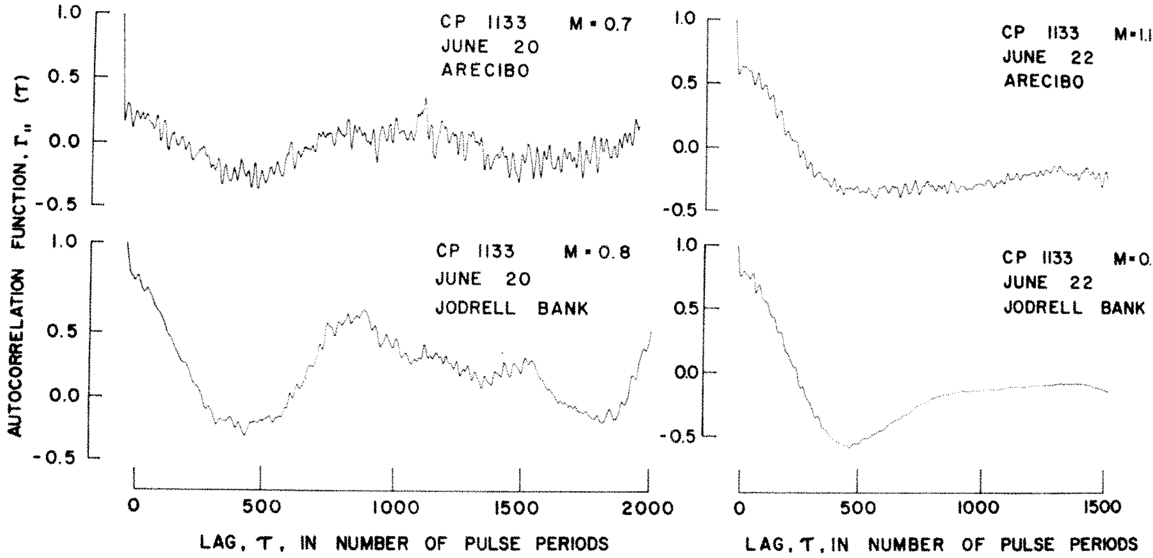


Fig. 1. Autocorrelation functions of smoothed pulse intensities detected at Arecibo and Jodrell Bank on two different days. The pulse intensities were smoothed by taking running means over ten pulses.

σ_1 and σ_2, are not significantly different from unity. From this we conclude that the long term fluctuations in the intensity, as observed at the two observatories, describe the same real function and hence their cross-correlation is an even function. Consequently, the origin of the cross-correlation function is defined by the abscissa of its centroid, as marked by the letter C in Fig. 2. This origin is displaced by 50 pulse periods from the zero lag. The r.m.s. error in determining the centroid, σ_T, was estimated by measuring the peak-to-peak value, P, of the undulations in the cross-correlation function outside the main peak shown in Fig. 2. Using $\sigma_T \approx$[width of main peak/10π] [$\tan^{-1}$ $(P/\Gamma_{12}(\mathrm{O}))$], the June 20 and June 22 measurements give $\sigma_T \approx 12$ and 8 respectively. As the observed lag of 50 pulse periods is roughly five times the r.m.s. error in determining the centroid, the lag is most probably real and indicates that a scintillation explanation for the long term fluctuations of CP 1133 is highly likely.

The 6,400 km baseline was at an angle of 60° to the line of sight giving a projected baseline $d=5{,}500$ km. In order to interpret these observations, we will assume that the observed intensity fluctuations are caused by randomly distributed phase changing irregularities giving rise to a stable diffraction pattern of scale size $r_p = a/\varphi_0$, where a is the irregularity size and φ_0 is the r.m.s. value of the phase fluctuations. If the stable diffraction pattern is moving with a velocity v at an angle θ to the projected baseline,

$$r_p = a/\varphi_0 = v\,\tau_0 \qquad (3)$$

where $\Gamma_{11}\ (\tau_0) = 1/\mathrm{e}$. The observed time shift, T, is related to v by the equation

$$v = d|\cos\theta|/T \qquad (4)$$

Because the variations are highly correlated we deduce that

$$r_p > d|\sin\theta| \qquad (5)$$

These three equations specify the following limits for r_p, v, and θ in terms of the observed τ_0, T and d.

$$\begin{gathered} \tau_0 d/T \gtrsim r_p \gtrsim \tau_0 d\ (T^2+\tau_0^2)^{-1/2} \\ d/T \gtrsim v \gtrsim d\ (T^2+\tau_0^2)^{-1/2} \\ \operatorname{arc\,tan}\ (\tau_0/T) \gtrsim |\theta| \gtrsim 0 \end{gathered} \qquad (6)$$

From our observations we therefore deduce that the parameters of the assumed stable diffraction pattern lie in the following approximate ranges:

$$\begin{gathered} 2{\cdot}7\times10^4\ \mathrm{km} \gtrsim r_p \gtrsim 5\times10^3\ \mathrm{km} \\ 92\ \mathrm{km/s^{-1}} \gtrsim v \gtrsim 22\ \mathrm{km/s^{-1}} \\ 80^\circ \gtrsim |\theta| \gtrsim 0^\circ \end{gathered} \qquad (7)$$

The known motions of the pulsar and/or medium with respect to the Earth, differential galactic rotation, solar motion and the Earth's rotation, predict velocities which lie well within the range given by (7). In addition, the limits placed on r_p can be compared with the predictions of interstellar scintillation theory. B. J. R. (unpublished) analysed the frequency and time decorrelation widths in the fading of ten pulsars, to which was fitted a model of strong scintillation in an extended interstellar scattering medium. If the pulsar is at a distance z kpc and is observed at a frequency f MHz, his model predicts

$$r_p = 0{\cdot}75\times10^2 f\, z^{-1/2}\ \mathrm{km} \qquad (8)$$

Taking the distance of CP 1133 to be 0·15 kpc, equation (8) gives $r_p = 8\times10^4$ km, which is of the same order of magnitude as that specified by (7).

The success of the experiment in detecting a time shift between the two sites should open the way for further probing of the diffraction pattern, preferably at three sites simultaneously. This will lead to interesting deductions concerning the motion and size of the interstellar scattering irregularities themselves.

We thank M. H. Cohen and F. D. Drake for useful discussions. The Arecibo Observatory is operated by Cornell University under contract to the US National Science Foundation and with partial support from the Advanced Research Projects Agency.

KENNETH R. LANG

Arecibo Observatory,
Cornell–Sydney University Astronomy Center.

B. J. RICKETT*

University of Manchester,
Nuffield Radio Astronomy Laboratories,
Jodrell Bank.

Received November 17, 1969.

---

* Now at Department of Applied Physics and Information Science, University of California, San Diego, La Jolla, California.

[1] Scheuer, P. A. G., *Nature*, **218**, 920 (1968).
[2] Salpeter, E. E., *Nature*, **221**, 31 (1969).
[3] Rickett, B. J., *Nature*, **221**, 158 (1969).
[4] Lang, K. R., *Science* (in the press).

Long Baseline Interferometer Measurements of CP 0329

THE pulsar CP 0329 has been observed with an interferometer of $4{\cdot}2\times10^6$ λ baseline at a frequency of 408 MHz. The results place an upper limit of 0·02 arc s on the size of the emitting region in position angle 93°. Although the angular size expected from current models[1] and estimates of distance[2,3] is several orders of magnitude smaller than this, the present limit has been established by direct measurement.

The observation was made with the 46 m paraboloid of the Algonquin Radio Observatory and the 26 m paraboloid of the Dominion Radio Astrophysical Observatory using a technique similar to one described previously[4,5]. Signals received in the band 404 to 412 MHz were folded about the local oscillator frequency and recorded in the video band from 0 to 4 MHz. At one station the second local oscillator was adjusted under computer control to give a fringe frequency of 0·1 Hz.

A gating waveform, recorded at one station in time coincidence with the pulsar signal, was used to remove noise from the interpulse interval when the signals were cross-correlated. Fig. 1 shows the result of cross-correlating signals with the optimum delay. The envelope of pulses has a quasi-sinusoidal variation at the expected frequency of 0·1 Hz. Variations in the pulse amplitude combine with receiver noise to cause deviations from a sine wave.

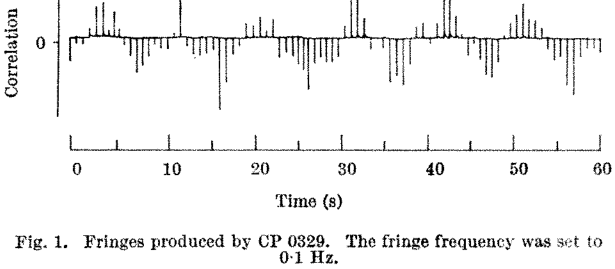


Fig. 1. Fringes produced by CP 0329. The fringe frequency was set to 0·1 Hz.

We thank Dr A. Hewish of the Mullard Radio Observatory for kindly supplying accurate coordinates of the source.

J. A. GALT

Dominion Radio Astrophysical Observatory,
Penticton, British Columbia.

N. W. BROTEN
T. H. LEGG
J. L. LOCKE

Radio and Electrical Engineering Division,
National Research Council,
Ottawa, Ontario.

J. L. YEN

University of Toronto,
Toronto, Ontario.

Received January 12, 1970.

[1] Gold, T., *Nature*, **221**, 25 (1969).

[2] Bridle, A. H., and Venugopal, V. R., *Nature*, **224**, 545 (1969).

[3] Mills, B. Y., *Nature*, **224**, 504 (1969).

[4] Broten, N. W., Legg, T. H., Locke, J. L., McLeish, C. W., Richards, R. S., Chisholm, R. M., Gush, H. P., Yen, J. L., and Galt, J. A., *Science*, **156**, 1592 (1967).

[5] Broten, N. W., Clarke, R. W., Legg, T. H., Locke, J. L., Chisholm, R. M., Yen, J. L., and Galt, J. A., *Mon. Not. Roy. Astron. Soc.*, **146**, 313 (1969).

The Extinction Law in the Magellanic Clouds

THE Magellanic clouds contain only small amounts of obscuring matter compared with our galaxy. The maximum reddening observed is of the order of $E_{B-V}=0{\cdot}5$ magnitudes, and *UBV* photometry suggests that the extinction law in the clouds follows the $1/\lambda$ law[1].

The peculiarity of the interstellar extinction law found in the galaxy—a change in slope in the blue region of the spectrum of the linear relation between Δm and $1/\lambda$ (refs. 2 and 3)—provides a useful clue to the composition and size distribution of the interstellar obscuring grains[4,5]. It is therefore of interest to observe the detailed extinction law in the Magellanic clouds particularly in the blue region of the spectrum.

For this purpose, spectra of reddened stars in the large and the small Magellanic clouds were obtained at a dispersion of 86 Å mm^{-1} at H_γ with the 72-inch telescope at Pretoria. Each plate (emulsion IIa0) included spectra of an MC star and a galactic star of the same spectral type. The MC stars were chosen from the list of Feast *et al.*[1] and the galactic stars from those of Gascoigne[6] and Willstrop[7]. The four cloud stars were independently reclassified for this programme by one of us (R. W.), who found agreement within 1/10 of a subclass with the previous classification. In order to avoid spurious effects caused by atmospheric extinction the galactic stars and the time of their observation were chosen so that their zenith distance should be the same as that of their partner MC star. In addition, each plate included a set of spectra of another galactic star taken at different zenith distances to monitor atmospheric extinction. Finally, photometric calibration was imposed on each plate by a multiple slit calibration spectrograph.

Stellar and calibration spectra were recorded using a digitized Joyce-Loebl microdensitometer, from which fifteen calibration curves were drawn by computer in the range 4000 Å to 5000 Å and applied to the stellar spectra which were recorded at fifty points within the same wavelength range. A number of spectra of four Magellanic cloud stars and three galactic comparison stars yielded four sets which are listed in Table 1.

The magnitude differences were normalized so that the colour excess between $1/\lambda=2{\cdot}0$ μm^{-1} and $1/\lambda=2{\cdot}25$ μm^{-1} is one magnitude and the extinction is zero at $2{\cdot}0$ μm^{-1}.

Table 1. SUMMARY OF THE DATA

Reddened star (Magellanic)			Comparison star		
HD	Sp	*B–V*	HD	Sp	*B–V*
4976	B6I	0·14	6882	B6V	–0·10 (galactic)
5291	B6Ia	0·05	6882	B6V	–0·10 ,,
270754	B1. 5Ia	0·10	160578	B2IV	–0·23 ,,
270086	A1 Ia–0:	0·24	221006	Aop	–0·18 ,,
4976	B6I	0·14	5291	B6Ia	0·05 (Magellanic)
270086	A1 Ia–0:	0·24	268675	A0Ia	0·05 ,,

Because the Magellanic cloud stars were all supergiants, which could not be matched readily by similarly luminous unreddened stars in the galaxy, main sequence stars had to be used for comparison and a correction for luminosity class applied to the magnitude differences. The correction, based on Ardeberg's data[8], ranged for our case from 0 to 0·12 m in the interval 2·25 μm^{-1} to 2·5 μm^{-1}, and had the effect of increasing the difference at the shortwave end. The corrected magnitude differences are shown by filled circles in Fig. 1. The combined errors due to uncertainty in spectral class and photometry at various wavelengths are also shown; the relatively large errors are due to the normalizing factors required because of the small reddening. It should be pointed out that the observed extinction may include a contribution from the galactic foreground which, however, is small ($E_{B-V}\sim 0{\cdot}05$ m) (refs. 9 and 10) compared with the average reddening in the MC stars we have used ($E_{B-V}\sim 0{\cdot}3$ m).

Our data also include two pairs of Magellanic cloud stars of the same spectral type and luminosity class but of different reddening. By differencing these pairs, shown as open circles in Fig. 1, the difficulties of luminosity class discrepancy and foreground reddening are avoided; on account of the smaller difference in reddening, however, the errors are larger than for the other pairs.

The observations are compared in Fig. 1 with the galactic extinction laws in the directions of Cygnus[11] and Perseus[12]. These preliminary observations suggest that the extinction law in the Magellanic clouds resembles the Perseus rather than the Cygnus law and that the change in slope in the neighbourhood of $1/\lambda=2{\cdot}3$ μm^{-1} is similar to that found in the galactic extinction laws. This result, if confirmed by later observations, is important. The interstellar features of our galaxy are represented in the visible wavelength region by two extinction laws—the Cygnus reddening law representing the majority of stars in the Cygnus and Cassiopeia regions, and the Perseus law representing the stars in the direction of the galactic anticentre[13]. Other evidence of a major discontinuity in stellar and interstellar features in the galaxy in the direction of Perseus points to the possibility that the composition and size distribution of grains in this region are distinctly different from those in the Cygnus region[13,14].

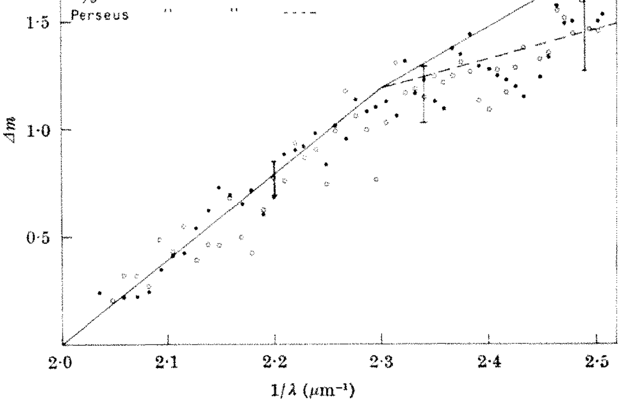


Fig. 1. The extinction law in the Magellanic clouds is compared with the galactic extinction laws for the direction of Cygnus and Perseus.

The present result indicates that the composition, size and optical properties of dust grains in the Magellanic clouds resemble those in the anticentre region of the galaxy.

M. T. Brück
L. C. Lawrence
K. N. Nandy

Royal Observatory,
Edinburgh.

A. D. Thackeray
R. Wood

Radcliffe Observatory,
Pretoria.

Received January 15, 1970.

[1] Feast, M. W., Thackeray, A. D., and Wesselink, A. J., *Mon. Not. Roy. Astron. Soc.*, **121**, 337 (1960).
[2] Whitford, A. E., *Astron. J.*, **63**, 201 (1958).
[3] Nandy, K., *Pub. Roy. Obs. Edin.*, **3**, 142 (1964).
[4] Wickramasinghe, N. C., *Interstellar Grains* (Chapman and Hall, 1967).
[5] Hoyle, F., and Wickramasinghe, N. C., *Nature*, **223**, 459 (1969).
[6] Gascoigne, S. C. B., *Mon. Not. Roy. Astron. Soc.*, **110**, 15 (1950).
[7] Willstrop, R. V., *Mon. Not. Roy. Astron. Soc.*, **69**, 83 (1965).
[8] Ardeberg, A., *Astron. Astrophys.*, **3**, 257 (1969).
[9] Thackeray, A. D., *Adv. Astron. Astrophys.*, **2**, 264 (1963).
[10] Walraven, Th., and Walraven, J. H., *IAU Symp. No. 20*, 321 (1964).
[11] Nandy, K., *Pub. Roy. Obs. Edin.*, **5**, 13 (1965).
[12] Nandy, K., *Pub. Roy. Obs. Edin.*, **6**, 169 (1968).
[13] Brück, M. T., Ireland, J. G., Nandy, K., and Reddish, V. C., *Nature*, **218**, 662 (1968).
[14] Reddish, V. C., *Nature*, **213**, 1107 (1967).

Observation of Structure in Čerenkov Pulses from Extensive Air Showers using Fast Techniques

Light pulses from the night sky were first observed in 1952 by Galbraith and Jelley[1] following Blackett's suggestion[2], and then by other authors (see ref. 3 for a review). These light flashes are due to the Čerenkov radiation produced essentially by the electronic component of extensive air showers (EAS) traversing the atmosphere.

In an experiment on the temporal structure of the Čerenkov flashes carried out at the Testa Grigia Laboratory (altitude 3,500 m above sea level) we detected three anomalous Čerenkov pulses. In two cases (A and B) the pulse was double, and there were two light pulses with a short relative delay (3 and 4 ns). The rise-time of the second pulse was slower than usual. Event A is reproduced in Fig. 1. In one case (C) we observed a light pulse slower and longer than usual.

We used two very high-speed RCA photomultipliers C 70045 D with their cathodes at the focal points of two parabolic mirrors with parallel optical axes, diameter 60 cm, focal length 29 cm and angular acceptance ±3° from the zenith. We then recorded the correlation between the two RCA systems and a coincidence from three 56 AVP Philips photomultipliers at the foci of three spherical mirrors of diameter 50 cm, angular acceptance ±2° and positioned on the vertices of a triangle of area 30 m². The outputs from the two fast RCA photomultipliers were fed to two Tektronix 519 oscilloscopes, and the pulses were recorded photographically. The vertical sensitivity was 10 V cm^{-1} and the sweep speeds 5 ns cm^{-1}. The response of the detecting apparatus to a light pulse of 0·1 ns had a rise-time of 0·5 ns.

The photocathode was screened with an ultraviolet filter (a 50 per cent aqueous solution of $CoSO_4.7H_2O$ in a quartz container). This arrangement improves the signal to background ratio. The threshold sensitivity of our apparatus was estimated using Čerenkov pulses of known intensity produced by cosmic ray muons in distilled water and lucite layers 3 and 15 cm thick. This gives an EAS threshold energy for detection $\simeq 10^{12}$ eV.

We observed 455 EAS Čerenkov flashes by coincidences of the five photomultipliers in a run of 70 h on moonless clear nights. Table 1 lists the characteristics of the three anomalous pulses as observed by the two RCA photomultipliers, together with those of normal pulses. In particular we notice that: (*a*) the first pulse of events A and B is larger in amplitude than the second; (*b*) the duration at half maximum of the first pulse (σ_1) of events A and B is in good agreement with the mean value $<\sigma>$ for normal pulses, while in event A $\sigma_2 > \sigma_1$; (*c*) in event C $\sigma_c \gg <\sigma>$.

Table 1. THE THREE ANOMALOUS PULSES

	E		W		E		W		E	W
	h_1	h_2	h_1	h_2	σ_1	σ_2	σ_1	σ_2	delay	(ns)
Mean value from 455 EAS	2·5		3		1·8		1·7			
Event A	2	1·5	2	1·8	1·5	7	2	6	4	4
Event B	2	1·5	2	1·5	2	2	2	2	3	3
Event C	1·5		2		8		9			

h_i = pulse height (V); σ_i = Half-maximum duration (ns).

E, W refer respectively to the two independent RCA photomultipliers in coincidence; subscripts 1, 2 refer respectively to the first and second pulse.

These anomalous pulses may have a physical or an instrumental explanation. We considered two instrumental causes: (1) the probability of having two spurious after pulses within 4 ns on two independent RCA tubes turned out to be $\sim 10^{-5}$; (2) the probability of casual coincidences due to light pulses produced by scintillation or Čerenkov effects in the photocathode glass or in the filter by EAS particles is $< 10^{-6}$. In any case, the photomultipliers look downward and only detect Čerenkov radiation after reflexion.

One can also exclude the following physical effects: (1) casual coincidences from independent EAS of 10^{12} eV, the probability being $< 10^{-6}$; (2) the second pulse arising from a successive interaction of the primary proton with an energy above the threshold—the delay should be negligible compared with that measured; (3) the second pulse is unlikely to be due to a local effect in an ordinary EAS, such as a single particle lagging behind the shower front and producing Čerenkov light in air inside the cone viewed by the mirrors. The probability of this event is small and the second pulse should be very short, in disagreement with our observation.

A possible interpretation (apart from extreme fluctuations in a normal shower) may be derived from the successive interactions of a primary nucleus in the atmosphere. A simple Monte Carlo calculation shows the possibility of obtaining the observed delays if the energy of the nucleus is $\sim 10^{12}$ eV. The presence of a very massive particle,

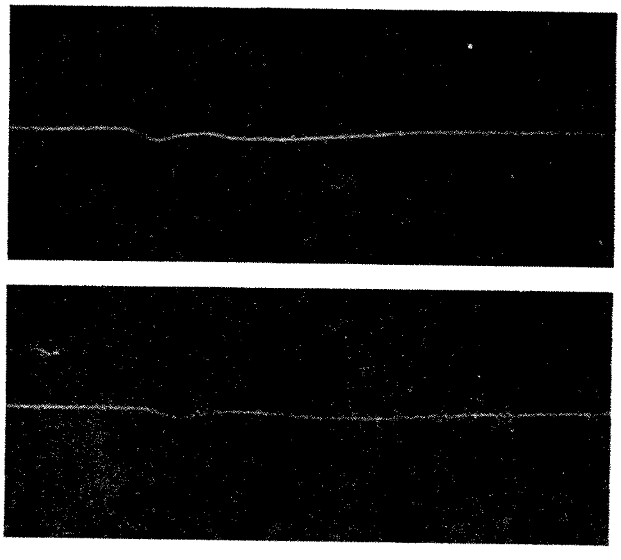


Fig. 1. Double Čerenkov pulse from an EAS (event A, Table 1) as observed by two independent fast channels. The heights of the first pulses are 2 V and of the second 1·8 V and 1·5 V respectively; relative delay 4 × 10⁻⁹ s.

generated in the first N–N collision, also could possibly explain the effect. A simple model shows that such a particle could produce a detectable shower with the measured delay observed in events A and B if the particle mass is ~ 10 GeV c^{-2} and its energy $E_0 \sim 10^{12}$ eV. Further work is under way at the same altitude (3,500 m).

It seems to us that the structure in the Čerenkov pulses which we have obtained with the fast technique opens a new approach to the study of air showers.

G. Bosia
C. Castagnoli
M. Dardo
G. Marangoni

Laboratorio di Cosmo-geofisica del
Consiglio Nazionale delle Richerche,
Istituto di Fisica Generale dell'Università,
10125 Torino.

Received November 18, 1969.

[1] Galbraith, W., and Jelley, J. V., *Nature*, **171**, 349 (1953).
[2] Blackett, P. M. S., *Phys. Soc. London Gassiot Committee Report*, 34 (1948).
[3] Jelley, J. V., *Prog. Elementary Particle and Cosmic Ray Phys.*, **9**, 41 (1967).

Topside Ionosphere of Venus and its Interaction with the Solar Wind

The abrupt termination of the daytime ionosphere of Venus at about 500 km observed with the Mariner V two-frequency occultation experiment provides an extremely interesting picture of the direct interaction of the solar wind with a planetary ionosphere[1,2]. It has been suggested that a pseudo-magnetopause is formed by magnetic fields carried along by the solar wind and forced to pile up on the topside of the highly conducting planetary ionosphere[3,4]. This magnetic obstacle then interacts with the super-alfvénic and supersonic solar wind to form a bow shock; evidence for such a bow shock has been obtained by experiments on Mariner V and Venera IV (refs. 2 and 5).

We have used the observed properties of the solar wind[2] to determine order of magnitude estimates of the characteristic parameters in this interaction.

A pressure balance at the stagnation point formed with the streaming pressure of the solar wind leads to a magnetic field build-up of approximately 50 γ. A balance between the streaming pressure of the solar wind $P_w = K\,n\,mv^2 \cos^2 \Psi$ and the plasma pressure $P_c = Nk(T_e + T_i)$ in the topside ionosphere of Venus is consistent with the sharp cut-off in the electron density profile. At $\Psi = 45°$ from the sub-solar point where the electron density of the topside ionosphere was observed to be $N \simeq 10^4$ cm^{-3} at the 500 km cut-off ("ionopause")[1], we calculate the streaming pressure of the solar wind to be about $8 \cdot 8 \times 10^{-9}$ dynes cm^{-2} using an accommodation coefficient $K = 1$ as is frequently done for the Earth's magnetosphere[6]. With this solar wind pressure and the observed electron density of the topside ionosphere, we obtain about 6,000 K for the plasma temperature $(T_e + T_i)$ of the topside ionosphere. In the following we shall discuss the important constraints which the solar wind interaction places on a self-consistent model of ionospheric temperatures and densities.

The presence or absence of a magnetic field within the ionosphere has important consequences for the structure of the Venus ionosphere. There are two extreme cases: (1) where the presence of an essentially horizontal magnetic field inhibits thermal conduction across field lines, and (2) where either because of the complete absence of a magnetic field or the presence of a tilted magnetic field the thermal structure is controlled by parallel heat conduction. An induced magnetic field due to the interaction of the solar wind with the conducting ionosphere or the presence of a small intrinsic planetary magnetic field can lead to a non-horizontal field within the Venus ionosphere. The upper limit for such a magnetic field inside the ionosphere is estimated to be about 40 γ (J. L. Blank and W. R. Sill at the IAGA Symposium, Madrid, 1969). The following discussion assumes the more likely case of a non-horizontal magnetic field within the Venus ionosphere using a thermal conduction coefficient of $0 \cdot 25\, K_{\|}$. It is not our intention to discuss the detailed ionic composition, so we selected a relatively simple photochemical model for the lower regions of the ionosphere based on the work of McElroy[7]. We have taken CO_2 to be the dominant heavy neutral species at 100 km, and H and He are taken to be the important light neutral constituents. We also have investigated the effect of the presence of H_2 or deuterium (D), but their presence does not affect our results significantly. As a result of using hydrostatic equilibrium for the distribution of the neutral gas density, H and He become the dominant species at higher altitudes. It therefore follows that the ionization peak at about 130 km will be controlled by the neutral CO_2, while the charged particle pressure in the topside is strongly influenced by H and He. For the light ions H^+ and He^+ we have solved the usual balance equations of hydrostatic pressure including gravitational and electrical forces. Combining these with the equations arising from the thermal balance in a multicomponent plasma (including the neutral gases) a self-consistent solution for the temperatures and densities has been obtained[8].

We have selected several boundary conditions based, wherever possible, on published values. At the lower boundary of 100 km we used a neutral temperature of $T_n = 250°$ and a CO_2 density of 2×10^{13} cm^{-3} comprising at least 99 per cent of the total density. The remaining 1 per cent or less was adjusted so that the corresponding topside plasma pressure balances that of the solar wind and simultaneously approximates the observed scale height of electron density. Because the light ion composition is experimentally unknown, we have assumed H^+ (or D^+) and He^+ to be the main constituents of the topside ionosphere. The electron density $N = [He^+] + [H^+] + [CO_2^+]$ was selected to match the observed electron density at 500 km.

Fig. 1 shows the computed ionospheric model with He^+ as the dominant ion. The electron temperature has the value of $T_e = 4{,}090$ K, as compared with the ion temperatures which have a value of $T_i \simeq 1{,}900$ K, considerably higher than the neutral gas temperature $T_n = 550$ K. The

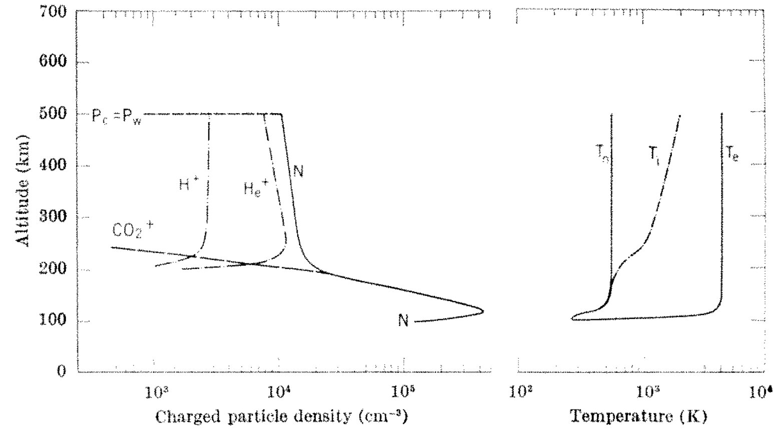


Fig. 1. Charged particle densities and temperatures for the Venus ionosphere when the electron thermal conductivity is $0 \cdot 25\, K_{\|}$. The dashed line $P_c = P_w$ represents the cut-off in electron density ("ionopause") arising from the pressure balance between the solar wind and the charged particles of the Venus ionosphere. The actual ionopause is expected to be a layer whose thickness should be of the order of a proton gyroradius, instead of the abrupt cut-off shown.

peak of electron density and the scale height of topside electron density for this model are in good agreement with the Mariner occultation data.

A similar ionospheric model satisfying the observed conditions is one where H^+ (or D^+) is the dominant ion in the topside ionosphere. Although such a model also gives reasonable agreement with the observed values, we argue that the high value required for the H^+ density (and/or D^+ density) in the topside ionosphere seems unlikely, because the observed density of neutral hydrogen is thought to be relatively low[9] and, in contrast to the Earth, H^+ is produced by photoionization rather than by charge exchange. We therefore favour the model in which He^+ is the dominant topside ion with relatively hot electrons compared with the ion and neutral gases.

In the other limiting case of a horizontal rather than a slightly inclined magnetic field, the thermal structure of the topside ionosphere is significantly different because heat conduction does not play a significant part due to the inhibiting effects of this horizontal magnetic field. In this case, the electron temperature is higher and the ion temperature is lower than in the model presented in Fig. 1.

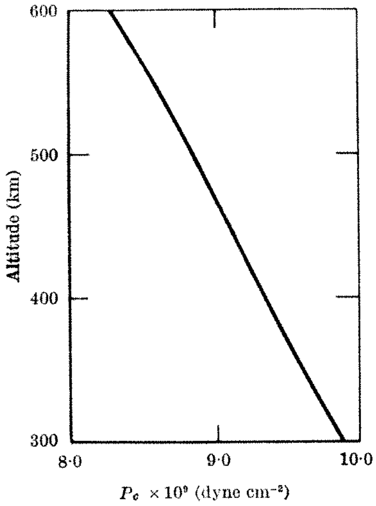


Fig. 2. Charged particle pressure $P_c = Nk(T_e + T_i)$ in the topside Venus ionosphere. At the altitude of the ionopause (500 km), $P_c = 8.8 \times 10^{-9}$ dyne cm^{-2}.

Fig. 2 shows the total pressure distribution based on the density and temperature distributions of Fig. 1. The pressure curve extended to 600 km was obtained by solving the density and temperature equations simultaneously with pressure balance between solar wind and ionospheric plasma pressure at 500 km. The very slow pressure decrease with altitude suggests that the altitude at which the ionosphere terminates (ionopause or anemopause[2]) is a strong function of solar wind pressure. If the solar wind pressure changes by a small amount, our model predicts a substantially unchanged ionosphere with an ionopause at a different altitude. It also suggests that the ionospheric boundary is flat-nosed at the sub-solar wind point and somewhat bulged out near the terminator due to the effect of the aspect angle in the pressure balance equation. Because the streaming pressure of the solar wind on the night side of Venus is negligible, the ionosphere would there extend to considerable altitudes as reported by Mariner V (ref. 1).

S. J. Bauer
R. E. Hartle
J. R. Herman

Laboratory for Planetary Atmospheres,
NASA, Goddard Space Flight Center,
Greenbelt, Maryland.

Received October 24; revised November 17, 1969.

[1] Mariner Stanford Group, *Science*, **158**, 1678 (1967).
[2] Bridge, H. S., Lazarus, A. J., Snyder, C. W., Smith, E. J., Davis, L., Coleman, P. J., and Jones, D. E., *Science*, **158**, 1669 (1967).
[3] Dessler, A. J., in *Atmospheres of Mars and Venus* (edit. by Brandt, J. C., and McElroy, M. B.), 241 (Gordon and Breach, 1968).
[4] Johnson, F. S., *J. Atmos. Sci.*, **25**, 658 (1968).
[5] Dolginov, S. S., Yeroshenko, E. G., and Zhuzgov, L. N., *Kosmich. Issled.*, **6**, 561 (1968).
[6] Spreiter, J. R., and Alksne, A. Y., *Revs. Geophys.*, **7**, 11 (1969).
[7] McElroy, M. B., *J. Geophys. Res.*, **74**, 29 (1969).
[8] Herman, J. R., and Chandra, S., *Planet. Space Sci.*, **17**, 815 (1969).
[9] Barth, C. A., Wallace, L., and Pearce, J. B., *J. Geophys. Res.*, **73**, 2541 (1968).

Hoyle–Narlikar Quantization of Wheeler–Feynman Electrodynamics

Hoyle and Narlikar[1] (HN) have proposed a quantized version of the Wheeler–Feynman[2,3] (WF) theory of classical electrodynamics. It is the purpose of this report to point out that the HN treatment of spontaneous emission deviates from the WF prescription. This is true even in the classical limit; however, in this limit the numerical effects of two different deviations cancel.

The WF theory is based on the postulates that charged particles interact via time symmetric ($\frac{1}{2}$ retarded + $\frac{1}{2}$ advanced) electromagnetic fields, and that there is no self interaction. The theory then takes into account the response of all charged particles in the universe to the field of each accelerated charge. The response field accounts for radiation reaction; it also re-establishes the full retarded field around each accelerated particle and defines a universal direction of time in which all the retarded potentials point.

The WF prescription for computing the response of the universe[2] to the field of an accelerated particle a is to let each charged particle b in the universe be excited by the full retarded and attenuated field of a, then let b react on a with one half its advanced field, unattenuated. (The full retarded and attenuated field is used for the action of a on b in order to allow for fields of particles other than a on b. No such allowance is necessary in the case of the action of b on a, because a summation on the contributions of all charged particles b in the universe is carried out explicitly.) The reaction field in the HN treatment is the full advanced field, attenuated.

HN quantize by Feynman's method of summation over all classical trajectories. The interaction term in their Lagrangian is $\int A^{(a)}_{\mathrm{ret}}(b(t))\cdot\dot{b}(t)\mathrm{d}t$. This may be transformed

$$\mathrm{e}\int A^{(a)}_{\mathrm{ret}}(b(t))\cdot\dot{b}(t)\mathrm{d}t = 2\mathrm{e}^2\iint\delta((a(t')-b(t))^2)\dot{a}(t')\cdot\dot{b}(t)\mathrm{d}t'\mathrm{d}t = \mathrm{e}\int A^{(b)}_{\mathrm{adv}}(a(t'))\cdot\dot{a}(t')\mathrm{d}t' \qquad (1)$$

The reaction of b on a is therefore automatically the full advanced field. (We adopt the notation where a stands for the four-vector position of particle a. The middle expression in equation (1) describes retarded or advanced interaction according to the relative position of a and b. HN choose a segment of the world line of particle a and in the vicinity of its future light cone a segment of the world line of particle b. This choice makes the action of a on b retarded and the action of b on a advanced.) Because attenuation is introduced into the interaction before the summation on classical trajectories is completed, however, the reaction of b on a is also attenuated.

In the classical limit, the summation over all classical trajectories may be replaced by the use of the trajectory corresponding to stationary action. When this is done, we find particle *b* moving in the retarded and attenuated field of particle *a*, and particle *a* moving in the full advanced and attenuated field of particle *b*. The use of the full advanced field rather than half the advanced tends to double the radiation reaction. The attenuation tends to cut it down—as it turns out—exactly in half.

The reason for the last point is that the response field from a layer of optical thickness $d\tau$ turns out to be proportional to $e^{-\tau}d\tau$ (ref. 2). The factor $e^{-\tau}$ is due to the attenuation of the field of *a* at *b*. When the reaction is also attenuated, an extra factor $e^{-\tau}$ is required, and the contribution becomes proportional to $e^{-2\tau}d\tau$. In the final result for the response field, the inclusion of attenuation has the effect of replacing $\int_0^\infty e^{-\tau}d\tau = 1$ by $\int_0^\infty e^{-2\tau}d\tau = \frac{1}{2}$.

Whether the effects of doubling the response field and of attenuating it cancel also outside the classical limit should be made clear.

Amnon Katz
Ezuz Ornan
Moshe Schwartz

Department of Physics,
Weizmann Institute,
Rehovoth, Israel.

Received November 18, 1969.

[1] Hoyle, F., and Narlikar, J. V., *Ann. Phys.*, **54**, 207 (1969).
[2] Wheeler, J. A., and Feynman, R. P., *Rev. Mod. Phys.*, **17**, 157 (1945).
[3] Wheeler, J. A., and Feynman, R. P., *Rev. Mod. Phys.*, **21**, 425 (1949).

Microbarograph Observation of Acoustic Gravity Waves

MAY I comment on the recent letter in *Nature* by Murty and Curry[1] on microbarographic observation of acoustic gravity waves.

Events such as these authors describe are rather commonplace in Victoria, Australia, and one such occurrence has been recorded[2] at some length, while Pothecary[3] has noted an English case. Although it would be rash to claim that the processes responsible for exciting gravity waves in the atmosphere are fully understood, it is recognized here that pronounced and sustained pressure oscillations, of amplitude of the order of a millibar, are almost invariably associated with a shallow layer (1–2 km) of cold air, underlying much warmer air. This commonly occurs when a shallow but intense cold front moves north or north-eastward over Melbourne and becomes almost stationary. In most cases it is also possible to find evidence of precipitation, which may be associated with thunder, originating in the warmer air and penetrating the inversion, somewhere within, say, a hundred miles. The conclusion has been drawn that excitation by precipitation, or its concomitant downdraughts, of gravity waves on a marked low level inversion is responsible for the observed oscillations of surface pressure. Ramm and Warren[4] have given a quantitative treatment of the processes involved.

It is suggested that your correspondents should pay some attention to the vertical and horizontal density and wind distribution at the time of the phenomenon they describe.

R. H. Clarke

CSIRO,
Division of Meteorological Physics,
Aspendale, Victoria 3195,
Australia.

Received December 12, 1969.

[1] Murty, R. C., and Curry, M. J., *Nature*, **224**, 169 (1969).
[2] Clarke, R. H., *Quart. J. Roy. Meteorol. Soc.*, **88**, 459 (1962).
[3] Pothecary, I. J. W., *Quart. J. Roy. Meteorol. Soc.*, **80**, 395 (1954).
[4] Ramm, P., and Warren, F. W. G., *Quart. J. Roy. Meteorol. Soc.*, **89**, 349 (1963).

Concentration Wave Propagation in Two-dimensional Liquid-phase Self-oscillating System

OSCILLATING chemical reactions are interesting, not only in themselves but as models of a number of important biological processes[1–5]. Oscillating reactions have been described in which cerium (or manganese) ions catalyse the oxidation of analogues of malonic acid by bromate[6–8]. Oscillations in the concentrations of oxidized and reduced forms of the catalyst cause oscillations in the solution colour, while stirring leads to forced synchronization of oscillations throughout the whole volume. In the absence of stirring, periodic propagation of concentration waves occurs in certain conditions, and such a phenomenon in a one-dimensional system (a long tube) has been described[9,10]. Our work deals with patterns in a thin layer of solution (two-dimensional system).

Fe (1,10-phenanthroline)$_3$ is a convenient indicator for observation of spatial effects in our system. Ferroin and Fe (2,2′-dipyridyl)$_3$ could also be used, not only as indicators but also as catalysts, and it seems that a number of variable valence ions with one-electron transition and standard potential between 0·9 and 1·6 V may be used as catalysts in the system. In the present work, the system bromate, bromomalonic acid (BMA), ferroin was used. BMA was prepared by bromination of malonic acid by bromate–bromide mixture in acidic aqueous solution.

$$3\ CH_2(COOH)_2 + 2\ HBr + HBrO_3 = 3\ CHBr(COOH)_2 + 3\ H_2O$$

Similar results are obtained with BMA prepared by the method described by Conrade[11]. The reaction was performed at room temperature (about 20° C) in Petri dishes about 100 mm in diameter, with a solution layer about 2 mm deep.

Fig. 1 shows a series of sixteen photographs taken at one-minute intervals. In the first photograph, the catalyst is completely reduced, and subsequent photographs show it starting to be oxidized at particular points (leading centres), from which circular waves of oxidation are propagated. The fourth photograph shows oxidation taking place in areas not reached by these waves. The time when these transitions take place is determined by the oscillation period (T_0) of the main part of the space, which is equal to the system oscillation period in the case of forced synchronization by stirring. The next photographs show waves coming from leading centres (with $T < T_0$) oxidizing all the space step by step. When the waves coming from different leading centres collide, mutual annihilation takes place, with the point of collision, situated on a straight line between two leading centres, gradually moving towards the leading centre with the longer period. When a wave from a high-frequency leading centre reaches a low-frequency centre, the latter disappears, and, in the ideal case, a single leading centre with the highest frequency eventually synchronizes the whole space. In most experiments, however, some fronts break at the end of the process and space disorganization takes place (photographs 12–16).

Qualitatively distinctive patterns, for example, radial symmetric and small-cell patterns, are observed at other initial reactant concentrations. Those shown in Fig. 1 resemble Liesegang rings (LR), but it should be emphasized that the phenomenon described differs from LR in two significant features. First, in this case, there are progressive concentration waves while the sediment rings in the LR phenomenon are motionless. Second, LR-pattern is a fixed imprint of a reaction which has proceeded, but in our case the space structure is supported at the expense of redox reaction energy.

We consider here the simplest model of wave propagation in the system. In a one-dimensional system (a thin tube with length L, filled by solution), see Fig. 2, let all

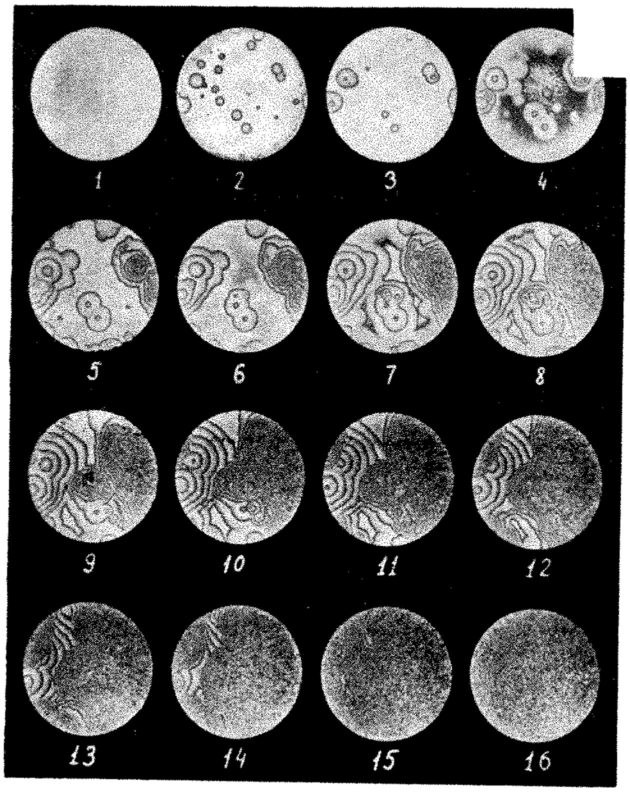


Fig. 1. Concentration wave propagation in two-dimensional self-oscillatory chemical system. Ring diameter equals 100 mm. Initial reactant concentrations (in mol/l.): [BMA] = 0·125; [$NaBrO_3$] = 0·3; [$Fe(phen)_3$] = 0·003; [H_2SO_4] = 0·37.

the points in the system (except X_A and X_B) possess a period of self-oscillation equal to T_o. Leading centres (A and B) have periods equal to T_A and T_B

$$T_A < T_B < T_O$$

The following features of the system, obtained experimentally, form the basis of the model. Relaxation oscillations take place at every point in the space. The period of oscillation is distinctly divided into two phases: an oxidation phase and a reduction phase. The catalyst exists in oxidized form during a small part of the oscillation period. In Fig. 2 the oxidized state is represented by black lines (in the same manner as in Fig. 1) and the line width corresponds to the duration of the oxidation phase. The velocity of wave propagation (V_o) is constant throughout the space and oscillation periods (T_A, T_B and T_o) are constant. When two waves collide, they cancel each other out.

In this model, waves spread in the following manner. All the space is initially in the reduced state, and at $t = 0$ all the points pass into the oxidized state and then the system returns to the reduced state. At $t = T_A$ transition into the oxidized state occurs at the point X_A from which a wave begins to spread with velocity equal to V_o. At $t = T_B$ the same process occurs at X_B. At $T = T_O$, transition into the oxidized state occurs in all parts of space free from waves coming from leading centres, and this annihilates one (external) wave from every leading centre. In the absence of this general oxidation, the velocity of propagation of the zone synchronized by one particular leading centre equals V_o. With general oxidation, the mean velocity of propagation of the zone by leading centres is given by

$$V_i = V_o\left(1 - \frac{T_i}{T_o}\right) \text{ ; with period } T_i$$

At

$$t = \frac{T_o}{V_o} \cdot \frac{X_B - X_A}{2T_o - T_A - T_B}$$

waves from centres A and B collide, and the point of collision then moves towards X_B with mean velocity

$$V = V_o \frac{T_B - T_A}{T_B + T_A}$$

The complete development of the picture in time is accompanied by the appearance of some characteristic points in X,t-space (Fig. 2). At point G, time t_G, general oxidation ceases to the left, from centre A.

$$X_G = 0; \; t_G = T_O \frac{X_A}{V_o(T_o - T_A)}$$

At C, general oxidation ceases between A and B.

$$X_C = \frac{X_B(T_o - T_A) + X_A(T_o - T_B)}{2T_o - T_B - T_A}; \; t_C = \frac{T_o(X_B - X_A)}{V_o(2T_o - T_B - T_A)}$$

At D, the centre B is captured by higher-frequency centre A.

$$X_D = X_B \; ; \; t_D = \frac{T_B(X_B - X_A)}{V_o(T_B - T_A)}$$

It should be noted that the time at which this takes place (t_D) does not depend on general oxidation being present in the system. At E, despite the disappearance of the centre B, its waves continue to propagate in the triangle DEH, and waves from centre A begin to interact with the general oxidation patterns.

$$X_E = \frac{X_B(T_O - T_A) - X_A(T_O - T_B)}{T_B - T_A} \; ; \; t_E = \frac{T_O(X_B - X_A)}{V_O(T_B - T_A)}$$

At F, waves from the higher-frequency centre A fill the whole tube.

$$X_F = L; \; t_F = \frac{T_O(L - X_A)}{V_O(T_O - T_A)}$$

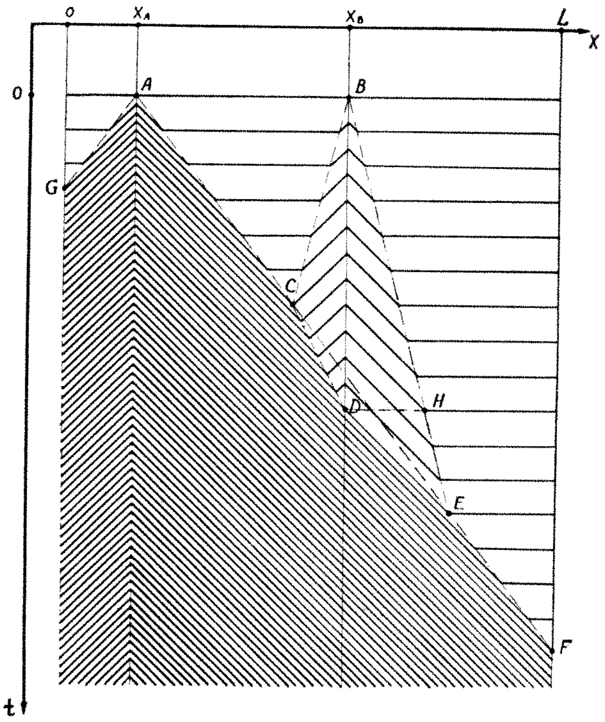


Fig. 2. Wave propagation in one-dimensional model.

It should be noted that t_F does not depend on the existence of the centre B.

All these events may be observed in a two-dimensional model on the straight line passing through two leading centres.

Similar models have been applied to the explanation of impulse propagation in cardiac muscle[12-14].

We thank Dr V. I. Krinsky for useful discussion.

A. N. ZAIKIN
A. M. ZHABOTINSKY

Institute of Biological Physics,
Academy of Sciences of the USSR,
Puschino, Moscow Region.

Received August 4; revised November 3, 1969.

[1] Christiansen, J. A., *Adv. Enzymol.*, **23**, 83 (1961).

[2] Goodwin, B. C., *Temporal Organisation in Cells* (Academic Press, London and New York, 1963).

[3] Bak, T. A., *Contributions to the Theory of Chemical Kinetics* (W. A. Benjamin, NY, 1963).

[4] Turing, A. M., *Phil. Trans. Roy. Soc.*, B, **237**, 37 (1952).

[5] Prigogine, J., and Nicolis, G., *J. Chem. Phys.*, **46**, 3542 (1967).

[6] Zhabotinsky, A. M., *Dokl. Akad. Nauk SSSR*, **157**, 392 (1964).

[7] Degn, H., *Nature*, **213**, 589 (1967).

[8] Vavilin, V. A., Zhabotinsky, A. M., and Zaikin, A. N., *Biochemical Oscillators* (edit. by Chance, B., Hess, B., and Selkov, E.) (Academic Press, Philadelphia, in the press).

[9] Zhabotinsky, A. M., *Biochemical Oscillators* (Academic Press, Philadelphia, in the press).

[10] Zhabotinsky, A. M., *Oscillatory Processes in Biological and Chemical Systems*, 149 (Science Publ., Moscow, 1967).

[10a] Busse, H. G., *J. Phys. Chem.*, **73**, 750 (1969).

[11] Conrade, M., and Reinbach, H., *Chem. Ber.*, **35**, 1814 (1902).

[12] Wiener, N., and Rosenblueth, A., *Arch. Inst. Cardiol. de Mexico*, **16**, 205 (1946).

[13] Gelfand, I. M., and Zetlin, M. L., *Dokl. Akad. Nauk SSSR*, **131**, 1248 (1960).

[14] Krinsky, V. I., *Biofizika*, **11**, 676 (1966).

Solid State Chemistry and Colloid Chemistry

THE properties of the solid state are often ignored in studies of lyophobic colloidal phenomena. Colloid chemists concentrate on the liquid phase when trying to interpret experiments, and consider the solid phase as relevant only in the role of an adsorbent or of a charged surface.

In 1947, Grimley and Mott[1] pointed out how solid state properties, in particular the type and the number of lattice defects, might influence colloidal phenomena. Until recently, very little attention was paid to their article, except by Mackor[2] and by Ottewill and Woodbridge[3], who rekindled the interest in Grimley and Mott's article. Recent experiments carried out in our laboratory have shown that the introduction of divalent anions or cations to AgBr crystals causes a considerable shift of the point of zero charge (p.z.c.) of AgBr[4]. The p.z.c. of AgBr is defined as that concentration of silver ions in solution (Ag_s^+) for which the crystals carry no net charge. According to Grimley and Mott the net charge in the crystals depends on the equilibrium $Ag_s^+ \rightleftarrows Ag_i^+$, or the p.z.c. depends on the concentration of interstitial silver ions (Ag_i^+) in the crystals. The introduction of S^{2-} in the crystals increases $[Ag_i^+]$ and hence the p.z.c. is expected to shift to a higher $[Ag_s^+]$ (lower $pAg^{p.z.c.}$). Conversely, the introduction of Pb^{2+} in the crystals decreases $[Ag_i]$, and the p.z.c. is expected to shift to a lower $[Ag_s]$, in agreement with the experimental data presented in Table 1.

A second property of the solid phase, which has to be considered, is the potential difference which exists between the surface and the bulk of the crystal due to a surface charge in the crystal, as pointed out by Lehovec[5], Kliewer[6] and Trautweiler[7]. We note that the origin of this surface charge is not the same as the surface charge usually assumed in colloid chemistry. The "colloid chemical" version of the boundary charge is an adsorption or desorption of ionic species on the surface from the solution. The "solid state chemical" version of the boundary charge is a different concentration of interstitials and vacancies in the surface region compared with the bulk of the crystals.

Table 1. POINTS OF ZERO CHARGE AT 25° C OF SULPHUR AND LEAD-DOPED AgBr

Dope	—	S	S	Pb	Pb	Pb	Pb
Dope, mole fraction (units 10^{-4})	—	0·44	4·7	1·35	1·45	1·45	5·0
$pAg^{p.z.c.}$	5·40	3·47	2·63	9·61	9·56	9·62	10·37

The existence of this surface charge leads to a modification of Grimley's theory[4,8,9]. From measurements of the p.z.c. of AgBr it is then possible to calculate the concentrations of lattice defects and the surface charge of AgBr. It turned out that the fraction of the lattice defects at 25° C in pure AgBr is $10^{-7\cdot2}$ and the surface charge is $-0\cdot011$ μCi/cm². From measurements of the latent image Trautweiler found $10^{-7\cdot6}$ and $-0\cdot03$ μCi/cm² respectively, while the extrapolated value from conductivity measurements is $10^{-7\cdot5}$ for the fraction of lattice defects. There is fair agreement between these data. In our case it has thus been possible to deduce data of the "dry state" from "wet" experiments.

Generally speaking, the interaction between the disciplines of colloid chemistry and of solid state chemistry is two-fold. First, the properties of the solid phase do influence the colloidal properties when such a solid is dispersed in a liquid, and second the magnitude of certain parameters of the solid phase can be deduced from colloidal measurements. In the previous section this point of view was demonstrated: the p.z.c. of AgBr is determined by the concentrations of lattice defects in AgBr, and these concentrations can be calculated from the measured p.z.c. of AgBr.

A second example is the relationship between the net charge of AgI and the silver ion concentration in solution. Instead of the usual assumption of a Stern layer in the liquid phase, with a dielectric constant of about 5 and a capacity which is a function of charge, the experimental data could be explained fairly well by a theory based on lattice defects and a fixed surface charge in AgI[9]. The fraction of lattice defects of AgI at 20° C is $10^{-2\cdot8}$ and the surface charge is $-1\cdot2$ μCi/cm²—deduced from fitting the experimental data to the theory.

As a third example, consider measurements of the time necessary for equilibration in a colloidal system. If diffusion in the solid is the rate determining process, it should be possible to calculate the diffusion coefficient D of the most mobile type of lattice defects from the time for equilibration, which is roughly equal to $V\varepsilon RT/16\pi F^2D$, where V is the molar volume, ε the dielectric constant of the solid, and R, T and F have their usual significance[4].

As a fourth example, a better understanding of the magnitude of the zeta potential might be obtained by noting that only part of the total potential jump between the bulk solid phase and the bulk liquid phase is situated in the liquid[3,9].

Not only colloidal phenomena but also electrochemical phenomena are influenced by lattice defects, as pointed out by Awad[11], who measured the overpotential at silver anodes covered with anodically deposited AgBr layers, and by Hall and Bruner[12], who measured the electrical resistance of thin AgBr membranes between two $AgNO_3$ solutions.

Whether a secondary electrode of the type metal/insoluble metal salt behaves as a reproducible and accurate electrode probably also depends on the solid state properties of the salt. In particular the concentrations and the diffusion coefficients of the lattice defects must have a certain minimum value in order for equilibrium to be quickly established. Silver halides, for example, which make very good secondary electrodes, have a large concentration of lattice defects with high diffusion coefficients. It may be that other secondary electrodes can be improved by doping, thus increasing the number of lattice defects.

E. P. Honig

Philips Research Laboratories,
N.V. Philips' Gloeilampenfabrieken,
Eindhoven–Netherlands.

Received December 12, 1969.

[1] Grimley, T. B., and Mott, N. F., *Disc. Faraday Soc.*, **1**, 3 (1947).
[2] Mackor, E. L., *Rec. Trav. Chim.*, **70**, 763 (1951).
[3] Ottewill, R. H., and Woodbridge, R. F., *J. Colloid Sci.*, **19**, 606 (1964).
[4] Honig, E. P., and Hengst, J. H. Th., *J. Colloid Interf. Sci.*, **31**, 545 (1969).
[5] Lehovec, K., *J. Chem. Phys.*, **21**, 1123 (1953).
[6] Kliewer, K. L., *J. Phys. Chem. Solids*, **27**, 705 (1966).
[7] Trautweiler, F., *Phot. Sci. Eng.*, **12**, 98 (1968).
[8] Grimley, T. B., *Proc. Roy. Soc.*, A, **201**, 40 (1950).
[9] Honig, E. P., *Trans. Faraday Soc.*, **65**, 2248 (1969).
[10] Müller, P., *Phys. Stat. Sol.*, **12**, 775 (1965).
[11] Awad, S. A., *J. Electroanal. Chem.*, **21**, 483 (1969).
[12] Hall, J. E., and Bruner, L. J., *J. Chem. Phys.*, **50**, 1596 (1969).

Macro-lattice from Segregated Amorphous Phases of a Three Block Copolymer

THERMOPLASTIC elastomers represent a new and important family of polymeric materials. One of the most representative examples contains three blocks, styrene/butadiene/styrene (SBS), within a chain. At ambient temperatures the two chemical species separate. The *B* phase is rubbery, and provides the matrix for the highly dispersed *S* phase which is glassy. Owing to the molecular connexion between the two, the *S* phase acts like a cross-link and thus creates a network. At high enough temperatures this cross-linking effect due to the *S* phase is removed and the object becomes mouldable.

The dispersed *S* phase has been observed to consist of spherical or rod-like particles or to exist in the form of layers[1-5]. A high degree of ordering is indicated from electron micrographs of solvent cast films, in which a hexagonal array of circles can sometimes be seen[1,3,5]. Low angle diffraction patterns have been reported to show a discrete diffraction ring corresponding to a spacing of the same order as observed electron microscopically[2]. We report here a further low angle X-ray effect indicative of a quite unexpected degree of regularity in the arrangement of the dispersed particles, which we believe represents an unusual kind of ordering in solid substances. We report only the effect itself in its most pronounced form, leaving the particulars to be described elsewhere[6].

The material was an *SBS* polymer with 25 per cent *S* content as manufactured by Shell Co. under the trade name Kraton 102. The molecular weight of the *S* block is 10^4 and that of the *B* block $5\cdot5\times10^4$ (ref. 7). We obtained the polymer in the form of an extruded rod of 9 mm diameter. Discrete spot-like X-ray diffraction effects were observed at low angles, the pattern displaying hexagonal symmetry about the plug, that is, extrusion direction. The ordered appearance of the pattern was apparent from the outset but could be further enhanced by heat treatments. Fig. 1 shows an example with the beam along the extrusion direction. Here the hexagonal spacing corresponds to 260 Å. With the beam perpendicular to the extrusion direction the pattern consisted of a set of sharp reflexions along the zero layer line but nowhere else. It is clear that we have an ordered arrangement of the dispersed phase comparable with that of atoms in a crystal lattice except that the scale of the periodicity is in the range of 300 Å. The individual "crystal" thus defined can assume macroscopic dimensions and in the most regular case observed so far can make up the whole cross-section of the plug.

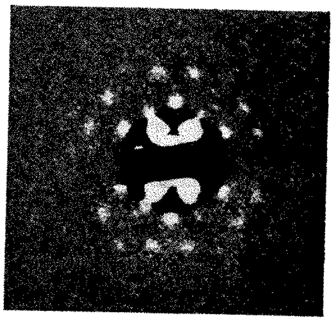


Fig. 1. Low angle X-ray diffraction pattern from an extruded Kraton cylinder with the beam parallel to the cylinder axis. Two of the six strongest reflexions obscured by backstop. Centre of pattern printed darker.

The patterns obtained along the two directions are consistent with a hexagonal packing of cylinders which are many times as long as wide and could well be of "infinite" length. The cylinder diameter could be assessed in two independent ways: (*a*) from the measured hexagonal spacing and from the known composition and macroscopic densities of the polymers; (*b*) from the missing second order of the strong hexagonal spacing in Fig. 1 interpreted as arising from the first zero of the first order Bessel function (the Fourier transform of a cylinder). Both assessments give a value of 150 Å within an uncertainty of about 10 per cent. This is half the centre-to-centre distance between the nearest neighbour cylinders.

Various experiments were performed by melting and stretching the material both uniaxially and biaxially. Such treatments altered and reformed the patterns in characteristic ways, the hexagonal axis always being along the stretch direction (or directions), which is consistent with the stress aligning the cylinders. Clearly this opens up a new field of texture studies in terms of the macro-lattice. This would differ from deformation studies in conventional crystals in as far as both the lattice and the dispersed units themselves could be affected by stress. The effect of the *SB* block ratio and that of the total chain length would also be expected to influence the "crystal", so examination of samples with different chemical composition should be of further interest.

It has been pointed out previously[2,3] that the size, shape and organization of the dispersed units are expected to influence the mechanical properties. We may add that in the case of a macroscopic "crystal" we would expect a pronounced directionality in property. Accordingly we should have an anisotropic elastomer, adding another significant factor influencing the mechanical properties.

Perhaps the major question is the origin of the forces which keeps the *S* particles in their exact lattice position. We suggest that on average the two *S* blocks of a given chain are situated in two different cylinders. Accordingly, the cylinders will be molecularly connected by *B* blocks which, being in a rubbery state, will act as an entropy spring. The different molecular springs connecting a given cylinder to its neighbours will be in different degrees of tension or compression depending on the end-to-end separation of the appropriate *B* block, and the cylinder will take up the position which minimizes these forces. This condition then defines the lattice. Accordingly we should have a situation where the lattice forces are represented by true material springs.

A. Keller
E. Pedemonte*
F. M. Willmouth†

University of Bristol,
H. H. Wills Physics Laboratory,
Royal Fort, Tyndall Avenue,
Bristol BS8 1TL.

Received October 16, 1969.

---

* Permanent address: Istituto di Chimica Industriale, Università, Genova, Italy.

† Present address: Imperial Chemical Industries Ltd, Plastics Division, Welwyn Garden City, Hertfordshire.

[1] Fischer, E., *J. Macromol. Sci. Chem.*, **A2**, 1285 (1968).
[2] Hendus, H., Illers, K., and Ropte, E., *Kolloid. Z. u. Z. Polymere* (in the press).
[3] Matsuo, M., *Japan Plastics*, **19**, 6 (1968).
[4] Matsuo, M., Ueno, T., Horino, H., Chugyo, S., and Asai, H., *Polymer*, **9**, 425 (1968).
[5] Lewis, P. R., and Price, C., *Nature*, **223**, 494 (1969).
[6] Keller, A., Willmouth, F. M., and Pedemonte, E., *Kolloid. Z. u. Z. Polymere* (in the press).
[7] Bianchi, V., Pedemonte, E., and Turturro, A., *Polymer* (in the press).

Field Emission from Silicon Carbide Whiskers

It is thought that most of the silicon carbide whiskers grown by the E.R.D.E. process do so by the vapour–liquid–solid (VLS) mechanism[1] where a metal is the principal "activator" in the process. Often a globule of frozen eutectic can be seen on the ends of the whiskers. Further, it has been observed that in many cases smaller whiskers had grown out from these balls. Some of the specimens, however, did not show such a eutectic residue on their ends and it is these that have been studied here.

The field emission microscope is ideally suited for the examination of the ends of these semiconducting needles. Not only is a highly magnified image of the whisker tip produced, but also it is possible by application of the equations describing the emission from semiconducting surfaces[2–4] to calculate the impurity concentration within the lattice, and further, by field evaporating the atoms from the whisker end, to measure the level of impurity down the whisker length. These results should therefore yield the amount of contaminant, presumably metallic, that is being deposited within the crystal lattice.

The whiskers used were 2–5 microns in diameter and were fastened to a typical field emission filament array with aquadag. Because we were interested in examining the whiskers as grown, the ends were not etched down to fine points as is the normal practice[5–7]. Two probe leads, spot welded to the heater filament for monitoring the temperature of the heater loop, were connected to a Kelvin bridge network[8]. This was calibrated at 77 K and room temperature, and at higher temperatures with an optical pyrometer. The microscope was connected to a typical UHV line which was pumped by a 50 ls^{-1} diffusion pump and a 10 ls^{-1} ion pump to pressures of 3 to 5×10^{-10} torr. Current–voltage characteristics were monitored rapidly and automatically by the previously described system[9].

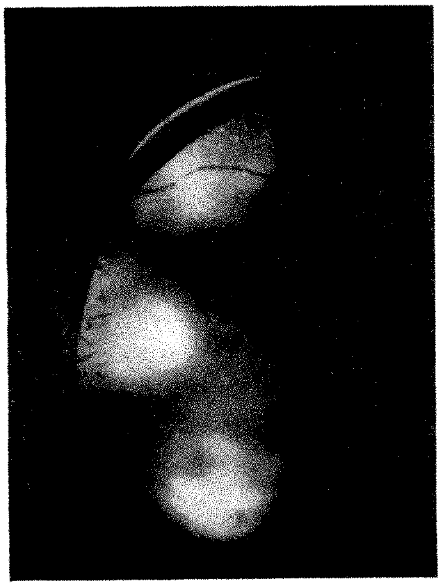


Fig. 1. Typical emission pattern from an unetched SiC whisker. Applied voltage 3 kV.

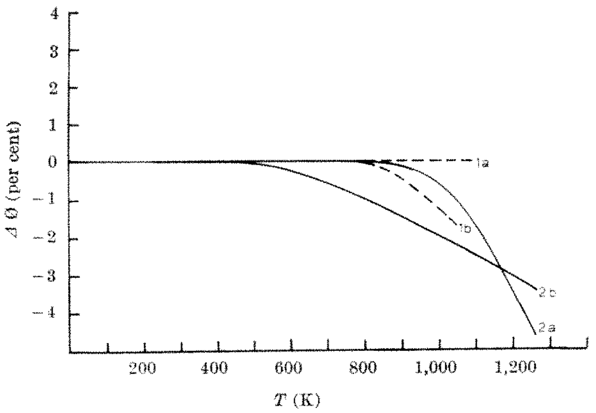


Fig. 2. Change in work function with temperature at a fixed applied voltage for two whiskers; 1a and 2a before field evaporation, 1b and 2b after field evaporation.

A typical field emission pattern obtained at an operating voltage of 3 kV is shown in Fig. 1. In view of the low voltage applied and the fact that the whisker is 2 microns in diameter, it is likely that these patterns originate from small projections present on the surface of the whisker tip. A closer examination of such images revealed that there was a degree of overlap in the patterns and in certain cases there was evidence of four-fold symmetry indicative of a cubic structure which indeed silicon carbide can possess.

These observations seem to indicate that the projections might be, indeed, "microwhiskers" analogous to those seen on the whiskers with a ball of eutectic on their ends. Experiments being carried out in which the tip is progressively removed by field evaporation should help to clear up this point.

The current–voltage characteristics of the emitter–anode diode were measured for a temperature range of 77 K to 1,200 K. The percentage change in work function with temperature was calculated from the ratio of the slopes of the Fowler–Nordheim equation at the different temperatures to that at room temperature, Fig. 2. It should be pointed out that for a given temperature the equation was obeyed well and only deviated markedly in the 1,200 K region.

In certain cases (for example, whisker 1a, Fig. 2), the work function did not change at all, even up to 1,200 K, and the Fowler–Nordheim equation was obeyed very closely indeed. With others (for example, whisker 2a, Fig. 2), the work function remained constant with temperature only up to some critical value, after which it changed in the manner depicted. It is therefore clear that the emission of the electrons is temperature dependent and this

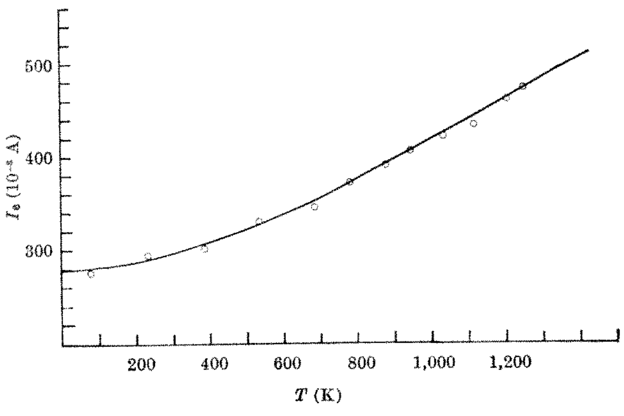


Fig. 3. Current versus temperature plot for whisker 2b. Applied field constant.

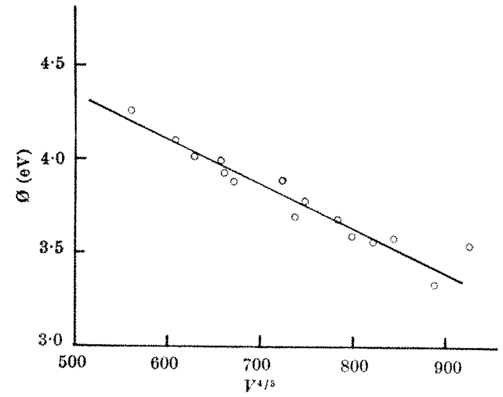


Fig. 4. A plot of work function against $V^{4/5}$, showing that field penetration has taken place.

relationship of emission current with temperature is plotted in Fig. 3 for whisker 2b.

These results lead to the conclusion that the electrons are being emitted from the conduction band and that the whisker is behaving as an n-type semiconductor. This being so, then from the equations given by Stratton[2,3] and Gomer[4] it should be possible to calculate the number of electrons in the conduction band and hence the concentration of impurity donor atoms in the lattice. On attempting to do this, however, it was found that the experimental current–temperature relationship did not comply with the theoretical equations. At this stage, therefore, it has not been possible to calculate the contamination level. It has been possible, however, to demonstrate that there is a gradation of properties down the shanks of the "microwhiskers", for on field evaporation the temperature dependence changed as shown in curves 1b and 2b of Fig. 2. The curve 1a to 1b can be interpreted qualitatively to mean that the whisker tip was highly contaminated, perhaps originally metallic, in character, but on field evaporation this metal was removed and the whisker began to demonstrate semiconducting properties. Similarly with curves 2a and 2b there is a change from a contaminated to a less contaminated whisker. It would seem therefore that the concentration of impurity atoms, possibly the metal from the eutectic, is decreasing down the shank of the "microwhiskers".

It has been assumed, from the observations made here, that the "microwhiskers" are behaving as n-type semiconductors with emission occurring from the conduction band. If this assumption is correct, then not only should there be a temperature dependence but also a field dependence should become evident. If the field is increased to such an extent that field penetration becomes so great that the conduction band edge is depressed well below the bulk Fermi-level, then Fermi–Dirac statistics apply. In such a case the Fowler–Nordheim equation holds with the correction that the observed work function φ_m is given now by

$$\varphi_m = \varphi_0 - A V^{4/5}$$

where φ_0 (4·2 eV)[10] is the depth of the conduction band, A is a constant involving the dimensions of the whisker and its dielectric constant, and V is the applied voltage. Experiments were performed to test this condition. As shown in Fig. 4 the relationship was obeyed, and strongly supports the assumption derived from the current–temperature observations.

The observations are consistent with the idea that small projections or "microwhiskers" are present on the tip of whiskers which do not possess a little ball of frozen eutectic on their ends and that these "microwhiskers" are behaving as n-type semiconductors. There is also a decrease in the concentration of impurity atoms down the shank of these projections. Because there is a discrepancy between experiment and theory, the degree of contamination could not be estimated. This anomaly could be a result of the contamination being excessively high and/or the band structure in these very tiny crystals differing markedly from those assumed in the theoretical calculations.

In conclusion, the experiments have shown that the end structure, observed on the macro-scale in many cases, consisting of smaller whiskers growing out of the eutectic ball, exists on a micro-scale for the whiskers examined here. It has become apparent also that an estimation of the impurity content within the microwhiskers cannot be made. More work on the temperature relationship of the emission current for both the microwhiskers and etched whiskers will have to be carried out to clarify this situation. Such an estimation of the impurity content would help to give a better understanding of the growth mechanism.

I thank D. Henshaw and A. R. Osborn for their helpful discussions.

FRANCIS S. BAKER

Ministry of Technology,
Explosives Research and Development Establishment,
Waltham Abbey,
Essex.

Received November 12; revised December 16, 1969.

[1] Wagner, Ellis, *Appl. Phys. Lett.*, **4**, 89 (1964).
[2] Stratton, R., *Phys. Rev.*, **125**, 67 (1962).
[3] Stratton, R., *Proc. Phys. Soc.*, B, **68**, 746 (1955).
[4] Gomer, R., *Field Emission and Field Ionization*, pp. 21–29 (Oxford University Press, 1961).
[5] Nishida, J., *J. Appl. Phys.*, **38**, 5417 (1967).
[6] Smith, D., *J. Sci. Instrum.*, E, **2**, 106 (1969).
[7] Busch, G., and Fisher, T., *Brown Boveri Rev.*, **45**, 532 (1958).
[8] Zimmerman, Gomer R., *Rev. Sci. Instrum.*, **36**, 1046 (1965).
[9] Chambers, J., and Baker, F. S., *J. Sci. Instrum.*, **44**, 1051 (1967).
[10] Dillon, jun., J., and Goffaux, R., in *Silicon Carbide, a High Temperature Semiconductor*, 236, 463 (Pergamon Press, 1960).

Ribbed Growth of Graphite in Ni-C Solutions containing Boron

WE report here on some effects due to boron in Ni-C melts. A number of interesting and hitherto unnoted features of graphite growth have been observed. The significance of these observations is that they demonstrate

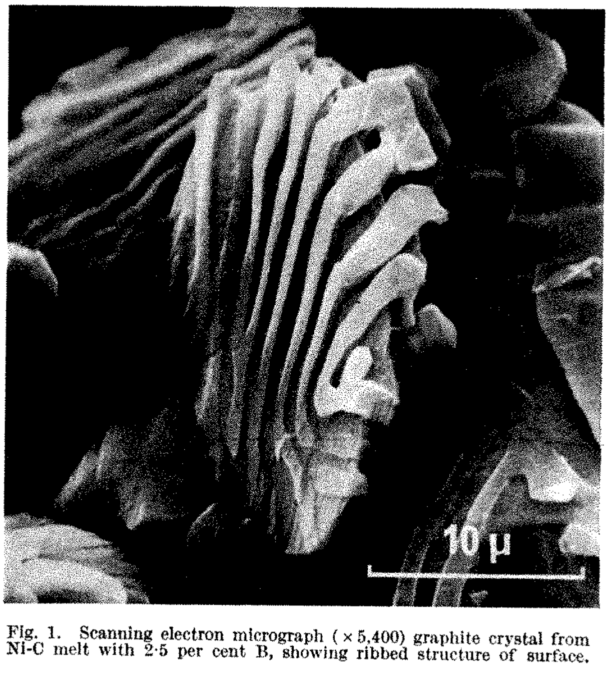


Fig. 1. Scanning electron micrograph (×5,400) graphite crystal from Ni-C melt with 2·5 per cent B, showing ribbed structure of surface.

an important mechanism of a morphology change in graphite growing from solution. We show that the growth changes can be attributed to a concentration of boron in a boundary layer at the liquid-solid interface. Growth interfaces break down and morphology changes result. In particular, we have observed both scroll and spherulitic forms.

In previous investigations in the Ni-C system, the growth of dendritic graphite[1] and effects resulting from the interaction between lanthanum and growing graphite[2] were described. In the present experiments, we have noted completely new effects when boron is added to Ni-C solutions.

The experiments we carried out used pure nickel and boron added as nickel boride. The nickel was melted in graphite crucibles by induction heating, under an atmosphere of CO and equilibrated with the carbon of the crucible for 5 min at 1,550° C. Pure nickel boride was then added in each experiment in amounts to give melt concentrations of 0·25, 0·5 and 2·5 weight per cent boron. The melt was stirred and retained at this temperature for 3 min before cooling. Freezing rates in the solidification range were approximately 45° C/min.

The ingots were sectioned and one half of each specimen was prepared for metallographic examination. Sections taken from the second half were dissolved in dilute HCl to obtain separated graphite crystals for examination by scanning electron microscopy. These crystals were coated with a thin evaporated gold film and examined in the normal manner with a Cambridge 'Stereoscan' instrument.

The metallographic examination showed a gradual reduction in the graphite content as the boron concentration increased. At the same time, a non-graphitic eutectic phase increased in volume.

A specimen structure of a graphite crystal observed in the scanning electron microscope is shown in Fig. 1. It shows ribbed steps developing on the crystal surface. This structure, which initiated at small boron concentrations, was observed to accentuate with increasing boron and, in the highest boron content alloy (2·5 weight per cent B), ribs grow independently from the crystal surface into the melt. Crystals of this character are shown in Fig. 2. The dimension of these ribs is approximately 0·5 microns in cross-section.

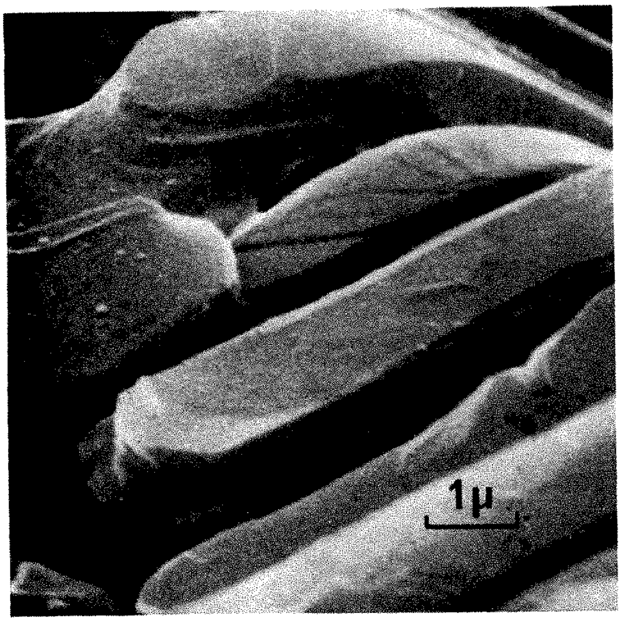


Fig. 2. Scanning electron micrograph (× 46,000). Ribs of graphite growing from crystal surface.

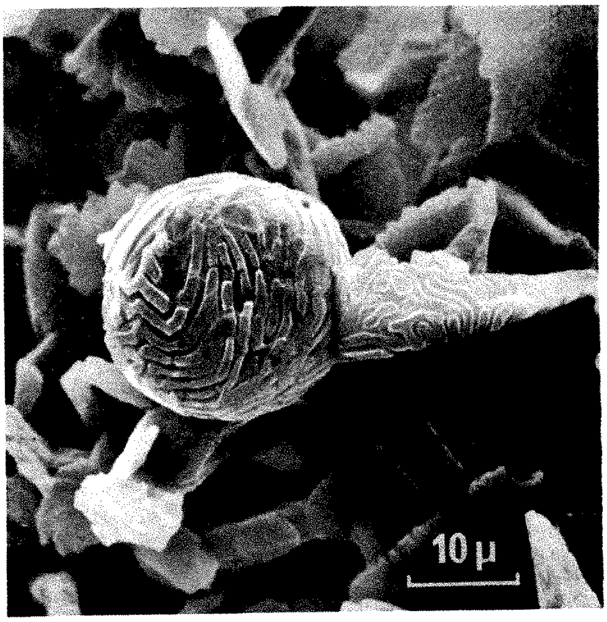


Fig. 3. Scanning electron micrograph (× 5,400). Spherical crystal of graphite joined to crystal of scroll type.

Two types of crystal other than flake were noted. These were in the forms of sphere and a scroll. Fig. 3 shows a spherical crystal joined to one of scroll type.

The experimental evidence is that the ribbed growth is due to the boron in solution and the ribs are formed in the following manner. Steps, which normally traverse the crystal surface along plane fronts, break down to form step protuberances which eventually dominate the growth. The concentration of 2·5 weight per cent B at which the increasing change in growth characteristics occurs is far in excess of normal growth poison effects[3]. This suggests that boron forms a solute boundary layer at the growing graphite interface (due to its low solubility in graphite) which produces a breakdown of step movement on its surface. All forms observed grow ribbed, whether plate, sphere or scroll. The ribbed crystals have a remarkable ability to adopt curved geometries.

It is of interest to compare the action of boron and its effect on graphite with the previously observed effect of lanthanum[2]. The latter element has a pronounced effect at a melt concentration of approximately 0·01 weight per cent La. Its specific action is to block the movement of a growth step by physical adsorption at the step. This might be expected of an element in the metallic solution which is very reactive with the carbon at this temperature. It has been noted that the melt, if the adsorbing element is present, has to undercool for further growth to occur[2] and this growth is then accentuated in the direction normal to (0001) planes. In the case of boron, the solute is rejected in the melt and swept ahead of the interface. Step growth on crystal surfaces is principally as protuberances in the (0001) plane. When the boron content is sufficiently high, these protuberances dominate growth.

A comparative effect in polymer growth, discussed by Keith and Padden[4], termed "fibrillation" has been described as having origin in causes similar to those described here. The planar interface solidification which leads to plate forms breaks down due to impurity concentration at a boundary layer. The grooves formation leads to needle like growth forms.

Fibrillar spherulites in polymers grow from a centre and branch outwards. The fibril of graphite growing in the presence of boron, however, is observed to grow circumferentially round the sphere. In both the case of polymer growth and graphite growth with boron, the action of the impurity is similar in that planar interface growth is prevented and crystals of a different geometry can then grow.

It is of interest to recall that scroll forms of graphite were observed by Bacon[5], growing in boules from arced carbon electrodes. The scroll forms observed here growing in solution do not develop from one continuous sheet as in the special case noted by Bacon, but grow from a series of ribs which develop over the crystal surface.

This work was sponsored by the Centre de Recherche, Institut Battelle, Geneva.

I. MINKOFF*
B. LUX

Institut Battelle,
Geneva.

Received September 23; revised December 22, 1969.

---

* On leave from Department of Materials Engineering, Technion, Haifa, Israel.

[1] Minkoff, I., and Einbinder, I., *Nature*, **194**, 765 (1962).
[2] Minkoff, I., and Nixon, W. C., *J. Appl. Phys.*, **37**, 4848 (1966).
[3] Buckley, H. E., *Crystal Growth* (John Wiley, NY, 1951).
[4] Keith, H. D., and Padden, F. J., *J. Appl. Phys.*, **34**, 2409 (1963).
[5] Bacon, R., *Growth and Perfection of Crystals*, 197 (John Wiley, NY, 1958).

Magnetic Relaxation in Ordered Systems

NUCLEAR magnetic resonance (NMR) spectroscopy is one of the most promising techniques to be recently applied to the study of lipid, protein and water interactions in biological and other ordered and semi-ordered systems. NMR studies of the water in many hydrated biological systems have been interpreted in terms of the existence of "structured", "ice-like" or "bound" water[1-6]. Likewise, line broadening in ordered liquid crystalline and biological systems has often been taken as an indication of restriction of molecular motion[7-13]. We should like to point out some possible pitfalls in interpreting the results of limited relaxation or line width data in terms of changes in molecular mobility arising from specific interactions, in systems which are not isotropic liquids and are not always homogeneous. We have carried out a spin-echo NMR study of the dimethyldodecylamine oxide–deuterium oxide system—a system in which anisotropic ordered structures exist and one for which considerable X-ray and continuous wave NMR data are available[9,14]. This system is an ideal one for the study of the effects of molecular ordering and anisotropy on the measured NMR parameters because the structure can be changed from isotropic to anisotropic simply by a slight change in composition or temperature.

In this system, high resolution NMR lines are observed for both the surfactant and water in the micellar and the cubic (viscous isotropic) phases, and much broader, non-Lorentzian lines are observed for the surfactant in the anisotropic hexagonal (middle) and lamellar (neat) phases[9]. The lines from the ^2H_2O in the anisotropic structures manifest residual quadrupolar coupling[9]. The line broadening and line shape for surfactant protons in the anisotropic phases were attributed by Lawson and Flautt[9] to a decrease in the rate of molecular reorientation and to the existence of a distribution of correlation times describing the reorientation processes. Penkett *et al.*[15] have attributed the line broadening observed in similar anisotropic systems —potassium laurate in ^2H_2O and lecithin in ^2H_2O—to the existence of a "magnetic anisotropic effect". They cite the observed magnetic field dependence of the line width and the decrease in line width produced by sonication as evidence for this effect. Thus they interpret the line broadening as resulting from the structure of the mesophase rather than from the rate of molecular reorientation. More recently, Sheard questions the interpretation of the sonication experiments[16]. He believes that the changes in line width result from a change in internal structure produced by the ultrasonic irradiation. The results we present here are pertinent to the interpretation of the line broadening observed at the isotropic–anisotropic phase transitions in liquid crystalline systems and have significance for studies carried out on other ordered, anisotropic, non-homogeneous systems.

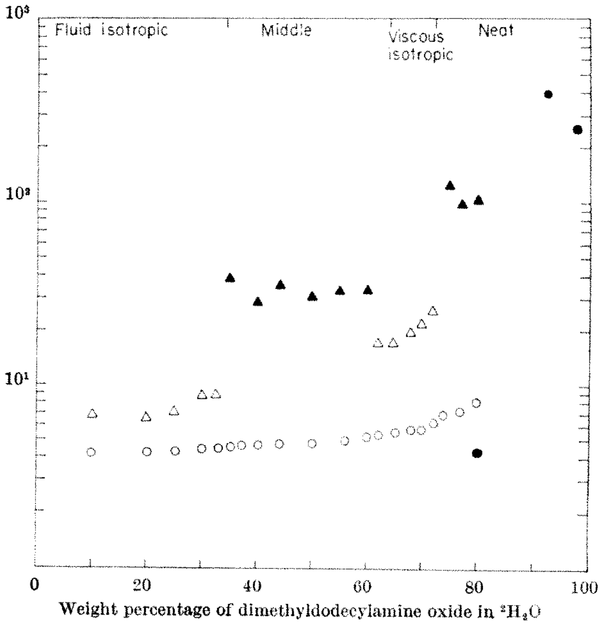


Fig. 1. Spin–spin and spin–lattice relaxation rates for surfactant protons as a function of weight per cent dimethyldodecylamine oxide in ^2H_2O. △, R_2; ▲, R_2(eff.), defined in text; ○ R_1.

Sample preparation has been described elsewhere[14]. Relaxation data were obtained on a Magnion ELH-15 pulsed spectrometer, operating at 36 and 6 MHz and equipped with a Varian 12 inch electromagnet with flux stabilizer. Both spin–spin and spin–lattice relaxation rates, $R_2(=T_2^{-1})$ and $R_1(=T_1^{-1})$, respectively, were measured. R_2 is a more direct and easily interpretable measure of the magnetic interactions, in a system in which the absorption line shape is neither Lorentzian nor Gaussian, than is the line width, and R_1 is a measure of the rate, and the distribution of rates, of molecular reorientation. The measurements of R_2 were made using the Carr–Purcell method[17] as modified by Meiboom and Gill[18]. The R_1 values were obtained by means of repeated 180°–90° pulse sequences[17] but from the slope of a semilog plot of the magnetization recovery rather than from a visual estimate of the null[19].

The proton relaxation rates R_1 and R_2 observed for dimethyldodecylamine oxide as a function of concentration in ^2H_2O are shown in Fig. 1. Discontinuities in the R_2 plot are observed at the phase boundaries, R_2 being much larger in the anisotropic than in the isotropic phases. Single exponential relaxation (one relaxation time) is observed for both R_1 and R_2 in the isotropic phases but only for R_1 in the anisotropic phases. The observed "effective" R_2 value in the anisotropic phases, R_2(eff.), is defined as the time at which the transverse magnetization has decayed to e^{-1} times the thermal equilibrium value. If the spin–spin relaxation were exponential, then R_2(eff.) $=R_2$. The observation that $R_2 \approx R_1$ in the isotropic phases is typical of liquids with isotropic molecular motion[20] and is consistent with the observation of high resolution NMR spectra for these phases[9]. In the anisotropic phases the spin–spin relaxation is non-exponential and $R_2 \gg R_1$. If in these phases, the non-Lorentzian line shape results from a distribution of correlation times, as suggested[9], the spin–lattice relaxation should also be non-exponential[21,22]. This is not observed, so that other explanations must be sought

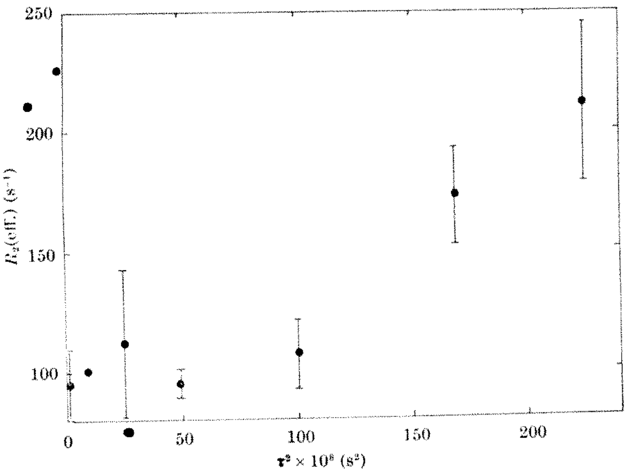


Fig. 2. Spin–spin relaxation rate for surfactant protons as a function of the Carr–Purcell pulse spacing, τ. The sample is 77 per cent by weight dimethyldodecylamine oxide in ^2H_2O (neat phase). The vertical lines through each point represent the r.m.s. errors.

for the line shape and relaxation data. The observations that R_1 has no discontinuities at the phase boundaries and remains essentially constant throughout the phases indicate that the rate of those molecular motions contributing to R_1 (probably hydrocarbon chain oscillations) does not change appreciably with concentration or with phase structure. Thus it seems that the changes in R_2 and in line width are not produced solely by changes in molecular motion. Evidently there is another relaxation mechanism operating in the anisotropic phases which contributes to R_2 but not to R_1, results in $R_2 \gg R_1$, and is responsible for the non-Lorentzian line shape.

In the anisotropic phases there is an observed dependence of R_2, for both the surfactant protons and the deuterium nuclei in the ^2H_2O, on the pulse spacing (τ) in the Carr–Purcell experiment[17]. Fig. 2 shows the proton R_2 as a function of τ^2 for a 77 per cent dimethyldodecylamine oxide–33 per cent ^2H_2O sample (neat phase). A similar dependence of R_2 on τ is found in the middle phase, but not in the isotropic phases. This dependence of R_2 on τ indicates the existence of another relaxation mechanism in addition to the dipolar relaxation resulting from molecular reorientation. There are two possible reasons for the τ dependence. It could be produced by exchange[23] of surfactant molecules between aqueous and hydrocarbon portions of the mesophase; but because R_2 is essentially independent of water content in a given phase this is apparently not the reason. The second and most likely reason is molecular diffusion. When relaxation is the result of molecular diffusion through magnetic field gradients, R_2 will be a linear function of τ^2 and will be greater than R_1. This phenomenon is the basis of the Carr–Purcell method of measuring self-diffusion coefficients using externally applied magnetic field gradients[17,24]. Spin–spin relaxation resulting from molecular diffusion through local magnetic field gradients produced by the microscopic heterogeneity of the sample has been observed for lithium in liquid dispersions[25], for benzene dissolved in solutions of high molecular weight polymers[26], and in binary solutions at their critical temperature[27]. If the structures of the anisotropic phases result in a "solution" of sufficient microscopic inhomogeneity, it is possible that diffusion of surfactant molecules through these regions could give rise to the relaxation data observed here and also to the "magnetic anisotropic effect" of Penkett *et al.*[15]. This inhomogeneity could result from orientation of large regions in different directions with respect to the applied field. The observed dependence of R_2 on τ^2 is not quite linear. This nonlinearity could result from the presence of a distribution of field gradients and from the fact that diffusion is restricted and anisotropic.

Field gradients resulting from magnetic susceptibility differences are field dependent and should increase with increasing applied field. If diffusion through the gradients contributes to the relaxation, then R_2 would be expected to increase with increasing field. Penkett *et al.*[15] have reported that the line widths of lamellar phases do increase with increasing field. Table 1 shows the field dependence of R_2 for a neat phase sample of dimethyldodecylamine oxide. R_2(eff.), previously defined, is measured by the Carr–Purcell method[17] with $\tau = 100$ μs (the shortest pulse spacing obtainable with our equipment). This method removes the effects on R_2 of molecular diffusion through field gradients. Thus if diffusion through field gradients is responsible for the anisotropic phase relaxation behaviour and for the observed[15] field dependence of line widths in lamellar phases, it is expected that the R_2 values measured by the Carr–Purcell method would be independent of the applied magnetic field strength. This is seen to be so, within experimental error, from the data in Table 1. R_2^*(eff.) is defined like R_2(eff.), except that it is measured from the free induction decay. By contrast with the Carr–Purcell method, this experiment gives R_2 values which reflect diffusion effects[17]. It is expected therefore that R_2^*(eff.) would increase with increasing applied field, which is so. Diffusion through local field gradients therefore seems to be partially responsible for the relaxation in the anisotropic phases, and these effects can be removed if a sufficiently small τ is used. The non-Lorentzian line shape in these phases seems consistent with the relaxation data; the absorption spectrum of a system whose spin–spin relaxation is non-exponential will be non-Lorentzian[28]. That this is not the only relaxation mechanism contributing to R_2 is indicated by the fact that even in the Carr–Purcell experiment with a τ of 100 μs the spin–spin relaxation is not completely exponential and by the fact that extrapolation of the data in Fig. 2 to $\tau = 0$ results in an R_2 that is larger than R_1. Further studies are being undertaken to determine the nature of these other relaxation mechanisms, which may involve anisotropic molecular reorientation.

Table 1. SPIN–SPIN RELAXATION RATES

H_0 kG	R_2^*(eff.), s^{-1}	R_2(eff.), s^{-1}
1·409	4,300 ± 350	126 ± 30
8·459	10,800 ± 900	95 ± 15

Measurements were made at two magnetic field strengths for surfactant protons in the 77 per cent dimethyldodecylamine oxide in ^2H_2O system (neat phase). R_2^*(eff.) and R_2(eff.) are defined in the text.

This study indicates the complexity of the relaxation processes in non-isotropic systems and points out the possible dangerous ambiguity which could be contained in interpretations based solely on line width measurements or on incomplete relaxation data. In systems like the lyotropic mesophases and in macromolecular biological systems it is possible that the diffusion of water, or other molecules, through microscopic inhomogeneities in the samples may result in line broadening and an increase in R_2, thus leading to erroneous conclusions about molecular mobility. Studies of such systems by NMR should include experiments along the lines we have used.

J. R. HANSEN
K. D. LAWSON

The Procter and Gamble Company,
Miami Valley Laboratories,
PO Box 39175,
Cincinnati, Ohio.

Received December 19, 1969.

[1] Cope, F. W., *Biophys. J.*, **9**, 303 (1969).
[2] Dehl, R. C., and Haine, C. A. F., *J. Chem. Phys.*, **50**, 3245 (1969).
[3] Kuntz, I. D., Brossfield, J. S., Law, G. D., and Purcell, G. V., *Science*, **163**, 1329 (1969).
[4] Glasel, J. A., *Nature*, **218**, 953 (1968).

[5] Kruger, G. J., and Helcke, *Magnetic Resonance and Relaxation* (edit. by Blinc, R., Hadzi, D., and Osredkar, M.), 1136 (North-Holland, Amsterdam, 1967).

[6] Hazelwood, C. F., Nichols, B. L., and Chamberlain, N. F., *Nature*, **222**, 747 (1969).

[7] Chapman, D., Kamat, V. B., de Gier, J., and Penkett, S. A., *Nature*, **213**, 74 (1967).

[8] Chapman, D., Kamat, V. B., de Gier, J., and Penkett, S. A., *J. Mol. Biol.*, **31**, 101 (1968).

[9] Lawson, K. D., and Flautt, T. J., *J. Phys. Chem.*, **72**, 2066 (1968).

[10] Jenkinson, T. J., Kamat, V. B., and Chapman, D., *Biochim. Biophys. Acta*, **183**, 427 (1969).

[11] Chapman, D., Leslie, R. B., Hirz, R., and Scanu, A. M., *Biochim. Biophys. Acta*, **176**, 524 (1969).

[12] Leslie, R. B., Chapman, D., and Scanu, A. M., *Chem. Phys. Lipids*, **3**, 152 (1969).

[13] Veksli, Z., Salsbury, N. J., and Chapman, D., *Biochim. Biophys. Acta*, **183**, 434 (1969).

[14] Lawson, K. D., Mabis, A. J., and Flautt, T. J., *J. Chem. Phys.*, **72**, 2058 (1968).

[15] Penkett, S. A., Flook, A. G., and Chapman, D., *Chem. Phys. Lipids*, **2**, 273 (1968).

[16] Sheard, B., *Nature*, **223**, 1057 (1969).

[17] Carr, H. Y., and Purcell, E. M., *Phys. Rev.*, **94**, 630 (1954).

[18] Meiboom, S., and Gill, D., *Rev. Sci. Instr.*, **29**, 688 (1958).

[19] Buchta, J. C., Gutowsky, H. S., and Woessner, D. E., *Rev. Sci. Instr.*, **29**, 55 (1958).

[20] Abragam, A., *The Principles of Nuclear Magnetism*, chap. 8 (Oxford Univ. Press, London, 1961).

[21] Odajima, A., *Progr. Theor. Phys. (Kyota), Suppl.*, **10**, 142 (1959).

[22] Connor, T. M., *Trans. Faraday Soc.*, **60**, 1574 (1964).

[23] Allerhand, A., and Gutowsky, H. S., *J. Chem. Phys.*, **41**, 2115 (1964).

[24] Woessner, D. E., *J. Phys. Chem.*, **67**, 1365 (1963).

[25] Zamir, D., Wayne, R. C., and Cotts, R. M., *Phys. Rev. Lett.*, **12**, 327 (1964).

[26] Anderson, J. E., and Liu, Kang-Jen, *J. Chem. Phys.*, **49**, 2850 (1968).

[27] Anderson, J. E., *J. Chem. Phys.*, **50**, 1474 (1969).

[28] Lowe, I. J., and Norberg, R. E., *Phys. Rev.*, **107**, 46 (1957).

Dye Lasers in the Ultraviolet

POTENTIAL applications of dye lasers in the fields of air pollution monitoring, photochemistry and biology have stimulated interest in operating dye lasers in the spectral region below 4500 Å. Many important molecular resonances and reaction thresholds are found at these higher photon energies. Using an AVCO C950 pulsed N_2 gas laser, with its output at 3371 Å in the ultraviolet, we have been able to obtain continuously tunable dye laser action as low as 3550 Å. This pumping technique has been described in some detail elsewhere[1]. The present scheme has a transverse pumping configuration where the rectangular N_2 laser beam is focused to a line within the dye cell and the dye laser's optical cavity. The pumping laser is capable of 100 kW peak power in a 10 ns pulse duration, and can be pulsed at rates as high as 100 pulses per second[2]. In the visible region of the spectrum, this system has produced dye laser peak powers as high as 30 kW and repetition rates up to 100 pulses s^{-1}. It is also significant that the visible dyes have attained these repetition rates in sealed dye cells without any external dye circulation.

In transverse pumping, the laser cavity and resulting dye laser beam are at right angles to the direction of propagation of the laser pump. With the N_2 laser, the dye gain is usually very large and superradiant oscillation is frequently observed. The output mirror of the cavity was a metallic coated, spectrally flat (57 per cent ± 2 per cent) transmitting mirror and the "high reflectivity" end was a diffraction grating. The grating enables one to tune the frequency of the laser selectively, and it also has the advantage that it can be used to "frequency pull" the response of a dye to wavelengths beyond the observed natural fluorescence of the dye[3]. The output pulse of the dye laser was monitored with an ITT F4000 photodiode with an S–5 spectral response and the wavelength response was measured with a Jarrell–Ash 0·5 m monochromator with an S–20 photomultiplier.

Table 1

Dye	Solvent	Spectral range
αNOPON	Benzene	4300–4550
POPOP	Tetrahydrofuran	4150–4300
Diphenylanthracene	Ethanol	4350–4500
Diphenyl stilbene	Toluene	4000–4150
	Benzene	4000–4150
αNPO	Benzene	3975–4100
	Cyclohexane	3850–4150
Sodium-salicylate	Ethanol	3950–4180
BBO	Ethanol	3640–3850
PPF	Ethanol	3650–3710
PPD	Ethanol	3830–3950
PBD	Ethanol	3550–3820
Aminobenzoic acid	Toluene	3980–4060
BOPOB	Tetrahydrafuran	4280–4500
p-Quaterphenyl	Toluene	3620–3900
BBP	Benzene	4000–4150

Table 1 is a list of the dyes and scintillators that have been observed to lase below 4500 Å, together with the solvents in which they were dissolved and the spectral range over which they could be made to lase. This information is also shown schematically in Fig. 1.

It should be pointed out that our search with a low Q optical cavity selected those dyes in which superradiant or near superradiant gain was obtainable. Other violet fluorescing compounds that we pumped but were unable to make lase with our present system and cavity optics were 4-dimethylamino antipyrine, 1-naphthylamine, and quinine sulphate.

Many of the absorption and fluorescence spectra of the above compounds are given by Bearlman[4]. Despite the fact that the reported absorption peaks of naphthylamine and quinine sulphate almost coincide with the pumping wavelength at 3371 Å, with high reported fluorescence efficiencies, we are unable to make these compounds lase. On the other hand, PPD and PBD are two compounds with absorption bands several hundred Ångstroms removed from 3371 Å, but they lase quite readily. Dye molecular limitations, such as low molecular extinction coefficients, slow excited singlet state relaxation rates, long spontaneous emission lifetimes or losses to the triplet states by radiationless transitions, may prevent the two former compounds from exhibiting high gain at any wavelength with the present N_2 laser pumping. Un-

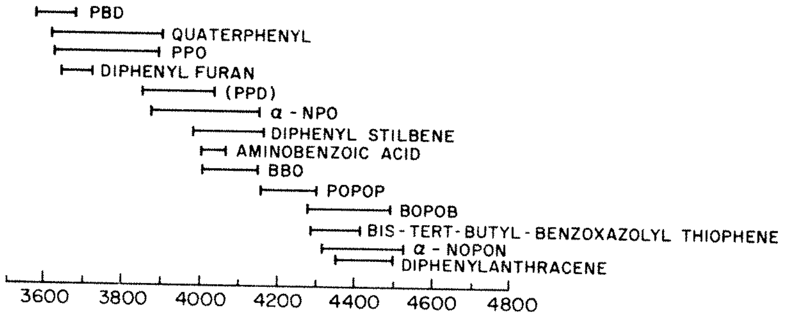


Fig. 1. Effective lasing ranges of dyes in the violet.

ambiguous interpretation of the relative importance of these effects would be extremely difficult without further molecular data. The fact that compounds such as PPD, which are not even expected to absorb 3371 Å radiation, show high gain suggests that dimers or excimers that absorb 3371 Å radiation may be forming in solution and lasing themselves, or their decomposition fragments may lase. This speculation is supported by the observation that some compounds, such as PPD or sodium salicylate, will only lase at quite high solution concentrations greater than 10^{-3} moles/l.

Solvents were included in the table of dyes, because the proper solvent determines, in many cases, whether or not a dye will reach lasing threshold. The effect of the solvent on lasing ability may be closely related to the singlet–triplet transition rate, which may be the limiting step in attaining threshold.

The ultraviolet dyes below 4000 Å are usually less efficient, and lase in a narrower spectral range than the visible dyes. Between 4000 and 4500 Å, conversion efficiencies of the order of 15–20 per cent have been obtained, while below 4000 Å the efficiencies so far obtained have been of the order of 1 per cent or less. Effective spectral ranges in this region are, on the average, of the order of 100–150 Å, while the visible dyes have in some cases spectral ranges as broad as 500–600 Å. Another disadvantage of a few ultraviolet dyes is that they have limitations on the rate at which they can be pulsed in our non-flowing dye cell. This indicates that these dyes may be decomposing (such effects were not observed for the visible dyes studied), and they must either flow or be cooled to perform as well as their visible counterparts.

While this list of scintillators and dyes is not complete, our success does indicate that tunable dye laser action is feasible down to, and probably well below, 3500 Å. We are continuing this work in order to improve ultraviolet dye laser performance.

JAMES A. MYER
IRVING ITZKAN
E. KIERSTEAD

Avco Everett Research Laboratory,
2385 Revere Beach Parkway,
Everett, Massachusetts 02149.

Received December 9, 1969.

[1] Myer, J. A., Johnson, C. W., Kierstead, E., Sharma, R., and Itzkan, I., *Appl. Phys. Lett.* (in the press).

[2] Leonard, D. A., *Appl. Phys. Lett.*, 7, 4 (1965). Gerry, E. T., *Appl. Phys. Lett.*, 7, 6 (1965).

[3] Soffer, B. H., and McFarland, B. B., *Appl. Phys. Lett.*, **10**, 266 (1967).

[4] Bearlman, I., *Handbook of Fluorescence Spectra of Aromatic Molecules* (Academic Press, New York, 1965).

BIOLOGICAL SCIENCES

Changes in Nucleoside Conformation

Arnott and Hukins[1], and Sundaralingam[2], have recently compared the conformational parameters of nucleosides and nucleotides with those found in polynucleotide structures. Two of the parameters they discuss are the relative orientation of the sugar component with respect to the purine or pyrimidine base, and the type of pucker in the sugar ring.

I should like to draw attention to the variation of these two parameters in three nucleosides that have been studied in this laboratory. The three nucleosides are 5-bromouridine, inosine and 5-iodouridine, and they are of interest because they show differences in conformation when in different environments in a crystal. The structure of 5-bromouridine has been determined when it is complexed with adenosine[3]; when it crystallizes by itself[4]; and when it is complexed with dimethylsulphoxide[5]. Inosine has been studied in two crystal forms[6-9], and in the case of 5-iodouridine there are two molecules in the asymmetric unit which have markedly different conformations[10].

The relative orientation of the sugar and base may be described in terms of a torsion angle φ_{CN} (ref. 11), or an angle χ (ref. 1), and, like most nucleosides, the three discussed here are in the *anti* conformation. Also, the pucker of the sugar rings is either C2′ *endo* or C3′ *endo*, the most common type of pucker observed in nucleosides and nucleotides.

Haschemeyer and Rich[12] have calculated the "allowed" ranges of φ_{CN} in nucleosides with different puckers, based on a consideration of intramolecular contacts. These calculations show that the *anti* ranges for both C2′ *endo* and C3′ *endo* pyrimidine nucleosides are not very different, and, although the ranges are larger for purine nucleosides, again the allowed ranges for C3′ and C2′ *endo* puckers are similar. It is therefore surprising to find, even in the three nucleosides discussed here, that the mean value of the φ_{CN} angles for the C3′ *endo* pucker has a smaller magnitude than the mean value of φ_{CN} for those with a C2′ *endo* pucker. This result is in agreement with the conclusion of Arnott and Hukins[1], based on a survey of nucleosides and nucleotides, and also that of Sundaralingam[2]. The φ_{CN} values are given in Table 1. The mean φ_{CN} value for the C2′ *endo* pucker is −69° and for the C3′ *endo* pucker it is −15°. In the refined molecular models of B-DNA and A-DNA structures[13] the φ_{CN} values are −80° and −19° respectively; in B-DNA the pucker is C2′ *endo* and in A-DNA it is C3′ *endo*. Thus the changes in the environments of the nucleosides in the crystal bring about similar changes in conformation to those occurring when DNA changes from one form to the other. Although there is this apparent correlation between the type of pucker and the mean value of φ_{CN} in nucleosides, there is an overlap of the ranges of the φ_{CN} values for the two types of pucker as may be seen from Table 1 of Haschemeyer and Rich[12].

Table 1. CONFORMATIONAL DATA ON NUCLEOSIDES

Nucleoside	φ_{CN}	Pucker	Ref.
Bromouridine			
Bromouridine + adenosine	−20°	C3′ *endo*	3
Bromouridine	−56°	C2′ *endo*	4
Bromouridine + dimethylsulphoxide	−63°	C2′ *endo*	5
Inosine			
Molecule I	−10·6°	C3′ *endo*	6, 7
Molecule IIA	−121°	C2′ *endo*	8, 9
Molecule IIB	−45°	C2′ *endo*	8, 9
Iodouridine			
Molecule I	−13°	C3′ *endo*	10
Molecule II	−59°	C2′ *endo*	10

I thank Dr Struther Arnott for showing me a draft of his manuscript with D. W. L. Hukins before publication, and Dr P. Tollin for discussion.

H. R. WILSON

Carnegie Laboratory of Physics,
University of Dundee,
Dundee, Scotland.

Received October 27, 1969.

[1] Arnott, S., and Hukins, D. W. L., *Nature*, **224**, 886 (1969).

[2] Sundaralingam, M., *Biopolymers*, **7**, 821 (1969).

[3] Haschemeyer, A. E. V., and Sobell, H. M., *Acta Cryst.*, **18**, 525 (1965).

[4] Iball, J., Morgan, C. H., and Wilson, H. R., *Proc. Roy. Soc.*, A. **295**, 320 (1966).

[5] Iball, J., Morgan, C. H., and Wilson, H. R., *Proc. Roy. Soc.*, A, **302**, 225 (1968).

[6] Tollin, P., and Munns, A. R. I., *Nature*, **222**, 1170 (1969).

[7] Munns, A. R. I., and Tollin, P., *Acta Cryst.* (in the press).

[8] Bugg, C. E., Thewalt, U. T., and Marsh, R. E., *Acta Cryst.* (in the press).

[9] Munns, A. R. I., Tollin, P., Wilson, H. R., and Young, D. W., *Acta Cryst.* (in the press).

[10] Rahman, A., and Wilson, H. R., *Nature*, **225**, 64 (1970).

[11] Donohue, J., and Trueblood, K. M., *J. Mol. Biol.*, **2**, 363 (1960).

[12] Haschemeyer, A. E. V., and Rich, A., *J. Mol. Biol.*, **27**, 369 (1967).

[13] Arnott, S., Dover, S. D., and Wonacott, A. J., *Acta Cryst.*, B**25**, 2192 (1969).

Electron Reactions in γ-Irradiated Thymidine and Cystamine

ALTHOUGH the technique of electron spin resonance (ESR) has been useful in the identification of some of the secondary radical species formed in γ-irradiated DNA and proteins, difficulties have been experienced in the interpretation of the spectra of the primary radiolytic products. Two of these secondary species are the thymine radical (with an additional H-atom on C-6 of the thymine ring) in DNA (refs. 1–3) and the radical R—CH$_2$—S$^\cdot$ in cystine-containing proteins[4–6]. Both have been postulated to be the result of electron reactions[7–10]. We have irradiated two model compounds, thymidine and cystamine, in frozen glasses of either ethanediol–water (1 : 1) or 6 M NaOH and, by comparing the ESR and optical absorption spectra, we have identified the anions produced.

All solutions were degassed and irradiated at less than 90 K with ^{60}Co γ-rays. Measurements were made in the dose range 0–400 krad. The first derivative ESR spectra were recorded at 77 K using a conventional X-band spectrometer[8]. Optical absorption spectra were recorded at about 90 K on an *SP*800 spectrophotometer using a spectrodewar similar to that described by Simons[11] and supplied by Jencons Ltd, England. All the spectroscopic details are summarized in Table 1.

Table 1. SPECTROSCOPIC DETAILS OF THE SPECIES OBSERVED IN γ-IRRADIATED GLASSES

Species observed	ESR g	N	Optical absorption λ_{max}	$G\varepsilon_{max}$	ε_{max}	Pulse radiolysis at room temp. in H$_2$O λ_{max}	ε_{max}	Ref.
Ethanediol-H$_2$O								
e_t^-	2·001	1	583	31,200	20,800	—	—	—
–(S–S)$^-$–	2·01	1	395	19,200	7,250	410	9,400	15
T	2·004	2	320	2,300	920	310	1,050–1,200	*
TH$^\cdot$	2·004	8	410	930	415 ‡	400	200	*
6 M NaOH								
e_t^-	2·001	1	588	34,000	28,000	—	—	—
T$^-$	2·004	2	330	3,010	2,150	—	—	—
TH$^\cdot$	2·004	8	470	1,920	1,010 ‡	475	770	* †
O$^-$	2·05	2	—	—	—	—	—	—

* Unpublished work of E. M. Fielden and G. Stevens.

† At *p*H 13·5.

‡ Measured after 10 min ultraviolet irradiation ($\lambda > 310$ nm).

g: g value of ESR spectrum; N: number of lines in ESR spectrum; λ_{max}: wavelength (in nm) of maximum optical absorption; G: number of species formed by 100 eV of absorbed radiation which can be calculated from the ESR data; ε_{max}: extinction coefficient at λ_{max}. In 6 M NaOH, thymidine is in the ionized form, which accounts for the shift in the absorption maxima of T$^-$ and TH$^\cdot$. A similar effect has been observed with the OH radical adduct of thymine in pulse radiolysis studies[17]. ε_{max} is calculated using G values taken from the ESR work. Quoted absolute radical yields vary considerably from one laboratory to another. Relative errors in the values of ε_{max} are ±10 per cent; the absolute errors are ±30 per cent.

In the absence of solutes the ESR spectrum of an irradiated ethanediol-H$_2$O glass consisted of a sharp singlet ($g = 2·001$) (from the trapped electron, e_t^-) (refs. 12, 13) superimposed on a triplet. Bleaching of the e_t^- caused it to react with the ethanediol to give an increase in the triplet spectrum. In the presence of 0·05 M cystamine the yield of e_t^- was reduced to 43 per cent although the yield of the triplet was unchanged. There was a new broad singlet in the ESR spectrum at $g = 2·01$ with an associated optical absorption at 395 nm. Bleaching e_t^- with white light did not affect this new absorption. The ESR spectrum of the free radical R—CH$_2$—S$^\cdot$ which has been observed in many irradiated sulphur compounds (see, for example, references 5, 10 and 14) was absent. The species observed in these studies was formed at the expense of e_t^- and we can therefore conclude that it is the cystamine anion, the high g value indicating that the electron is localized on the disulphide bond.

In the presence of 0·08 M thymidine, the yield of e_t^- was reduced to 10 per cent and a new optical absorption was observed at 320 nm. The ESR spectrum contained a doublet with a hyperfine splitting of 15 G together with a small trace of an eight line spectrum superimposed on the triplet from ethanediol (the yield of which was again unchanged by the presence of the additive). White light bleaching removed e_t^- with no noticeable effect on the other species, whereas on irradiation with ultraviolet light (wavelengths above 310 nm) the doublet disappeared and at the same time there was a large increase in the eight line ESR spectrum which has been identified as the thymine radical (TH$^\cdot$) (ref. 3). Comparison of the ESR and optical data showed that this radical had an absorption maximum at 410 nm. The doublet ESR spectrum together with the optical absorption at 320 nm must be attributed to the thymidine anion (*T*$^-$) because its formation leads to a large reduction in e_t^-.

Examples of spectra obtained in 6 M NaOH glasses are shown in Figs. 1 and 2. The ESR spectrum of a γ-irradiated 6 M NaOH glass revealed a sharp singlet at $g = 2·001$ superimposed on a broad doublet centred at $g = 2·05$. These spectra arise from e_t^- and O$^-$, respectively[12,13,16]. The addition of 0·08 M thymidine reduced the yield of e_t^- to 6 per cent and increased the yield of O$^-$ to 135 per cent. With ESR we observed the additional spectra of T$^-$ (a doublet) and TH$^\cdot$ (eight lines) (Fig. 1). The corresponding optical absorption spectra had maxima at 330 nm and 470 nm (Fig. 2). The 6 M NaOH glass was different from polycrystalline water[8] and ethanediol–H$_2$O glass, in that there was a large yield of TH$^\cdot$ immediately after irradiation. Removal of e_t^- with white light had no observable effect on the other species present. Subsequent irradiation at ultraviolet wavelengths above 310 nm removed T$^-$ (330 nm absorption and ESR doublet) and increased the yield of TH$^\cdot$ (470 nm absorption and ESR octet). For purposes of comparison, the absorption maxima and optical extinction coefficients for the same species observed in aqueous solution at room temperature are given in Table 1. These data have been obtained by others using pulse radiolysis.

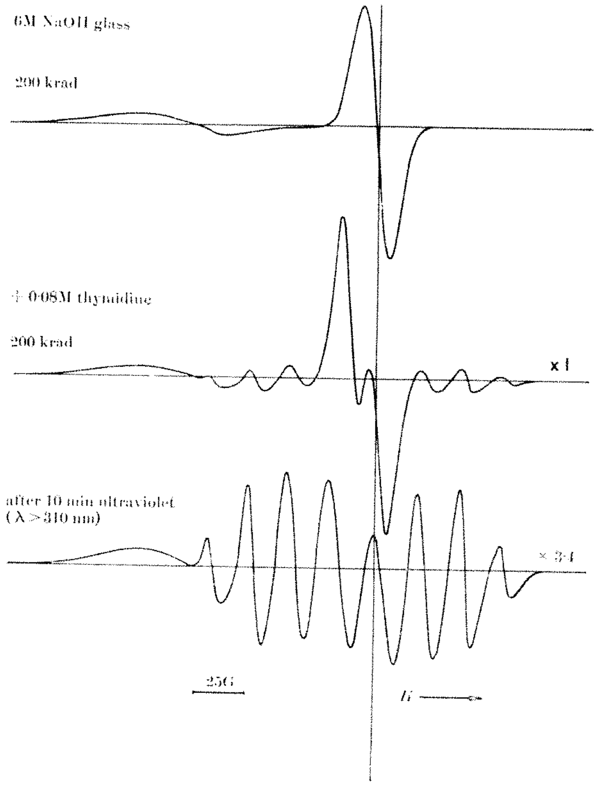


Fig. 1. ESR of 6 M NaOH glasses at 77 K. The samples were γ-irradiated at 77 K to a dose of 200 krad. The upper spectrum is of irradiated 6 M NaOH, the centre spectrums is with 0·08 M thymidine, and the lower spectrum shows the effect of 10 min ultraviolet bleaching (above 310 nm) on the latter sample. The vertical axis shows the resonance position of diphenyl picryl hydrazyl (DPPH). The relative amplifier gains are shown.

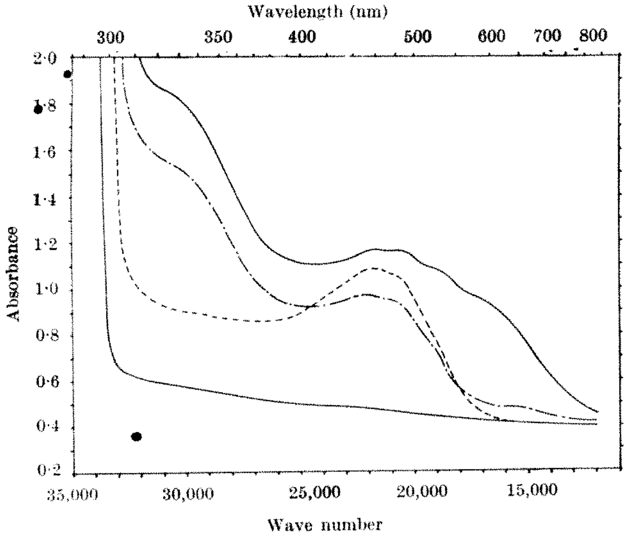


Fig. 2. Optical absorption spectra of 0·08 M thymidine in a 6 M NaOH glass at about 90 K. Upper solid line, after 400 krad γ-irradiation at about 90 K; broken line, same sample after 20 min white light bleaching; dashed line, same sample after 20 min ultraviolet bleaching (above 310 nm); lower solid line, unirradiated base line.

It has previously been postulated that the thymine radical ($TH^{\bullet}$) is formed in γ-irradiated thymidine, thymidylic acid and DNA, not by the direct addition of a hydrogen atom to the thymine ring, but by protonation of the thymine anion (T^-) (refs. 7, 8). Although there is good evidence to support this postulate, part of the argument is based on the assignment of the ESR doublet to T^-. Firm identification of this species confirms the previously published reaction mechanism[8]. Thymidine and cystamine compete for electrons in irradiated frozen solutions at 77 K (ref. 8), and in suitable conditions e_t^-, T^- and $(R{-}S{-}S{-}R)^-$ are all present. On warming, electrons appear to transfer to cystamine. The optical spectra can be used to follow these changes (these reactions will be described elsewhere).

We have shown here that the broad singlet at $g = 2{\cdot}01$ observed in the ESR spectrum of cystamine arises from a disulphide anion. Similar ESR spectra have been observed in oxidized glutathione and cystine-containing proteins irradiated at 77 K (refs. 10, 18). These spectra, which are reduced in intensity in the presence of electron scavengers, arise from disulphide anions. It has been clearly demonstrated that their reactions at room temperature leads to the formation of the radical $R{-}CH_2{-}S^{\bullet}$ (refs. 18–20). Our identification of the disulphide anion fully confirms the earlier postulate that the sulphur radicals observed in irradiated proteins are formed by the reaction of electrons produced by radiation.

We thank Professor A. Charlesby for his support and encouragement, Dr E. M. Fielden and Mr G. Stevens for making available their unpublished data and Mr Stevens for discussions. This work was partly supported by grants to the Chester Beatty Research Institute, Institute of Cancer Research, from the Medical Research Council, the British Empire Cancer Campaign for Research and the National Cancer Institute, US Public Health Service.

A. D. LENHERR

Department of Physics,
Royal Military College of Science,
Shrivenham, Wiltshire.

M. G. ORMEROD

Chester Beatty Research Institute,
Institute of Cancer Research,
Belmont,
Sutton, Surrey.

Received August 18; revised October 6, 1969.

[1] Ehrenberg, A., Ehrenberg, L., and Löfroth, G., *Nature*, **200**, 376 (1963).
[2] Salovey, R., Shulman, R. G., and Walsh, jun., W. M., *J. Chem. Phys.*, **39**, 839 (1963).
[3] Pruden, B., Snipes, W., and Gordy, W., *Proc. US Nat. Acad. Sci.*, **53**, 917 (1965).
[4] Gordy, W., and Shields, H., *Proc. US Nat. Acad. Sci.*, **46**, 1124 (1960).
[5] Kurita, Y., and Gordy, W., *J. Chem. Phys.*, **34**, 282 (1961).
[6] Henriksen, T., Sanner, T., and Pihl, A., *Rad. Res.*, **18**, 147 (1963).
[7] Ormerod, M. G., *Int. J. Rad. Biol.*, **9**, 291 (1965).
[8] Lenherr, A. D., and Ormerod, M. G., *Biochim. Biophys. Acta*, **166**, 298 (1968).
[9] Holroyd, R. A., and Glass, J. W., *Int. J. Rad. Biol.*, **14**, 445 (1969).
[10] Ormerod, M. G., and Singh, B. B., *Biochim. Biophys. Acta*, **120**, 413 (1966).
[11] Simons, J. P., *J. Sci. Instrum. Ser. 2*, **1**, 872 (1968).
[12] Schulte-Frohlinde, D., and Eiben, K., *Z. Naturforsch.*, **17**a, 445 (1962); **18a**, 199 (1963).
[13] Moorthy, P. N., and Weiss, J. J., *Adv. Chem. Ser.*, **50**, 180 (1965).
[14] Henriksen, T., *J. Chem. Phys.*, **37**, 2189 (1962).
[15] Adams, G. E., Armstrong, R. C., Charlesby, A., Michael, B. D., and Willson, R. L., *Trans. Faraday Soc.*, **65**, 732 (1969).
[16] Ayscough, P. B., Collins, R. G., and Dainton, F. S., *Nature*, **205**, 965 (1965).
[17] Myers, jun., L. S., Hollis, M. L., and Theard, L. M., *Adv. Chem. Ser.*, **81**, 345 (1968).
[18] Singh, B. B., and Ormerod, M. G., *Nature*, **206**, 1314 (1965).
[19] Singh, B. B., and Ormerod, M. G., *Int. J. Rad. Biol.*, **10**, 369 (1966).
[20] Singh, B. B., and Ormerod, M. G., *Biochim. Biophys. Res. Commun.*, **25**, 28 (1966).

Correlation of Bradykinin Structure with ability to increase Vascular Permeability

OF the chemical substances that possibly mediate the inflammatory response[1,2], bradykinin (H–L-Arg–L-Pro–L-Pro–Gly–L-Phe–L-Ser–L-Pro–L-Phe–L-Arg–OH) warrants special study because four of its pharmacological activities—the capacity to increase vascular permeability and to initiate vasodilation, pain and the emigration of leucocytes—are all encountered in acute inflammation[3]. We have sought a correlation of the structure of bradykinin with its ability to increase vascular permeability. The effect on activity was investigated when each of the nine amino-acids in the peptide chain was replaced in turn with L-alanine.

Reference bradykinin and its 1-alanine analogue (H–L-Ala–L-Pro–L-Pro–Gly–L-Phe–L-Ser–L-Pro–L-Phe–L-Arg–OH) were synthesized[4] by the Merrifield solid phase method[5]; samples of synthetic bradykinin and other analogues[6] were supplied by Dr E. Schröder, Hauptlaboratorium der Schering AG, Berlin.

The two synthetic bradykinin samples and the analogues were assayed against histamine and natural bradykinin (Sandoz) by intradermal injection in the dorsal skin of depilated albino guinea-pigs and closely shaven rabbits which had previously received intravenous Evans blue[7]. The dye becomes bound to plasma albumin, and local exudation of the dye–albumin complex registers increased vascular permeability which was estimated from the diameter and intensity of the blue lesions, 30 min after intradermal injection of the test factors[7,8]. Each point on the dose-response graphs (Fig. 1) represents the mean of eight lesions in four guinea-pigs or two rabbits for each test concentration of a permeability factor[7]. Relative permeability factor activity was estimated from reasonably parallel dose-response curves at lesion diameters of 7–8 mm. Fig. 1 illustrates the dose-response relationship in the guinea-pig for the most active alanine analogue, [Ala^3]-bradykinin, compared with those of the two standards.

Table 1 records the permeability factor potency of the nine alanine analogues, relative to natural bradykinin (which is assigned an arbitrary value of one). Bradykinin A synthesized by the Merrifield method had a relative potency of 0·5–0·8 which is similar to that (0·6–0·8) of bradykinin B synthesized by Dr Schröder according to classical methods. In both the rabbit and guinea-pig the

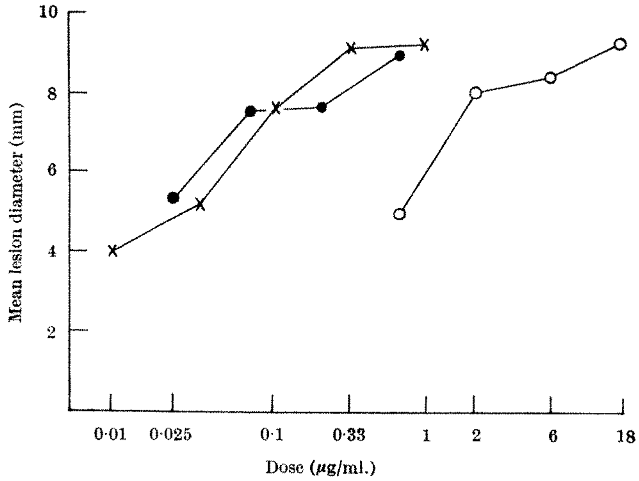


Fig. 1. Dose-response lines for permeability factor in the guinea-pig. ●, Bradykinin; ○, histamine; ×, [Ala^{3}]-bradykinin.

order of potencies is [Ala^{3}]-bradykinin > [Ala^{6}]-bradykinin > [Ala^{7}]-bradykinin; the remaining analogues have no significant activity. This order of potency resembles that from assays using the rat uterus, guinea-pig ileum and rabbit blood pressure[9], and suggests a similarity in mechanism of action or site of reaction in the two systems.

Table 1. RELATIVE PERMEABILITY FACTOR POTENCY OF BRADYKININ AND ANALOGUES IN THE GUINEA-PIG AND RABBIT

Permeability factor	Residue substituted	Guinea-pig	Rabbit
[Ala^{1}]-bradykinin	Arg	0·0025	0·002
[Ala^{2}]-bradykinin	Pro	0·002	0·0125
[Ala^{3}]-bradykinin	Pro	1	1
[Ala^{4}]-bradykinin	Gly	0·002	0·002
[Ala^{5}]-bradykinin	Phe	0·002	0·002
[Ala^{6}]-bradykinin	Ser	0·25	0·125
[Ala^{7}]-bradykinin	Pro	0·025	0·008
[Ala^{8}]-bradykinin	Phe	0·002	0·002
[Ala^{9}]-bradykinin	Arg	0·0025	0·002
Histamine	—	0·04	0·062

We conclude that there are three sites on the bradykinin molecule—the centre and the two ends—which contribute predominantly to its biological activity. In the centre, the substitution of a methyl group for hydrogen in the 4-alanine analogue, or a hydrogen for a phenyl group in the adjacent phenylalanyl residue, leaves no significant activity. This can be attributed in each case to a steric effect. Neither the size nor the polar nature of the hydroxyl group of the seryl residue in the 6-position seems to contribute significantly to the biological activity of the molecule, for the replacement of hydroxyl with hydrogen results only in a relatively small change of activity.

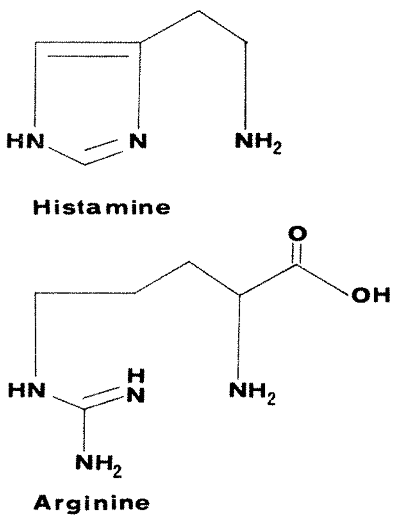


Fig. 2. Structural similarity between histamine and arginine.

The two ends of the molecule have in common a very basic arginyl residue, which exerts a dominant influence on activity. Structurally, arginine resembles histamine (Fig. 2) and this similarity may account, in part, for the similar biological activities of histamine and bradykinin. The replacement of the phenylalanyl and prolyl residues with alanyl in the 8 and 2-analogues, respectively, must bring about a substantial change in molecular conformation, probably a displacement of the neighbouring arginyl residues.

Thus bradykinin has an active site similar to histamine at each end of the peptide chain and at the centre, a site important for its active conformation; this is compatible with the suggestion[10] that bradykinin exists in a cyclic form.

G. J. H. Melrose
H. K. Muller
W. J. Vagg

Department of Applied Organic Chemistry
and School of Pathology,
University of New South Wales,
Sydney, Australia 2033.

Received August 25; revised October 21, 1969.

[1] Wilhelm, D. L., *Pharmacol. Rev.*, **14**, 215 (1962).
[2] Spector, W. G., and Willoughby, D. A., *Bact. Rev.*, **27**, 117 (1963).
[3] Lewis, G. P., *Ann. NY Acad. Sci.*, **104**, 236 (1963).
[4] Melrose, G. J. H., Muller, H. K., and Vagg, W. J., *Proc. Austral. Biochem. Soc.*, 51 (1968).
[5] Merrifield, R. B., *J. Amer. Chem. Soc.*, **85**, 2149 (1963); **86**, 304 (1964).
[6] Schröder, E., *Liebigs Ann. Chem.*, **679**, 207 (1964).
[7] Miles, A. A., and Miles, E. M., *J. Physiol.*, **118**, 228 (1952).
[8] Rawson, R. A., *Amer. J. Physiol.*, **138**, 708 (1943).
[9] Schröder, E., and Hempel, R., *Experientia*, **20**, 529 (1964).
[10] Spragg, J., Schröder, E., Stewart, J. M., Austen, K. F., and Haber, E., *Biochemistry*, **6**, 3933 (1967).

Biosynthesis of Insulin in Anglerfish Islets

It is now well established that insulin is synthesized according to the proinsulin route[1-4]. Our studies are concerned with the mechanisms of biosynthesis in anglerfish islets. The anglerfish insulin was isolated chromatographically on CM-cellulose as previously described[5,6]; a typical chromatogram is presented in Fig. 1. The main components of the first two peaks were found to be two peptides which have amino-acid compositions similar to that of hog glucagon.

Amino-acid analyses of the material recovered from effluent of peak 3 indicated that it had no apparent relationship with insulin. Insulin (peak 4) was eluted at 15–16 mΩ^{-1} and was eventually recovered in the hydrochloride form[5,6]. The specific activity of the anglerfish insulin was found to be 25 to 26 IU/mg according to the mouse convulsion assay. Amino-acid analyses after acid hydrolysis revealed a molar composition in excellent agreement with the theoretically expected values for anglerfish insulin[7].

Peak 5 was eluted after insulin; it was always very small and at times invisible. It could, however, be located easily in pulse labelled experiments because of the significant radioactivity.

The results of several incorporation experiments with fresh anglerfish islets incubated with L-^{3}H-proline can be summarized as follows: (*a*) The specific radioactivity of the isolated insulin (cpm/mg) was found to increase with the time of incubation. (*b*) Only after incubation of the islets for at least 40 min did radioactivity begin to appear in insulin. (*c*) L-^{3}H-proline was found to be incorporated into the material of peak 5. Incorporation of ^{3}H-proline into this protein preceded that into insulin and the specific activity of this material was several-fold higher than that of insulin (Fig. 1, I). (*d*) Further, it was found that arrest of protein synthesis by sparsomycin[8] and continuation of incubation after the addition of the antibiotic resulted in increased radioactivity in the insulin peak and a comparable decrease of radioactivity in the area of peak 5 (Fig. 1, II). The continuous increase of the specific radioactivity of insulin in the absence of protein synthesis indicates that the conversion of precursor(s) to insulin is not dependent on protein synthesis. Further, the decline in the radioactivity of peak 5 correlates with the increased radioactivity in the insulin peak, which strongly suggests that the material of peak 5 may be a precursor of insulin. Clearly, although the synthesis of this precursor has been arrested by the inhibitor, its conversion to insulin has not.

The material from peak 5 was recovered in the hydrochloride form and amino-acid analyses (Table 1) gave an amino-acid composition different from that of insulin but comprising all the amino-acids present in insulin.

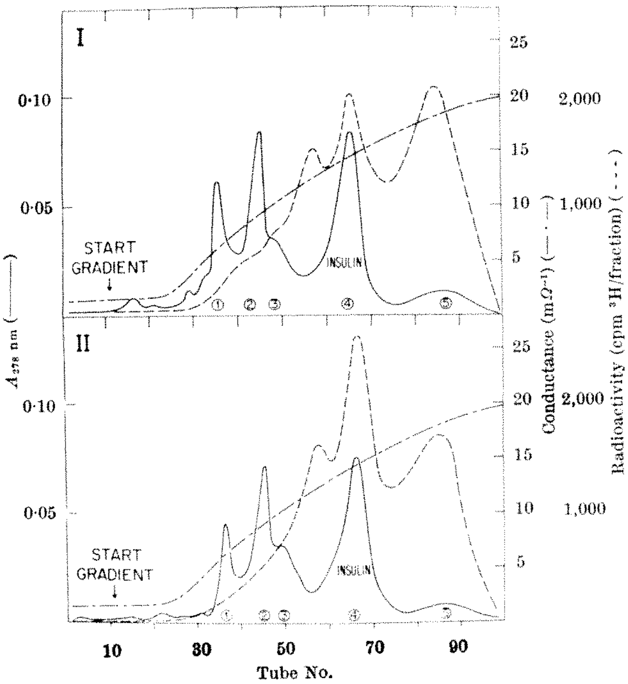


Fig. 1. I, Column chromatography of material obtained from 16 anglerfish islets incubated for 75 min in the presence of 0·5 mCi of ^{3}H-proline. The minced islets were incubated in 5 ml. of incubation medium at *p*H 7·4 under 95 per cent O_2/5 per cent CO_2 in the presence of L-^{3}H-proline in a Dubnoff metabolic shaker at 20° C. The incubation medium was Hanks salt solution containing 350 mg/100 ml. of glucose and 2 mg/100 ml. of each of the naturally occurring amino-acids, excluding proline. After 75 min of incubation, trichloroacetic acid (TCA) was added to a final concentration of 10 per cent and insulin was isolated as follows: (*a*) homogenization of islets in 10 per cent TCA; (*b*) extraction of the precipitate with HCl-ethanol; (*c*) evaporation in a rotary evaporator; (*d*) extraction of lipids with methylene chloride; (*e*) precipitation with TCA and extraction of the precipitate with acetic acid/NaCl; (*f*) precipitation with picric acid; (*g*) chromatography on a CM-cellulose column. Steps (*a*)–(*e*) have been described by Humble and Crestfield[7]. Steps (*f*) and (*g*) have been described previously[5,6]. During chromatography the effluent was monitored by a Gilford recording spectrophotometer and a conductivity meter. The effluent was collected in 2 ml. fractions with the aid of a fraction collector. 0·5 ml. of a fraction was pipetted into 14·5 ml. of Bray's scintillation liquid and the radioactivity was counted. II, Column chromatography of material obtained from sixteen anglerfish islets incubated for 75 min in the presence of 0·5 mCi of ^{3}H-proline. Then 0·1 mg of sparsomycin was added to the islet suspension and the incubation was continued for another 75 min. The incubation media and techniques are as described for I.

Table 1. AMINO-ACID COMPOSITION* OF ANGLERFISH INSULIN AND PROINSULIN

	Insulin		Proinsulin	
Amino-acid	Theory	Found	Found	Nearest integer
Lys	1	1·2	8·1	8
His	3	2·8	2·7	3
Arg	2	2·0	8·2	8
Asp	7	6·7	7·1	7
Thr	—	—	3·7	4
Ser	1	1·1	3·6	4
Glu	4	4·1	8·7	9
Pro	3	2·8	3·0	3
Gly	4	3·8	5·9	6
Ala	3	3·1	6·2	6
Val	4	3·7	4·1	4
Ile	2	1·7	3·1	3
Leu	5	4·8	6·7	7
Tyr	3	2·7	2·7	3
Phe	3	2·8	2·8	3
Cys	6	5·6	5·6	6
Total	51			84†

* Number of amino-acid residues per molecule according to analysis by the method of Spackman *et al.*[9] in a Beckman-Spinco analyser (model 120C) equipped with a digital readout system.

† Minimum possible number of amino-acid residues.

The N-terminal amino-acid was determined by the dansyl technique[10] and found to be valine, which is also the N-terminal residue of the B-chain of anglerfish insulin. This protein was incubated with 10 µg of trypsin in 0·05 M Tris buffer (*p*H 7·5) at 37° C for 2 h, and cold anglerfish insulin was added. On chromatographic separation most of the radioactivity was eluted with the insulin. We conclude that this protein represents the anglerfish proinsulin. According to the mouse convulsion assay method, the insulin-like activity of proinsulin was less than 2·5 IU/mg.

This work was supported by a grant from the US National Institutes of Health.

ANTHONY C. TRAKATELLIS
GERALD P. SCHWARTZ

Department of Biochemistry,
Mount Sinai School of Medicine of
The City University,
10 East 102nd Street,
New York.

Received July 21; revised September 19, 1969.

[1] Steiner, D. F., Cunningham, D., Speigelman, L., and Aten, D., *Science*, **157**, 697 (1967).
[2] Steiner, D. F., and Oyer, P. E., *Proc. US Nat. Acad. Sci.*, **57**, 473 (1967).
[3] Chance, R. E., Ellis, R. M., and Bromer, N. N., *Science*, **161**, 165 (1968).
[4] Grant, P. T., and Reid, K. B. M., *Biochem. J.*, **110**, 281 (1968).
[5] Katsoyannis, P. G., Trakatellis, A. C., Johnson, S., Zalut, C., and Schwartz, G., *Biochemistry*, **6**, 2642 (1967).
[6] Katsoyannis, P. G., Trakatellis, A. C., Zalut, C., Johnson, S., Tometsko, A., Schwartz, G., and Ginos, J., *Biochemistry*, **6**, 2656 (1967).
[7] Humble, R. E., and Crestfield, A. M., *Biochemistry*, **4**, 1044 (1965).
[8] Trakatellis, A. C., *Proc. US Nat. Acad. Sci.*, **59**, 854 (1968).
[9] Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1190 (1958).
[10] Woods, K. R., and Wang, K. T., *Biochim. Biophys. Acta*, **133**, 369 (1967).

Effect of L-Asparaginase from *Aspergillus terreus* on Ascites Sarcoma in the Rat

IT is well known that L-asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1) is the antitumour agent in guinea-pig serum[1]. The same antitumour activity has been demonstrated for the L-asparaginase from *Escherichia coli*[2] and recently for that from *Erwinia carotovora*[3]. L-Asparaginase preparations from other bacteria[2,4] or yeast[5] have no inhibitory action. After screening a number of microorganisms, a strain of *Aspergillus terreus* which produces an L-asparaginase active against Walker 256 ascites carcinosarcoma[6] has now been isolated.

The organism was grown for 48 h at 24° C in submerged culture on nutrient broth (*p*H 7) containing 5 per cent peptone, 1 per cent beef extract, 5 per cent mannitol in distilled water. The cells were harvested by filtration, washed with distilled water and disrupted with the 'X' press (Biox, Nacka, Sweden). The enzyme was extracted with 0·02 M phosphate buffer (*p*H 8), according to the procedure described by Roberts *et al.*[4] and partially purified through a column of 'Sephadex *G*-50' gel. This extract, tested according to the method of Roberts *et al.*[4], exhibited an activity of 66 I.U./g mycelial dry weight (0·6 I.U. per mg protein) in 0·1 M Tris-phosphate buffer (*p*H 7) at 37° C. The *A. terreus* asparaginase has optimum activity at *p*H 6–8 (Fig. 1).

The antitumour activity of this enzyme preparation was compared with that of guinea-pig serum on a Walker 256 carcinosarcoma growing in ascitic form in the peritoneal cavity of the rat. Male Wistar rats, weighing approximately 80 g, were divided into three groups each consisting of eighteen animals. All animals were inoculated intraperitoneally with about 5×10^5 tumour cells. Rats of one group then immediately received 2·5 ml. of sterile 0·154 M NaCl, intraperitoneally. Animals of the other two groups received 2·5 ml. of either guinea-pig serum or *A. terreus* L-asparaginase preparation dissolved in saline, both containing 4·0 I.U. of enzyme. The same treatments were repeated every 24 h for 8 days. All treatments were tolerated by the animals.

Table 1. EFFECT OF GUINEA-PIG SERUM AND OF L-ASPARAGINASE FROM *Aspergillus terreus* ON WALKER 256 ASCITES CARCINOSARCOMA IN THE RAT

Treatment	No. of rats	Healthy survivors after 60 days	Mortality in less than 60 days from Ascitic tumour	Combined ascitic and solid tumour	Solid tumour	Survival time (days)
Saline	18	0	2	16	0	21·7 ± 1·2
Guinea-pig serum	18	5	10	3	0	41·1 ± 1·0*
A. terreus asparaginase	18	4	1	2	11	41·0 ± 2·7*

* $P < 0·01$.

The results (Table 1) show that the asparaginase produced by *A. terreus* has a carcinostatic effect on Walker 256 ascites carcinosarcoma. As compared with guinea-pig serum, this preparation of *A. terreus* asparaginase has a greater carcinostatic effect on the ascitic tumour and a smaller effect on those solid tumours that frequently develop in the subcutaneous at the inoculation site.

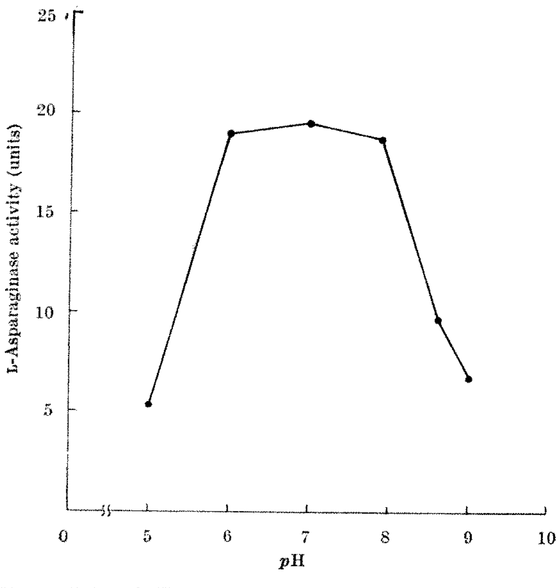


Fig. 1. Effect of *p*H on *A. terreus* L-asparaginase. Assays were carried out in 0·0285 M citric acid–barbital–KH_2PO_4–boric acid buffer, as described by Roberts *et al.*[7]. The *p*H was determined before and after incubation.

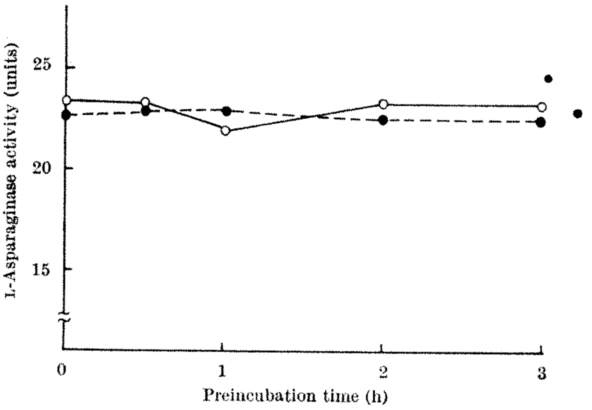


Fig. 2. Stability of *A. terreus* L-asparaginase. ●– – –●, 0·05 M phosphate buffer (*p*H 6·8); ○——○, rat ascitic fluid.

The *A. terreus* asparaginase was tested after preincubation at 37° C either in 0·05 M phosphate buffer (*p*H 6·8, the *p*H of the peritoneal fluid) or in rat ascitic fluid (Fig. 2). At intervals, aliquots of the incubation mixtures were assayed for L-asparaginase activity according to the method of Roberts *et al.*[7] with few modifications (the incubation mixtures contained 0·1 M Tris-HCl buffer, *p*H 7·0, instead of 0·05 M Tris-HCl buffer, *p*H 8·5). Because the *A. terreus* asparaginase is not inactivated either at the *p*H of the peritoneal fluid or by the ascitic fluid, the weak activity on solid tumours may be correlated with the low diffusibility of this enzyme preparation from the peritoneal cavity into the blood stream.

Although it seems improbable, we cannot exclude the possibility that the antitumour activity is to be attributed to other proteins present in *A. terreus* extracts, because of the low degree of purity of the product.

We thank Professor A. Ballio, Istituto Chimico, University of Naples, for his advice and encouragement.

LAVINIA CARTA DE-ANGELI
F. POCCHIARI
SERENA RUSSI
A. TONOLO
VICTORIA E. ZURITA

Laboratori di Chimica Biologica,
Istituto Superiore di Sanità,
Rome.

E. CIARANFI
A. PERIN

CNR Centro di Patologia Cellulare,
Istituto di Patologia Generale,
Università di Milano,
Milan.

Received June 10; revised August 11, 1969.

[1] Broome, J. D., *Nature*, **191**, 1114 (1961).
[2] Mashburn, L. T., and Wriston, J. C., *Arch. Biochem. Biophys.*, **105**, 450 (1964).
[3] Wade, H. E., Elsworth, R., Herbert, D., Keppie, J., and Sargeant, K., *Lancet*, ii, 776 (1968).
[4] Roberts, J., Burson, G., and Hill, J. M., *J. Bact.*, **95**, 2117 (1968).
[5] Broome, J. D., *J. Nat. Cancer Inst.*, **35**, 967 (1965).
[6] Kwak, K. S., Jameson, E., Ryan, A. M., and Kurtz, H. M., *Cancer Res.*, **21**, 44 (1961).
[7] Roberts, J., Prager, M. D., and Bachynsky, N., *Cancer Res.*, **26**, 2213 (1966).

Immunosuppression by L-Asparaginase

L-ASPARAGINASE, an enzyme which occurs in the serum of certain rodents and is produced by various micro-organisms, catalyses the hydrolysis of asparagine to L-aspartic acid and ammonia. It has been found useful in the treatment of acute lymphoblastic leukaemia and in occasional cases of other types of neoplasm in man[1].

It has been shown recently that the phytohaemagglutinin-induced transformation of normal human lymphocytes is inhibited by asparaginase *in vitro*[2] and *in vivo*[3]. I have studied the effects of asparaginase on antibody producing cells. Similar investigations were made independently during the course of this work by Dr L. G. Lajtha and Professor P. Alexander, and their findings accord with some of those described here.

Male Balb/C mice, weighing 15–20 g, were injected intraperitoneally with 0·2 ml. of a 10 per cent suspension in saline of formalized sheep red cells (Burroughs Wellcome). Spleens were removed 4 days later (except as described here) and the numbers of cells producing IgM haemolysin counted by Jerne's method[4]. The results are here expressed as fractions of the geometric mean control value (which varied in different experiments from 40,000 to 140,000 antibody-forming cells per spleen on day 4).

The initial experiments were carried out with Bayer asparaginase, which is prepared from *Escherichia coli*. It was at first assumed that asparaginase would be most effective 2 days after the antigen injection, when immunocytes would be proliferating most rapidly. Even doses of 5,000 IU per mouse (or about 250,000 IU/kg) given intravenously at that time had little effect, however, reducing the number of splenic haemolysin-producing cells to 0·7 of the control value. The effect of varying the time of injection of enzyme relative to that of the antigen was therefore investigated.

Mice were given single intravenous injections of 1,000 IU Bayer asparaginase at various times before or after sheep red cells. There was a clear-cut time dependence in the immunosuppressive effect, the enzyme being maximally effective when given during the period from 24 h before to 4 h after the antigen (Fig. 1).

Mice were given 1,000 IU asparaginase 24 h before sheep red cells and spleens examined 2, 4, 6, 9 and 12 days later. Depression of the antibody response was temporary—the number of antibody-forming cells approached control values by day 6 (Fig. 2). At an early stage of the investigation, Professor P. Alexander pointed out that the doses of enzyme required for immunosuppression were relatively enormous, and suggested two explanations for this. First, asparaginase preparations of bacterial origin have lower glutaminase activity, and immunosuppression could have arisen from this and not the asparaginase. Second, the enzyme has a half-life *in vivo* of only a few hours[1,5]. If cell susceptibility depends on the duration of exposure to the agent and only single injections are given, inordinately high doses might be needed to keep the *in vivo* concentration high enough over a long enough fraction of the cell cycle. These possibilities were tested by comparing the effects of asparaginase preparations with differing glutaminase activities and differing half-lives.

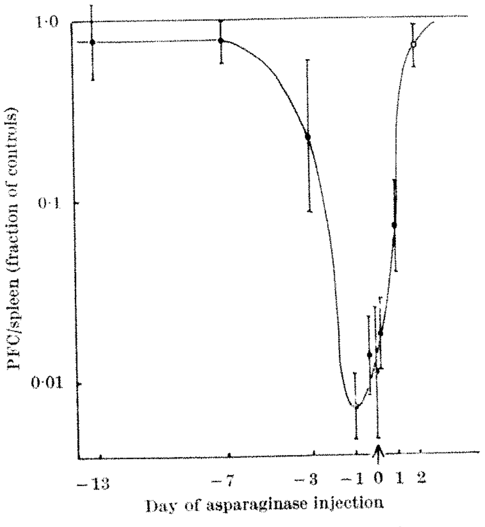


Fig. 1. Time dependence of immunosuppression by asparaginase. Sheep red cells given intraperitoneally on day 0. Asparaginase, 1,000 IU, given intravenously at various times before, on or after day 0 (5,000 IU given on day +2). Spleen plaque-forming cells (PFC) counted on day 4. (Eight mice per point.)

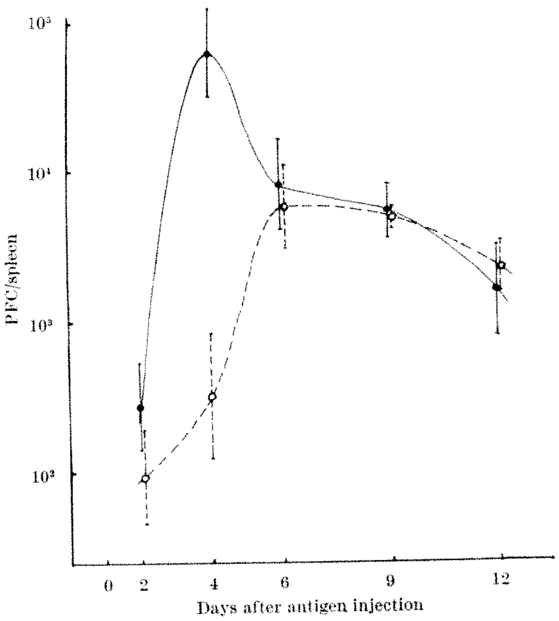


Fig. 2. Duration of immunosuppression by asparaginase. Sheep red cells given on day 0. ●——●, 0·5 ml. saline given intravenously on day −1; ○– –○, 1,000 IU asparaginase in 0·5 ml. saline given intravenously on day −1. (Five mice per point.)

The three materials used were the *E. coli* enzymes produced by Bayer and Squibb and the *Erwinia carotovara* enzyme produced by the Microbiological Research Establishment at Porton. Various doses of these in 0·5 ml. saline were given intravenously to mice 24 h before sheep red cells. The resulting degrees of immunosuppression are shown in Fig. 3.

All three preparations gave dose-response curves fitting the equation for a hyperbolic curve[6]: $F = [D_0/D]^{-\gamma}$, where F is the surviving cell fraction, D the dose, D_0, the threshold dose and $-\gamma$ the slope of the curve. The value of γ is not much different from unity for any of the preparations. The value of D_0 is about 15 IU for the Squibb, 25 IU for the Bayer and 200 IU for the Porton material. The Squibb enzyme is therefore about thirteen times and the Bayer eight times as potent as the Porton enzyme. The glutaminase activities of the three preparations are in the order Porton > Squibb > Bayer; the *in vivo* half-lives are in the order Squibb > Bayer > Porton (personal communication from P. Alexander). It is therefore unlikely that immunosuppression in this system is to be attributed to glutaminase, but the results are consistent with immunosuppressive effectiveness being a function of the half-life—the enzyme that persists longest in the circulation is the most effective. It is relevant that asparaginase from guinea-pig serum—which has a half-life in the mouse of 19 h—is very active against mouse lymphoma, whereas yeast asparaginase, with a half-life of less than 30 min, is inactive[7].

Although cell survival here seems to be related to the duration of exposure to enzyme, it is unlikely that asparaginase is a phase-specific agent in the sense of Bruce *et al.*[8], that is, one that destroys or sterilizes cells exposed to it above a threshold concentration while in a particular phase of the cell cycle. For such an agent, a plot of cell survival against dose of agent, both on linear scales, should in theory give a line which is straight[9], or very nearly straight[10], depending on what assumptions are made in the calculations. The curves in Fig. 2 would, however, not be even remotely linear when plotted in this way and therefore do not support the hypothesis that asparagine acts as a phase-specific agent, at least in the simple sense outlined here.

Further, the effectiveness of phase-specific or cycle-specific agents is characteristically greater for rapidly proliferating cells than for resting cells[11]. The effectiveness of asparaginase is, however, greatest if given just before the antigen, when there is relatively little cell proliferation, and it is practically ineffective if given 2 days after the antigen, when cell multiplication is most marked. This again throws doubt on the phase specificity of asparaginase, although it does not disprove it, for special factors may operate in this system.

If the relative ineffectiveness of the *Erwinia* enzyme in the experiments was the result of inappropriate timing of the injection in relation to its unduly short half-life—that is, if the critical time for exposure of mice to the enzyme began a few hours before injection of antigen and its *in vivo* concentration when injected 24 h previously had fallen too far by that time—then administration nearer the critical time should be more effective. Mice were accordingly given 1,000 IU of one or other enzyme preparation intravenously 15 h, 3 h or 1 min before an intraperitoneal injection of sheep red cells. Fig. 4 shows that the degree of immunosuppression hardly varied, so that this explanation for the lack of potency of the *Erwinia* enzyme does not hold.

The time dependence curve (Fig. 2) suggests that asparaginase affects the cells mediating the immune response at about the time of exposure to antigen and that effectiveness is lost once the cells begin to multiply rapidly, which may be relevant to the action of asparaginase in acute leukaemia. There are several possible explanations for this anomalous sensitivity: (*a*) rapidly proliferating lymphoid cells may normally synthesize asparagine, (*b*) production of asparagine synthetase when induced by lack of exogenous asparagine may be much more rapid in proliferating than in resting cells, (*c*) lymphocytes may be able to obtain exogenous asparagine from feeder cells in the changed milieu of an antigenically stimulated spleen, (*d*) asparaginase may act on macrophages and not on lymphocytes during ingestion and processing of antigen (although one might expect, in this case, that the preparation most rapidly removed from the circulation, that is, with the shortest half-life, would be the most active), (*e*) asparaginase might act on lymphocytes or a subpopulation of lymphocytes involved in the initial stages of antigen recognition, so that administration subsequent to this step would be ineffective.

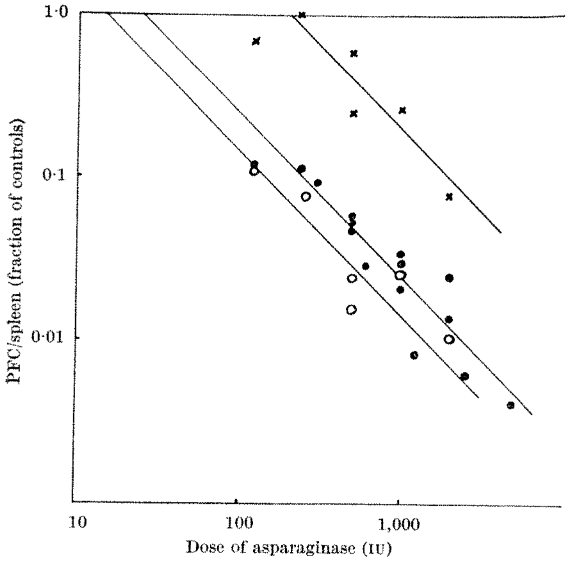


Fig. 3. Dose-response curves for three asparaginase preparations, given in various doses intravenously 24 h before sheep red cells. Spleen plaque-forming cells (PFC) counted on day +4. ×, *Erwinia carotovara* asparaginase; ●, *E. coli* asparaginase (Bayer); ○, *E. coli* asparaginase (Squibb). (Six mice per point.)

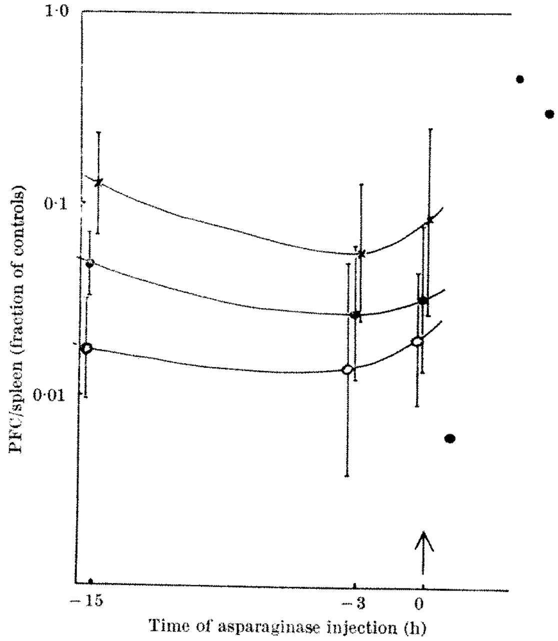


Fig. 4. Time dependence for different asparaginase preparations. Sheep red cells given intraperitoneally at time 0. Asparaginase, 1,000 IU, given intravenously at −15 h, −3 h or −1 min. Spleen plaque-forming cells (PFC) counted on day 4. ×, *Erwinia carotovara* asparaginase; ●, *E. coli* asparaginase (Bayer); ○, *E. coli* asparaginase (Squibb). (Six mice per point.)

Histological studies show that injection of 1,000 IU asparaginase leads to necrosis of lymphoid cells in the thymic cortex and the germinal centres of the mouse spleen and lymph nodes. These changes are greatest 4–8 h after the injection. Atrophy of the thymic cortex follows at 24–48 h.

I thank Professor P. Alexander, Professor R. M. Hardisty and Dr H. E. M. Kay for supplies of asparaginase and Miss L. Stephenson for assistance. This work was supported by the British Empire Cancer Campaign for Research, the Leukaemia Research Fund and the Nuffield Foundation.

M. C. BERENBAUM

Wellcome Laboratories of
Experimental Pathology,
Variety Club Research Wing,
St Mary's Hospital Medical School,
London W2.

Received October 13, 1969.

[1] Adamson, R. H., and Fabro, S., *Cancer Chemother. Rep.*, **52**, 617 (1968).
[2] Astaldi, G., Burgio, G. R., Krč, J., Genova, R., and Astaldi, jun., A. A., *Lancet*, i, 423 (1969).
[3] McElwain, T. J., and Hayward, S. K., *Lancet*, i, 527 (1969).
[4] Jerne, N. K., and Nordin, A. A., *Science*, **140**, 405 (1963).
[5] Broome, J. D., *Brit. J. Cancer*, **22**, 595 (1968).
[6] Berenbaum, M. C., *Brit. J. Cancer*, **23**, 426 (1969).
[7] Broome, J. D., *J. Nat. Cancer Inst.*, **35**, 967 (1965).
[8] Bruce, W. R., Meeker, B. E., Powers, W. E., and Valeriote, F. A., *J. Nat. Cancer Inst.*, **42**, 1015 (1969).
[9] Rall, D. P., and Homan, E. R., *Cancer Chemother. Rep.*, **51**, 247 (1967).
[10] Valeriote, F. A., Bruce, W. R., and Meeker, B. E., *Biophys. J.*, **6**, 145 (1966).
[11] Madoc-Jones, M., and Bruce, W. R., *Nature*, **215**, 302 (1967).
[12] Valeriote, F. A., and Bruce, W. R., *J. Nat. Cancer Inst.*, **38**, 393 (1967).

Maths Teaching

The liveliest job a graduate can tackle

That means an exciting future for a maths graduate like you. Developing the latest teaching techniques. Showing bright young minds how to solve the problems of an increasingly computer-minded, technological world. Enjoying the satisfaction of a career you can really make progress in; one that can fulfil your hopes and ambitions perhaps better than any other.

Certainly the need for mathematicians, scientists, engineers and technicians will be tremendous. People like you to teach them will always be in demand. And mathematics teaching need never be a frustrating, dull or dead-end job.

The scope is immense. Over 40% of maths and science graduates who started teaching seven years ago are already heads of departments. One in five graduates over 40 is a head teacher, while for others teaching leads to a further career in educational research or administration.

Jobs for mathematics teachers are available in almost every part of the country. And there are long holidays.

Pay and prospects. Starting salaries are competitive with industry, ranging for graduates from £965 to £1,170. Looking forward, as head of a large department you could earn over £2,600; a deputy headship could take you above the £3,000 mark. Promotion, like responsibility, is seldom long in coming when you're a mathematics teacher. There are also many good posts in colleges of education and as an inspector, as well as in administration. Want to find out more about teaching? Send for our free booklet 'Careers in Education for Graduates'. Available from: The Department of Education and Science (Dept. N.1, Room 107), Curzon Street, London, W1Y 8AA.

Are you already in employment? Before it's too late, measure what you're doing now against teaching. Is it as interesting as you expected? Are the prospects as good? Are you in charge of your own daily activities in the way a teacher is? You can still come into teaching—you'll be *very* welcome.

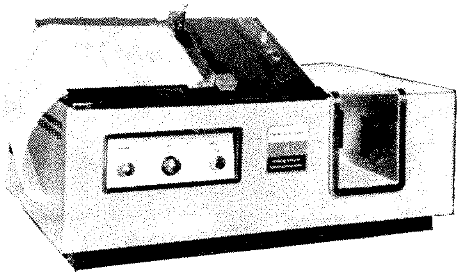
Looking forward?

TEACH MATHS

There are eleven good reason why over half of the Infrared Spectrophotometers in the world are from Perkin-Elme

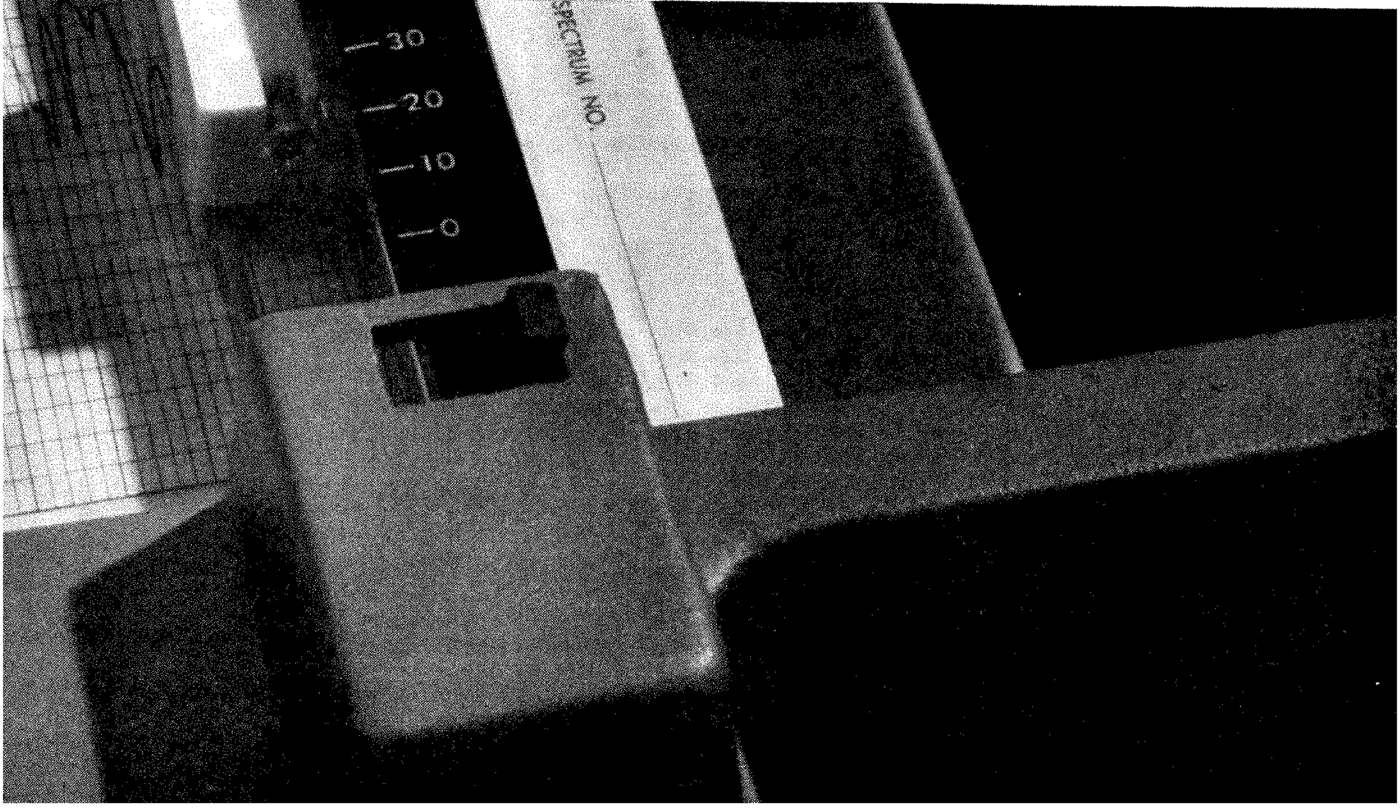
They are the Models 700, 137, 157, 257, 457, 21, 221, 421, 521, 621, 325. From this range of eleven instruments you can easily choose the one that is exactly right for you. The reliable low-cost 700 for teaching and routine work, the fast-scan 157 for high sample throughput and the 325 for its unequalled performance; these are some of the reasons for the universal use of Perkin-Elmer spectrophotometers. Together with a comprehensive range of accessories, from a new concept in liquid cells to such specialised items as 1 and 10 metre gas cells, wire-grid polarisers and the ultra micro KBr disc equipment.

Get details from
Perkin-Elmer Ltd (IRGB),
Beaconsfield, Bucks,
Tel: Beaconsfield 2571
Telex 83257.



Perkin-Elmer Instruments: Infrared, Ultraviolet, Fluorescence and Atomic Absorption Spectrophotometers, Gas and Liquid Chromatographs, Spectropolarimeters, Elemental and Thermal Analysers, Mass and NMR Spectrometers.

PERKIN-ELMER



Immunoglobulin Determinants on the Surface of Mouse Lymphoid Cells

THERE is increasing evidence that lymphocytes have antigen-specific receptors on their surface[1-3] and it seems reasonable to suppose that the receptors are immunoglobulins (Ig)[1]. The existence of Ig on the surface of lymphocytes has also been inferred from the transforming effect of anti-Ig sera[4] and the opsonic adherence to macrophages of lymphocytes treated with anti-Ig[5]. We report here the demonstration of Ig determinants on the surface of living mouse lymphocytes by the use of immunofluorescence and immunoautoradiography.

Fluorescein-labelled anti-Ig has commonly been used to demonstrate Ig within the cytoplasm of lymphoid cells[6]. In all of these studies the tissues have been frozen and/or fixed, and the fluorescence has been largely confined to the cytoplasm; the majority of cells stained have been plasma cells. Surface staining was first encountered by Möller[7] as an unavoidable background in his study of histocompatibility antigens by indirect immunofluorescence on living lymphocytes. More recently, surface fluorescence has been observed in cultured Burkitt lymphoma cells[8]. We have treated normal living mouse lymphoid cells with either fluorescein-labelled or ^{125}I-labelled rabbit anti-mouse immunoglobulin (fl-anti-M Ig or ^{125}I-anti-M Ig) and report here experiments designed to prove that the staining observed does indicate the presence of Ig on the cell surface and that only a certain proportion of the lymphoid cells become stained—a proportion which is characteristic of the tissue examined.

The anti-M Ig was prepared by immunizing rabbits with an Ig fraction from normal mouse serum for fl-anti-M Ig, and with myeloma protein 5563 (ref. 9) for ^{125}I-anti-M Ig, both in Freund's complete adjuvant. The rabbit antisera were fractionated on DEAE-cellulose and that fraction which was not adsorbed to the resin in 0·02 M phosphate buffer at *p*H 7·5 was used. It was conjugated with fluorescein isothiocyanate (FITC) by a modification of the dialysis method of Clark and Shepard[10]. The Ig fraction was adjusted to 10 mg/ml. and *p*H 9·5 in buffered saline (0·05 M phosphate–0·075 M NaCl) and was dialysed for 24 h at 4° C against 10 volumes of FITC solution at 0·125 mg/ml. in the same buffer. The conjugate was dialysed against phosphate buffered saline (PBS) at *p*H 7·3 until fluorescein was no longer detectable in the dialysate. The conjugate was absorbed with acetone dried mouse liver powder just before use. Iodination was carried out by the method of Hunter and Greenwood[11] as modified by Klinman and Taylor[12]. Details of the specificities of these labelled antisera will be published elsewhere (submitted to *Immunology*).

Cell suspensions were prepared as previously described by Raff[13] for immunofluorescence and by Klinman and Taylor[12] for autoradiography. The only significant difference between the two methods concerns the use of ammonium chloride to lyse the red cells for fluorescence studies. Immunofluorescent labelling of the cells was carried out by the method of Möller[7]. Equal volumes (0·025 ml.) of cells (20×10^6/ml.) in veronal buffered saline (VBS) with 5–10 per cent foetal calf serum (FCS) and fl-anti-M Ig (10 mg/ml.) were mixed and incubated for 15 min at room temperature. The cells were washed twice and resuspended in VBS with 5–10 per cent FCS and examined under a coverslip with ultraviolet light. 100–300 cells were counted and the percentage of cells showing surface fluorescence was determined.

For autoradiographic studies the cells were stained similarly, except that the ^{125}I-anti-M Ig was used at a much lower concentration (approximately 1 μg/ml.). The stained cells were diluted to 1×10^6/ml. and 0·1 ml. was mixed with 0·3 ml. of 0·6 per cent agarose in VBS and spread on an agarose-subbed slide. The slides were fixed for 10 min in 0·25 per cent glutaraldehyde in PBS, washed for 10 min in 0·5 per cent glycine (adjusted to *p*H 9) to inactivate the glutaraldehyde, and finally washed for 10 min in distilled water and dried in a current of warm air. The slides were then dipped in 1 : 3 dilution of Ilford *G*-5 emulsion and exposed for 1–7 days. The autoradiographs were developed in Ilford *D 19b* and stained with methyl green. 100–500 cells were scored and any cell judged to have more than background label was counted as bearing Ig. By 7 days' exposure, the difference between labelled and unlabelled cells became very obvious.

Various lymphoid tissues of normal adult CBA and Balb/c mice were examined and the results are shown in Table 1. The two methods gave similar results and the number of lymphocytes bearing Ig on their surface was approximately 40 per cent in spleen, 20 per cent in lymph node, and 15 per cent in thoracic duct and bone marrow. Titrations were performed with both methods and showed that the percentage of stained cells increased with increasing concentration of anti-M Ig up to a plateau beyond which no more cells could be labelled. The plateau was reached at 0·05 μg/ml. of ^{125}I-anti-M Ig and at 500 μg/ml. of fl-anti-M Ig. The fact that the two methods stained approximately the same percentage of cells in any one tissue, despite the very great difference in sensitivity, suggests that there are at least two distinct populations of lymphocytes, one bearing Ig on their surface and one having very much less or no surface Ig. With immunofluorescence the staining had a striking distribution, most of the labelled cells showing a clearly defined fluorescent "cap" occupying approximately one-fifth to one-third of the cell surface and usually overlying a pseudopod-like structure.

Table 1. PERCENTAGE OF IMMUNOGLOBULIN-BEARING CELLS IN VARIOUS TISSUES OF NORMAL MICE

Experiment No.	Spleen	Lymph node	Thoracic duct	Thymus	Bone marrow
A. Autoradiography					
1	41	17	14	0·31	15
2	36	—	—	—	—
3	43	16	—	0·14	—
4	44	18	—	0·21	—
Mean	41	17	14	0·22	15
B. Fluorescence					
1	38	13		0	
2	40	—		—	
3	39	13		—	
*4	36	23		0	
*5	—	16		—	
*6	33	22		0	
*7	—	15		—	
Mean	35	19		0	

* Experiments using Balb/c mice. In all other experiments CBA mice were used.

Control experiments were done to establish that the staining of cells with the anti-M Ig was the result of the presence of Ig on the cell surface. Ig fractions of normal rabbit serum were prepared and labelled with fluorescein and ^{125}I as before and did not stain normal mouse lymphoid cells. With both fluorescence and radiolabelling techniques, the staining could be strikingly inhibited by pretreating the cells with unlabelled rabbit anti-M Ig.

The morphology of the labelled cells has not been studied, but smears of cell suspensions of lymph nodes, spleen, thoracic duct and thymus stained with Wright's stain and methyl green pyronine all show that the great majority of the nucleated cells are lymphocytes[13]. Thus most labelled and unlabelled cells in these tissues must clearly have been lymphocytes. Although it might be expected that macrophages with cytophilic antibody on their surface would become labelled by these methods, they could only account for a small percentage of the labelled cells in most of the tissues examined.

The origin of the immunoglobulin on the surface of lymphocytes is uncertain. The three obvious possibilities are that it is adsorbed from the serum, transmitted from outside the cell to the cell surface by an active process, or it could be a product of the cell which bears it. The first hypothesis seems unlikely, because thymus lymphocytes failed to stain for surface Ig after incubation in normal mouse serum for 30 min, followed by washing, although this does not exclude the possibility of *in vivo* adsorption. Preliminary experiments with class-specific anti-M Ig suggest that the Ig on any one cell belongs to a single class. This result is most easily reconciled with the third hypothesis. The question of whether the surface Ig molecules function as antigen-specific receptors remains to be answered.

We thank Mr R. Lucken, Mr A. Savill and Miss J. Cressey for their technical assistance, and Dr B. A. Askonas for the anti-M Ig. This work was supported in part by the US National Multiple Sclerosis Society.

M. C. Raff
M. Sternberg
R. B. Taylor

National Institute for Medical Research,
Mill Hill,
London NW7.

Received September 18, 1969.

[1] Mitchison, N. A., *Cold Spring Harbor Symp. Quant. Biol.*, **32**, 431 (1967).
[2] Wigzell, H., and Andersson, B., *J. Exp. Med.*, **129**, 23 (1969).
[3] Sulitzeanu, D., and Noar, D., *Int. Arch. Allergy*, **35**, 564 (1969).
[4] Sell, S., and Gell, P. G. H., *J. Exp. Med.*, **122**, 813 (1965).
[5] Greaves, M. F., Torrigiani, G., and Roitt, I. M., *Nature*, **222**, 885 (1969).
[6] Coons, A. H., Leduc, E. H., and Connolly, J. M., *J. Exp. Med.*, **102**, 49 (1955).
[7] Möller, G., *J. Exp. Med.*, **114**, 415 (1961).
[8] Osunkoya, B. O., Mottram, F. C., and Isoun, M. J., *Intern. J. Cancer*, **4**, 159 (1969).
[9] Askonas, B. A., *Biochem. J.*, **79**, 33 (1961).
[10] Clark, H. F., and Shepard, C. C., *Virology*, **20**, 642 (1963).
[11] Hunter, W. M., and Greenwood, F. C., *Nature*, **194**, 495 (1962).
[12] Klinman, N. R., and Taylor, R. B., *Clin. Exp. Immunol.*, **4**, 473 (1969).
[13] Raff, M. C., *Nature*, **224**, 378 (1969).

Gas Chromatographic Determination of N-Alkyl Nitrosamines

The carcinogenicity, at very low concentrations, of many N-nitrosamines is well established[1,2]. There is a need for improved methods of specific sensitive and quantitative determination of these compounds in samples of interest. Polarographic methods[3-6] are sensitive to rather less than one part per million but they lack specificity. Thin layer chromatography[7-9] offers a measure of discrimination between N-nitrosamines, but the methods so far developed are not entirely satisfactory. A specific and sensitive gas chromatographic method for N-alkylnitrosamines is described below.

The study has been limited to simple N-alkylnitrosamines which are volatile in steam; the separation procedure used has been that of Heath and Jarvis[3]. At the 1 p.p.m. level dimethyl and diethylnitrosamines are recovered to 95 per cent and 97 per cent, respectively, when added to corned beef. The gas chromatographic method is directly applicable to aqueous samples. Using microporous polymer beads as a column packing, water is rapidly eluted in a narrow band which does not interfere with the determination of N-alkylnitrosamines. A typical chromatogram is shown in Fig. 1. A lower limit of detection of 1 p.p.m. (corresponding to 10^{-2} μg in a 10 μl. injection on the column) was achieved using standard solutions, but the practical detection limit for sample extracts would depend upon the degree of purification achieved during the extraction stage. With suitable concentration techniques, such as repeated distillation or evaporation of a suitable solvent, amounts of nitrosamine of the order 0·01 p.p.m. should be detectable. The method is capable of direct extension to the analysis of more complex nitrosamines, but an alternative separation procedure, for example, solvent extraction, is necessary for non-volatile nitrosamines.

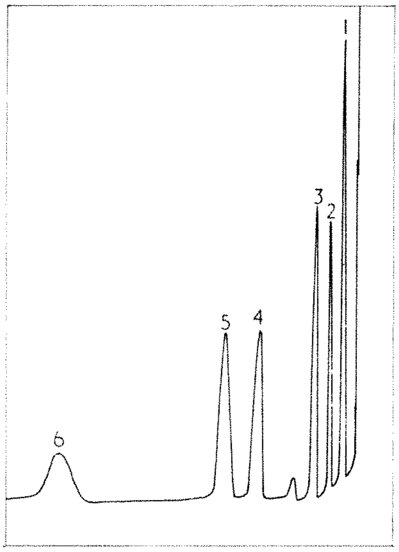


Fig. 1. Chromatogram of mixture of N-nitrosamines. 1, Dimethylnitrosamine; 2, methylethylnitrosamine; 3, diethylnitrosamine; 4, dipropylnitrosamine; 5, methylhydroxyethylnitrosamine; 6, dibutylnitrosamine. The gas chromatograph used was a Varian Aerograph 1200 with a 6 feet × ⅛ inch (outside diameter) stainless steel column packed with Chromsorb 101, at 200° C, with a flame ionization detector. The flow rate was 25 cc/min, chart speed 4 inch/h; 5 μl. samples were taken.

J. K. Foreman
J. F. Palframan
E. A. Walker

Laboratory of the Government Chemist,
Stamford Street,
London SE1.

Received July 14; revised August 14, 1969.

[1] Magee, P. N., and Barnes, J. M., *Brit. J. Cancer*, **10**, 114 (1956).
[2] Druckrey, H., Preussmann, R., and Schmahl, D., *Acta Union Intern. Contra Cancer*, **19**, 510 (1963).
[3] Heath, D. F., and Jarvis, J. A. E., *Analyst*, **80**, 613 (1955).
[4] Lydersen, D. L., and Nagy, K., *Z. Anal. Chem.*, **230**, 277 (1967).
[5] English, F. L., *Anal. Chem.*, **23**, 344 (1951).
[6] Smales, A. A., and Wilson, H. N., *J. Soc. Chem. Ind.*, **67**, 210 (1948).
[7] Preussmann, R., Neurath, G., Wulf Lorentizen, G., Daiber, D., and Hengy, H., *Z. Anal. Chem.*, **202**, 198 (1964).
[8] Serfontein, W. J., and Hurter, P., *Nature*, **209**, 1239 (1966).
[9] Neurath, G., Pirmann, B., and Dunger, M., *Chem. Ber.*, **97**, 1631 (1964).

Adaptation to Hamsters of Neurotropic Measles Virus from Subacute Sclerosing Panencephalitis

Subacute sclerosing panencephalitis (SSPE), a fatal neuropathy of children, has gained prominence recently because of its presumed aetiological association with measles virus[1]. Several successful isolations of the virus from affected brain tissues have been reported[2,3]. Preliminary characterization studies on these isolates have failed to show differences from classical measles virus strains.

This communication describes the adaptation of one of these SSPE measles isolates to brains of baby hamsters. Other strains of measles virus have been adapted to the brains of rodents in the past[4,5], but these strains were non-neural in origin. The virus used in our study was derived from the brain of a documented case of SSPE[6].

The Mantooth strain[2] of SSPE virus was obtained from Dr W. Zeman, Indiana University Medical Center. A pool of stock virus, which had been received as second passage fluids, was prepared in BSC-1 cells[7]; these cells were used throughout the study. To prepare hamster inocula, virus at a high multiplicity of infection was inoculated in bottles of cultured cells. After 24 h at 36° C, cells were scraped from bottles; cellular fluids were centrifuged for 15 min at 1,000*g*; and pellets were resuspended in a tenth of the original volume. Clumps were disrupted by pipetting and cells counted. Approximately 2×10^5 infected cells were injected intracerebrally into each of ten 2-day-old hamsters.

Except for one hamster found dead on the eleventh day of the infection and a second, stunted by the twelfth day, animals were unaffected clinically. The brain of the stunted hamster was removed and processed, and the suspension was inoculated both into a litter of hamsters and into cell cultures. The hamsters did not respond clinically except for one possibly stunted animal, but cytopathic changes characteristic of measles infection were seen in cultures by the sixth day.

The cells were scraped from bottles exhibiting these changes and suspensions of the infected cells were used for inoculation of a litter of hamsters. Each animal was injected intracerebrally with approximately 2×10^4 cells. Because no clinical effects were noted by the seventh day, the brains from two hamsters were removed and prepared for further passage in cell cultures. Syncytia were seen in 72 h and infected cultures haemadsorbed when simian erythrocytes were added. The infected cells were collected and passaged back into hamsters. Thus an alternating hamster-cell culture series was initiated. A total of four pairs of such "to and fro" passages were made with apparent enhancement of virulence occurring in each type of host. Four direct hamster brain to brain passages were then made and a pool of brain grown virus (HBS) was prepared for further study (representing the tenth passage in our laboratory).

Two-day-old hamsters, inoculated intracerebrally with HBS virus, uniformly developed neurological signs in about 3 days. Animals first showed ataxia and weakness, then opisthotonus and death 1 to 2 days after first appearance of illness. The virus titre was found to be $10^{4 \cdot 5}$ LD_{50}/0·02 ml. in baby hamsters. The virus had no clinical effects on neonatal rats and guinea-pigs.

Weanling hamsters (18 days old), similarly injected, responded with a less acute disease but with deaths occurring as early as the fifth day and sporadically thereafter until the twenty-second day after injection. Nineteen of twenty weanlings died. Posterior limb paralysis was noted in several hamsters before death.

Preliminary histological examinations of brains from acutely infected baby animals revealed a severe viral encephalitis with extensive cerebral necrosis. In older hamsters, lesions included glial cell infiltrations, perivascular cuffing, neuronal death and reduced pan-necrosis. Inclusion bodies were not observed in the brains studied so far.

HBS virus grew well in cell cultures, displaying the characteristics of measles effects, including typical nuclear and cytoplasmic inclusion bodies and production of haemagglutinins.

Identification of HBS virus as that of measles was carried out in several ways: (1) specific immunofluorescence was obtained in HBS infected cultures reacted with fluorescein-conjugated γ-globulin (prepared from rabbit antiserum) against classical Edmonston measles virus; (2) rabbit antiserum prepared with the HBS virus neutralized Edmonston virus in cell culture and inhibited Edmonston virus haemagglutination; (3) antiserum against Edmonston virus neutralized HBS virus in cell culture; (4) reference measles antiserum (used by the National Institutes of Health for vaccine strain identification) neutralized HBS virus when tested in cell cultures and inhibited HBS virus haemagglutination.

We are also studying another rodent brain-adapted measles virus variant (HNT) in this laboratory[4,8,9]. The properties of HNT are distinct from those of HBS and include: virulence for hamsters, mice and baby rats; and abortive infection in BSC-1 cells characterized by healing of cytopathic effects, absence of extracellular virus, nondetectable haemagglutinins and failure to haemadsorb. By contrast, HBS virus produces a virulent infection with haemadsorption, complete destruction of BSC-1 cell sheets, high yields of extracellular virus and haemagglutinins. Also, as indicated, clinical signs are not observed in rats injected with HBS virus.

The availability of an SSPE-derived neurotropic measles variant that has been adapted to an experimental host should provide a model for approaching the elucidation of the role of measles virus in SSPE.

This research was supported by a grant from the US National Institute of Neurological Diseases and Stroke.

D. P. Byington
A. E. Castro
T. Burnstein

Department of Veterinary Microbiology,
School of Veterinary Medicine,
Purdue University,
Lafayette, Indiana 47907.

Received October 28, 1969.

[1] Freeman, J. M., *Neurology*, **18**, 176 (1968).
[2] Horta-Barbosa, L., Fuccillo, D. A., Sever, J. L., and Zeman, W., *Nature*, **221**, 974 (1969).
[3] Payne, F. E., Baublis, J. V., and Itabashi, H. H., *New Engl. J. Med.*, **281**, 585 (1969).
[4] Burnstein, T., Jensen, J. H., and Waksman, B. H., *J. Inf. Dis.*, **114**, 265 (1964).
[5] Imagawa, D. T., and Adams, J. W., *Proc. Soc. Exp. Biol. and Med.*, **98**, 567 (1958).
[6] Chen, T. T., Watanabe, I., Zeman, W., and Mealey, J., *Science*, **163**, 1193 (1969).
[7] Hopps, H. E., Bernheim, B. C., Nisalak, A., Tjio, J. H., and Smadel, J. E., *J. Immunol.*, **91**, 416 (1963).
[8] Burnstein, T., and Byington, D. P., *Neurology*, **18**, 162 (1968).
[9] Castro, A. E., and Burnstein, T., *Bact. Proc.*, 186 (1969).

Similar Polymorphism of Taste Sensitivity to PTC in Mice and Men

INDIVIDUAL differences in taste sensitivity to phenyl thiocarbamate in human populations, first demonstrated in 1931, were soon shown to be due to segregation at a single autosomal locus[1]. Although this phenomenon was initially very interesting, its dependence on a synthetic compound relegated it to a laboratory demonstration of single locus determination of perceptual differences. More recent work concerning the antithyroid activity of PTC and similar compounds in several naturally occurring foodstuffs, however, has prompted renewed interest in this phenomenon[2].

There is some evidence of individual differences in taste sensitivity to PTC in subhuman primates and rodents. Fisher, Ford and Huxley[3] found that the proportion of tasters to non-tasters among the anthropoid apes was similar to that previously found in human populations[1], and they postulated a stable polymorphism which antedated the separation of the anthropoid and hominid stocks. Richter and Clisby[4] later determined individual taste thresholds for PTC in both rat and human populations and found similar distributions. Differences in taste sensitivity to PTC among inbred strains of mice have also been reported[5].

Although these results suggest that individual differences in taste sensitivity to PTC in subhuman species are also hereditary, their mode of inheritance has not been elucidated. We now have evidence that PTC taste sensitivity is determined by a single locus in mice, similar to that in man[6]. Such evidence raises interesting questions about the maintenance of similar polymorphisms in widely divergent species.

In an initial experiment to assess strain differences of mice, six males and six females from each of five inbred strains (A/Ibg, BALB/cIbg, C3H/Ibg, C57Bl/Ibg and DBA/2Ibg) were presented tap water and PTC solutions in a two-choice situation. Thirteen concentrations, ranging from 0·1 to 409·6 mg PTC/l. of tap water, were presented to each subject in ascending order. The concentration of PTC was doubled every second day and cylinder position was reversed daily. A daily preference ratio was calculated for each subject by dividing the amount of PTC solution consumed by the total liquid ingested. Thus a preference ratio of 0·5 indicates no preference, whereas a value less than 0·5 indicates avoidance. An inverse relationship of PTC taste sensitivity and preference ratio between 0·0 and 0·5 was inferred. To control for possible position preferences, a mean preference ratio for each 2 day period was computed. These data were transformed (Arcsin $\sqrt{\%}$) before statistical analysis.

The threshold concentration for a subject was defined as that concentration at which the subject's preference ratio was below 0·20 and remained below that value for all higher concentrations. The resulting threshold concentration distribution for each of the strains is presented in Fig. 1. It is interesting that the mode of the threshold distribution for BALB mice occurs at a concentration similar to that reported for human non-tasters[6]. Although qualitatively different, the mode of the threshold distributions of the other four strains occurs at a lower concentration than that for human tasters.

The mean preference ratios (untransformed) for each strain as a function of concentration are presented in Table 1. Transformed preference ratio data were subjected to analysis of variance, repeated measures design. Highly significant effects due to concentration ($F=157·5$, $P<0·005$, df$=12/480$) and strain by concentration interaction ($F=2·495$, $P<0·005$, df$=48/480$) were observed. These results suggest that BALB mice taste PTC at lower concentrations than the other strains. Although olfaction may have a role in the avoidance of PTC solutions, additional research has indicated that odour alone could not account for the observed differences.

Before the initiation of a genetic analysis of the strain difference in PTC taste sensitivity, we tried empirically to identify a concentration which yields maximum discrimination between "taster" and "non-taster" strains. For this purpose, the C57Bl strain was selected as representative of non-tasting strains. Three groups, each consisting of ten members of this strain and ten members of the BALB strain, were presented tap water and a single concentration of PTC solution in the two-choice situation for 12 days. On the basis of within-strain reliability, the subject's mean preference ratio on days 9 and 10 was used as the measure of taste sensitivity. Little or no difference was found between the strains at concentration five (1·6 mg/l.). At concentration six (3·2 mg/l.), most BALB mice avoided the PTC solution. In contrast, C57Bl mice began avoiding at concentration seven (6·4 mg/l.). Two other groups of ten BALB and ten C57Bl mice were tested at concentrations 6·25 and 6·5 (3·8 and 4·5 mg/l. respectively). Because the largest strain difference was obtained at concentration 6·5, this part of the experiment was repeated. On the basis of these results, the mean preference ratio on days 9 and 10 at concentration 6·5 was chosen as the phenotype for genetic analysis.

Corresponding data were obtained from additional members of the two parental strains and their F_1, backcross, F_2 and F_3 generations. There were several unusually high scores, which indicated almost perfect discrimination between PTC and tap water, but which resulted in preference rather than avoidance. To maintain an interpretation of these data consistent with that applied to the results of the initial investigation, the rejection rule of Anscombe and Tukey[7] was applied to these data. (The selection of a value of P (the percentage chance one is willing to take of rejecting an observation which is valid) of 2·5 per cent led to the rejection of one BALB, two B_1, one B_2, two F_2 and one F_3 subjects whose untransformed preference ratios were 0·951, 0·957, 0·980, 0·981, 0·973, 0·976 and 0·904 respectively.) The distribution of preference ratios of the remaining subjects in each generation is shown in Fig. 2. The bimodality observed in the B_2, F_2 and F_3 generations suggests single-locus determination with dominance for avoidance. The method of Collins and Fuller[8] was applied to these data to test the suitability of the single locus model. In general, the expected frequency within each preference ratio class interval of the segrating generations was calculated from the corresponding frequencies in the isogenic groups. These expected frequencies are also indicated in Fig. 2. None of the χ^2 values differed significantly from that predicted by the single locus model.

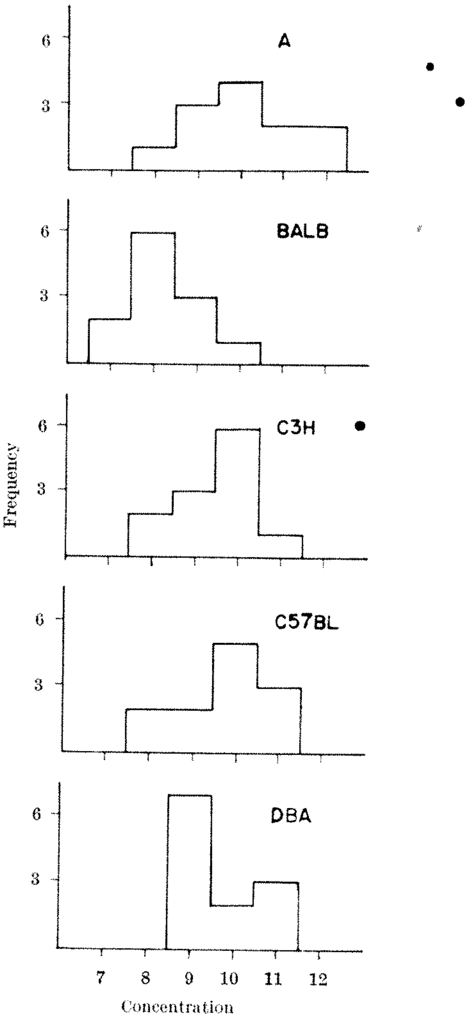


Fig. 1. PTC taste threshold concentration distributions.

To analyse the data of this experiment as a threshold character, a preference ratio of less than 0·20 was used as a criterion for tasting. The method of Collins and Fuller

Table 1. MEAN PTC PREFERENCE RATIO AS A FUNCTION OF CONCENTRATION

Strain	Concentration No. (mg/l.) 1 (0·1)	2 (0·2)	3 (0·4)	4 (0·8)	5 (1·6)	6 (3·2)	7 (6·4)	8 (12·8)	9 (25·6)	10 (51·2)	11 (102·4)	12 (204·8)	13 (409·6)
A	0·43	0·48	0·50	0·44	0·44	0·40	0·45	0·44	0·25	0·14	0·13	0·05	0·05
BALB	0·50	0·52	0·58	0·47	0·61	0·46	0·33	0·19	0·13	0·04	0·05	0·05	0·05
C3H	0·48	0·58	0·50	0·39	0·45	0·49	0·50	0·43	0·27	0·09	0·04	0·05	0·04
C57Bl	0·41	0·50	0·48	0·55	0·53	0·55	0·46	0·39	0·28	0·14	0·10	0·06	0·06
DBA	0·53	0·44	0·48	0·51	0·53	0·50	0·47	0·41	0·26	0·16	0·06	0·06	0·06

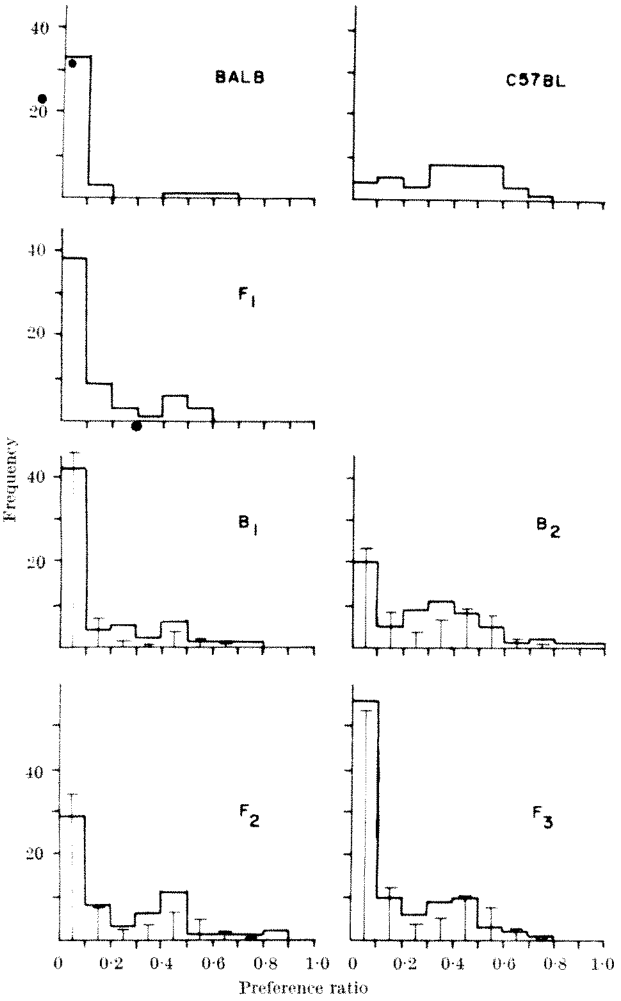


Fig. 2. Distribution of mean PTC preference ratios on days 9 and 10 at concentration 6·5 for each of two inbred strains and their derived generations. For each segregating generation the expected distribution based on a single locus model is indicated by vertical lines. (N = 39 BALB, 40 C57Bl, 60 F_1, 62 B_1, 63 B_2, 62 F_2 and 97 F_3 subjects.)

was also applied to these data and no significant χ^2 value was found. These results support the hypothesis of determination by a single locus.

Wright's methods[9] for estimating the number of independently segregating Mendelian units were applied to the preference ratio data. Although different assumptions are required for each of the estimates and although data from different generations are used, the resulting estimates are in very close agreement (1·04, 1·05 and 1·07). Finally, the method suggested by Scott and Fuller[10] for estimating the number of loci involved in a threshold character was applied to the preference ratio data. This method, which uses only first degree statistics, is especially suitable for characters in which the distributions of the isogenic parental strains are not clearly dichotomous. Resulting values were highly consistent with single locus determination.

Although the methodology used precludes its proof, it is very likely that individual differences in taste sensitivity to PTC in mice are due largely to a single autosomal locus. The existence of this polymorphism, similar to that in man, raises interesting questions about the maintenance of polymorphisms across a wide phyletic distribution. Although Fischer[2] has discussed the possible fitness value of taster and non-taster phenotypes and has proposed that this polymorphism may be maintained by heterozygote advantage, the adaptive significance of individual differences in taste sensitivity to PTC and related compounds remains unknown.

This work was supported in part by a grant from the National Institute of General Medical Sciences, National Institutes of Health, US Public Health Service.

T. W. KLEIN
J. C. DEFRIES

Institute for Behavioral Genetics,
University of Colorado,
Boulder, Colorado 80302.

Received November 7, 1969.

[1] Fox, A. L., *Proc. US Nat. Acad. Sci.*, **18**, 115 (1932); Blakeslee, A. F., and Salmon, M. R., *Eugen. News*, **16**, 105 (1931); Snyder, L. H., *Science*, NS, **74**, 151 (1932); Blakeslee, A. F., and Salmon, M. R., *Proc. US Nat. Acad. Sci.*, **21**, 84 (1935).

[2] Fischer, R., in *The Chemical Senses and Nutrition* (edit. by Kare, M. R., and Maller, O.) (Johns Hopkins, Baltimore, 1967).

[3] Fisher, R. A., Ford, E. B., and Huxley, J., *Nature*, **144**, 750 (1939).

[4] Richter, C. P., and Clisby, K. H., *Amer. J. Physiol.*, **134**, 157 (1941).

[5] Hoshishima, K., Yokoyama, S., and Seto, K., *Amer. J. Physiol.*, **202**, 1200 (1962).

[6] Harris, H., and Kalmus, H., *Ann. Eugen.*, **15**, 24 (1949); Kaplan, A. R., Fischer, R., Karras, A., Griffin, F., Powell, W., Marsters, R. W., and Glanville, E. V., *A. Ge. Me. Ge.*, **16**, 229 (1967).

[7] Snedecor, G. W., and Cochran, W. G., *Statistical Methods*, sixth ed., 322 (Iowa State University Press, Ames, 1967).

[8] Collins, R. L., and Fuller, J. L., *Science*, **162**, 1137 (1968).

[9] Wright, S., *Evolution and the Genetics of Population*, **1** (University of Chicago Press, Chicago and London, 1968).

[10] Scott, J. P., and Fuller, J. L., *Genetics and the Social Behavior of the Dog* (University of Chicago Press, Chicago and London, 1965).

Bark Beetle Pheromones

KINZER *et al.*[1] have recently reported the identification and synthesis of the bicyclic compound, frontalin. We wish to question the validity of some of their conclusions in the light of earlier work by this group at the Boyce Thompson Institute and Battelle Memorial Institute.

In connexion with the volatiles from oleoresin in the population aggregating pheromone of *Dendroctonus frontalis* they state: "The attack generates host odours as the beetles penetrate the phloem tissue and stimulate resin flow" and "the response to the synthetic attractant [frontalin] was increased by adding oleoresin freshly tapped from *Pinus taeda*". Yet in another article[2] they state: "The results demonstrate the existence of a mechanism which enables certain bark beetle species [*D. frontalis*] to release a population aggregating pheromone immediately upon arrival at a new host, prior to actual infestation". In another report[3], they state for the same insect: "At peak attractiveness of a tree, relatively few of the attacking beetles had begun gallery construction. The majority had barely reached the phloem tissue". Thus whereas two papers reported that the attractants are produced before the xylem oleoresin is released, in ref. 1 Kinzer *et al.* state that xylem oleoresin, which the beetle must release through its boring activity, is a synergist to the identified compound, frontalin. If frontalin is attractive only in mixture with oleoresin, then their observation that the population aggregating pheromone is released "immediately upon arrival at a new host, prior to actual infestation" must be in error. If this observation is not in error, however, then the role of frontalin in the aggregation pheromone is not as clear as they state.

A fraction attractive to pedestrian *D. frontalis* beetles in the laboratory bioassay has been isolated by Renwick and Vité[4], but this fraction could not be identified because "an estimated million beetles seemed barely enough to yield a single milligram of this substance"[5]. Subsequently, Kinzer *et al.*[1] isolated and identified a compound from *D. brevicomis* males which had the same ring structure (bicyclic ketal) as did brevicomin, previously isolated from *D. brevicomis* females by Silverstein *et al.*[6]. The structure of brevicomin was announced in November 1967 (ref. 7). The unknown compound in *D. frontalis* females was then found to have the same gas chromatographic properties as the bicyclic ketal identified from *D. brevicomis* males which was named frontalin (contrary) to the usual practice of assigning trivial names descriptive of the source from which they were identified). The compound was then synthesized, but "walking beetles [*D. frontalis*] failed to show any reaction to the new material"[5], in contrast to earlier bioassay results with that fraction isolated from female *D. frontalis* hindguts[4]. When frontalin was mixed with oleoresin, however, it was found to be active in the field[1]. We question the rationale of a procedure by which a compound is identified from the males of one species, searched for and found to be present in the females of another and then designated as the "principal component of the aggregating pheromone"[1]. Any number of similar or different compounds might be more significant as attractants for *D. frontalis*.

In their field tests, Kinzer *et al.* compare the relative attractiveness of frontalin to crushed males and females[1]. Crushed insects can hardly be considered a natural phenomenon, particularly in view of previously published work in which they show that female-infested billets and trees under attack are very attractive[3]. To our knowledge, the relative attractiveness of crushed beetles combined with oleoresin to infested billets and/or trees under attack has not been established. We would conclude that such a tree or billet is the more logical and meaningful control than crushed males and females. Compounds present in the emerged insect, in, for example, their hindguts, plus xylem oleoresin[1] do not necessarily add up to the pheromones of these insects. Many other sources of compounds are possible; for example, compounds produced through feeding, compounds in the bark and phloem, and compounds in the xylem not associated with oleoresin.

Their attempt to associate *trans*-verbenol with the pheromone of *D. frontalis* is doubtful[1]. In two series of three 10 min field tests, the following materials were exposed: frontalin, *trans*-verbenol, and oleoresin; frontalin and oleoresin; *trans*-verbenol and oleoresin; frontalin; oleoresin; and crushed male and female beetles and oleoresin. Apart from the brief exposure times and their dubious use of crushed beetles as a control (see earlier remarks), no test results were shown for a mixture of frontalin and *trans*-verbenol, nor was the mixture of frontalin, *trans*-verbenol and oleoresin compared with the mixture of frontalin and oleoresin and the mixture of *trans*-verbenol and oleoresin in either series. Thus no assessment of the function of *trans*-verbenol can be made from their data.

In reporting the identification of frontalin, they state that "two analogous compounds have been previously identified from natural sources"[1]. These two "analogous compounds", however, should not be considered in the same light, biologically speaking, because one was isolated from hop oil and the other from *D. brevicomis* females, certainly a close relative of *D. brevicomis* males. Silverstein *et al.*[6] actually identified two bicyclic ketals from the frass of *D. brevicomis* females (exo- and endo-brevicomin).

D. L. WOOD

Department of Entomology and Parasitology,
University of California,
Berkeley, California.

R. M. SILVERSTEIN*

Stanford Research Institute,
Menlo Park, California.

Received June 11; revised July 30, 1969.

---

* Present address: Department of Chemistry, State University College of Forestry at Syracuse, Syracuse, New York.

[1] Kinzer, G. W., Fentiman, A. F., Page, T. F., Foltz, R. L., Vité, J. P., and Pitman, G. B., *Nature*, **221**, 477 (1969).

[2] Vité, J. P., and Renwick, J. A. A., *Contrib. Boyce Thompson Inst.*, **24**, 61 (1968).

[3] Vité, J. P., and Crozier, R. G., *Contrib. Boyce Thompson Inst.*, **24**, 87 (1968).

[4] Renwick, J. A. A., and Vité, J. P., *Contrib. Boyce Thompson Inst.*, **24**, 65 (1968).

[5] Vité, J. P., *Texas Industry*, **36**, 14 (1968).

[6] Silverstein, R. M., Brownlee, R. G., Bellas, T. E., Wood, D. L., and Browne, L. E., *Science*, **159**, 889 (1968).

[7] *Chem. Eng. News*, November 6, 21 (1967).

Actin-like Properties of Colchicine Binding Protein isolated from Brain

THE isolation from mammalian brain of a protein with properties similar to those of muscle actomyosin was recently reported[1] and it was suggested that the protein was composed of actin-like and myosin-like subunits. There have also been references[2] to the possibility that colchicine binding protein isolated from the microtubules of sea urchin sperm tails[3], mitotic spindles[4,5] and the cilia of *Tetrahymena*[6] may be composed of an actin-like protein. The ability to bind colchicine also seems to be a characteristic of the microtubules of the central nervous system[7] and a colchicine binding protein has been isolated from mammalian brain[8]. It was therefore of interest to isolate colchicine binding protein from brain and test its actin-like properties.

Colchicine binding protein was isolated from porcine brain by the batch procedure[8]. Ammonium sulphate was removed from the final preparation by passage through 'Sephadex *G*-25'. Myosin was prepared from fresh, long back muscles of the cat by a modification of the method of Asai[9,10]. Brains were delivered in ice within a few hours after removal from the animals. The leptomeninges and the associated blood vessels as well as the choroid plexus were removed before extraction. Isolation of the protein was followed by ^{3}H-colchicine binding studies performed essentially as described by Weisenberg *et al.*[8]. Before the viscosity and adenosine triphosphatase (ATPase) experiments the protein was passed through 1·5 × 10 cm columns of 'Sephadex *G*-25' to remove the GTP used in the isolation procedure. ATPase activity was assayed by the Marsh procedure[11] adapted for the determination of as little as 0·1 μg of P_i released from ATP (for details see Table 1).

Table 1. EFFECT OF COLCHICINE BINDING PROTEIN ON THE ATPase ACTIVITY OF MUSCLE MYOSIN

	μmoles Pi/mg protein/min		
Cation	Myosin	Colchicine binding protein	Myosin and colchicine binding protein
Mg^{2+}	0·024	0	0·65
Ca^{2+}	0·37	0	0·48

The myosin was isolated from cat striated muscle and the colchicine binding protein from porcine brain. Phosphate release was calculated on the basis of the amount of myosin in the assay medium. The incubation medium (1 ml.) contained 0·03 M imidazole-HCl (*p*H 6·8), 0·06 M KCl, 5×10^{-4} M ATP, 10^{-3} M Mg^{2+} or Ca^{2+}, 10^{-4} M ouabain and 0·01 mg of myosin and 0·1 mg of colchicine binding protein. Incubation was for 30 min at 37° and was terminated by the addition of 0·4 ml. of 20 per cent trichloroacetic acid. The blanks were protein solutions inactivated by boiling for 3 min or by trichloroacetic acid treatment before the addition of ATP.

Protein was determined by the procedure of Lowry *et al.*[12], with bovine serum albumin as standard.

Colchicine binding protein increased the Mg^{2+}-ATPase activity of muscle myosin approximately twenty-seven-fold (Table 1); the Ca^{2+}-activated ATPase activity of the myosin was enhanced only slightly. The Mg^{2+}-stimulated activity of the myosin increased from levels much below that for the Ca^{2+} activation to values above it. Addition of colchicine binding protein to myosin resulted in an increase in the relative viscosity of the myosin and a marked increase in sensitivity to ATP (Fig. 1). The addition of ATP to the mixture of myosin and colchicine binding protein resulted in a decrease in relative viscosity which increased again over a period of 30–60 min. GTP did not have this effect.

Disc gel electrophoresis (Canalco apparatus) performed in 7·5 per cent acrylamide gel containing 8 M urea showed a single major band similar to that described previously[8]. A sedimentation study of colchicine binding protein in the model E Beckman analytical ultracentrifuge revealed a single peak ($s_{20,w}$ 8·7) with negligible contamination. An analysis for 80 min after reaching speed confirmed its homogeneity. Similarities between colchicine binding protein and actin have been described[2] and recently reviewed[6]. These include the characteristics of extraction at low ionic strength, molecular weight, amino-acid composition, electrophoretic mobility and nucleotide binding properties.

Our study demonstrates that colchicine binding protein from brain reacts with muscle myosin and that the interaction occurs not only between proteins from different organs but also from different species. Its effects on the ATPase activity and viscosity of the muscle myosin indicate that it has actin-like properties. It may well be, therefore, that microtubules in brain function in the saltatory transport of material and vesicles from their site of formation in the cell body to their site of utilization at the synaptic endings (compare ref. 2).

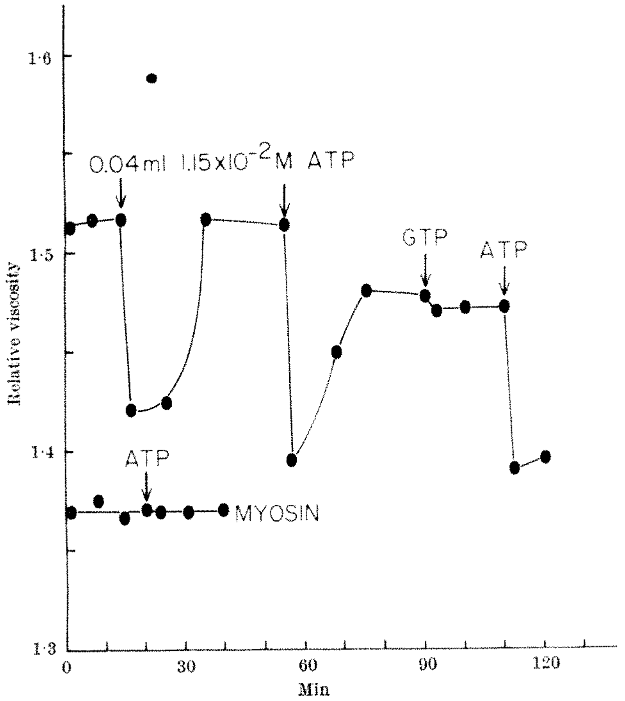


Fig. 1. Effect of ATP on the relative viscosity of myosin alone (lower curve) and in combination with colchicine binding protein (upper curve). Measurements were performed at 21° in a Cannon–Ubbelohde dilution viscometer size 50 on a minimum volume of 6 ml. The viscosity of protein solutions relative to the buffer (η_{rel}) was determined in 0·05 M Tris-HCl (*p*H 7·2)—0·6 M KCl. The response to ATP (η_{rel} ATP) was measured by the addition of 0·04 ml. of $1 \cdot 15 \times 10^{-2}$ M ATP in the same buffer adjusted to *p*H 7·2. The arrows indicate times of addition of 0·04 ml. $1 \cdot 15 \times 10^{-2}$ M ATP or 0·05 ml. 5×10^{-3} M GTP. Myosin (2 mg/ml.) was isolated from cat striated muscle and colchicine-binding protein (0·70 mg/ml.) from porcine whole brain. The relative viscosity of the colchicine binding protein alone was approximately 1.

This work was supported in part by a US Public Health Service grant and a career development award to S. B., and grants from the Cerebral Palsy Education and Research Foundation, the Clinical Research Center for Parkinson's and Allied Diseases and the Parkinson Information Center. We thank Dr M. Shelanski and Dr H. Rosenkrantz for the ultracentrifugal analysis and Dr D. D. Clarke and Dr Elena Puszkin for discussions.

S. PUSZKIN
S. BERL

Columbia University,
College of Physicians and Surgeons,
New York,
New York 10032.

Received September 18, 1969.

[1] Puszkin, S., Berl, S., Puszkin, E., and Clarke, D. D., *Science*, **161**, 170 (1968).
[2] Schmitt, F. O., and Samson, jun., F. E., *Neurosci. Res. Program Bull.*, **6**, 117 (1968).
[3] Shelanski, M. L., and Taylor, E. W., *J. Cell Biol.*, **34**, 549 (1967).
[4] Sakai, H., *Biochim. Biophys. Acta*, **112**, 132 (1966).
[5] Kiefer, B., Sakai, H., Solari, A. J., and Mazia, D., *J. Mol. Biol.*, **20**, 75 (1966).
[6] Renaud, F. L., Rowe, A. J., and Gibbons, I. R., *J. Cell Biol.*, **36**, 79 (1968).
[7] Borisy, G. G., and Taylor, E. W., *J. Cell Biol.*, **34**, 525 (1967).
[8] Weisenberg, R. C., Borisy, G. G., and Taylor, E. W., *Biochemistry*, **7**, 4466 (1968).
[9] Asai, H., *Biochemistry*, **2**, 458 (1963).
[10] Richards, E. G., Chung, C.-S., Menzel, D. B., and Olcott, H. S., *Biochemistry*, **6**, 528 (1967).
[11] Marsh, B. B., *Biochim. Biophys. Acta*, **32**, 357 (1959).
[12] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

Population Analysis of Somatosensory Thalamus in the Cat

THE responses to both natural and electrical stimulation of peripheral structures, and to electrical stimulation of the cerebral cortex, were determined for a representative sample of forepaw-responsive neurones in the nucleus ventralis posterolateralis (VPL) of the cat thalamus. Information from all the cells studied permits a reconstruction of the behaviour of the total population of neurones responding to a given input (that is, forepaw stimulation). The spatio-temporal distribution of unit neuronal activity occurring within the somatosensory thalamus following application of discrete stimuli to the body surface, once reconstructed, can be understood in terms of the contributions of various kinds of neuronal elements to the overall population response.

Relatively few cells (ten to twenty) could be isolated and studied in any one animal; it was necessary to synthesize the total population by pooling data from individual animals, with unit stereotaxis locus providing a basis for collating the data. Correspondence between the stereotaxic locus of units and actual regions of the nucleus in which they were located was checked by gross potential mapping of the forepaw responsive area in each animal and by histological confirmation of penetration positions in some cases. An imaginary grid was constructed, its boundaries being the minimal rectangular volume enclosing the VPL (established by planes at A8 and A11, L5 and L8, and H3 and H-2·5). Vertical microelectrode penetrations were made through this volume of tissue at millimetre intervals on the grid, and each neurone encountered (regardless of the particular animal in which it was found) was represented on the grid at its stereotaxic locus. Data were collected from each unit in the form of extracellular recordings of its discharge of impulses evoked by peripheral and central stimulation, utilizing NaCl-filled glass micropipettes of 1·5–2·0 μm tip diameter.

Attention was given in the analysis to a correlation of the response properties of neurones with their position within the volume of tissue studied. The aggregation of neurones sharing a particular natural stimulus sensitivity or possessing receptive fields of comparable extent has been noted by other investigators studying dorsal column nuclei (DCN)[4,7,10] and somatosensory cortex[6]. One would suppose that a separation of hair and touch sensitive cells begun in the DCN would be maintained as projections pass through the thalamus on the way to the cortex, where hair and touch cells also reside preferentially in different regions. Also, one might expect to see wide-field neurones at the thalamic level which correspond to those observed in pericruciate regions of cortex[12] if the convergence of input involved in elaborating these wide fields occurs at the thalamic rather than the cortical level. For these reasons, the reconstructed population of thalamic neurones was examined for evidence of aggregation of neurones according to their natural stimulus sensitivities and/or their receptive field sizes.

Cats were anaesthetized with α-chloralose (60 mg/kg) and paralysed with 'Syncurine' or 'Flaxedil'. Stimulus pulses were administered through bipolar needle electrodes placed in the central footpad of each of the four paws to evoke discharge of, isolate, and identify thalamic cells. Bipolar ball-tipped stimulating electrodes on the cortical surface in postcruciate, coronal and postdimple areas were used to study cortical influences on peripherally evoked thalamic activity and to identify cortical projection areas of thalamic cells by antidromic activation. Units isolated in response to electrical stimulation of the contralateral forepaw (CFP) were then tested for responsiveness to electrical stimulation of the other appendages. Their natural stimulus was determined, including modality, location and extent of receptive field. Finally, responses to electrical stimulation of CFP and other inputs were photographed for measurement of unit response characteristics, including latency and number of spikes per discharge. Other functional properties (threshold stimulus intensity, ability to follow repetitive stimuli at various rates, and changes in excitability arising from conditioning shocks applied to the cortex) were assessed directly.

Units were partitioned into an *s* set, responding only to stimulation of CFP, and an *m* set, responding to CFP and at least one other appendage as well. They were further classified according to their natural stimulus modality, using operational definitions of hair and touch sensitivity. Hair cells discharged in response to deflexion of a few hairs by air puffs or with a forceps, while touch cells failed to discharge to such stimuli but responded to light taps applied directly to the skin. Other types of cells (claw sensitive, nociceptive, vibration sensitive, and so on) were also found. In forty-six experiments on chloralose-anaesthetized animals, 640 neurones were isolated within the volume of tissue under consideration and studied. Of these, approximately 30 per cent were classified as *m* neurones. These neurones usually had large but discontinuous receptive fields with portions in the form of "gloves" and "stockings" on the contralateral forelimb and one or more other limbs. They were found scattered throughout the nucleus, often in the same tracks with *s* neurones (even in the CFP focus defined by gross potential mapping), although they predominate in a particular region. Various combinations of effective inputs were found for *m* neurones, such as both forelimbs, both contralateral limbs, or all four limbs. Most of them had ipsilateral inputs. The *s* neurones accounted for about 68 per cent of the total population. (An additional seventeen neurones were lost before the extent of their receptive fields could be determined and were classified as responding to CFP at least.) The *m* and *s* sets were compared with respect to their composition in terms of the natural stimulus sensitivities of their constituent elements; for both subsets the hair cells outnumber the touch cells, but there is a higher proportion of hair cells among the *s* set (50 per cent) than the *m* set (30 per cent). Hair and touch cells together account for 70 per cent of

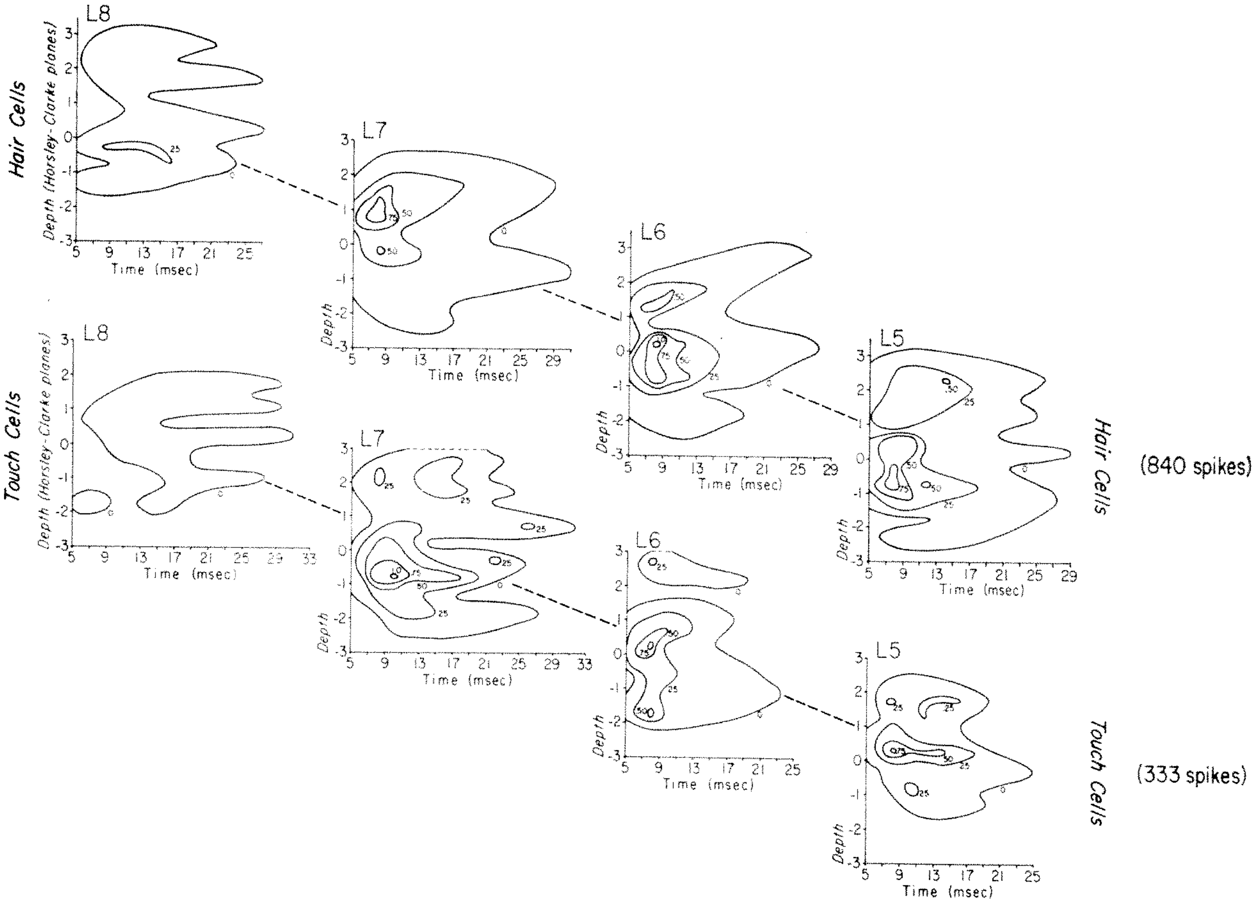


Fig. 1. Spike density plots for activity sustained by hair cell and touch cell subsets in response to stimulation of contralateral forepaw (0·1 ms, 7 V pulse). Each plot in the series of four for each subset depicts spike distribution as a function of depth and time in a particular parasagittal plane, moving from L5 (most medial) at the right to L8 (most lateral) at the left. Information about the anterior–posterior distribution of spike activity is unfortunately lost in these plots; all spikes occurring in a particular parasagittal plane were treated as if they had been found in a single "compressed penetration at the corresponding distance from the midline, no account being taken of where they occurred along the A–P axis. (This information is available, but not as useful as their distribution along the M–L axis.) Contour lines enclose regions in which the spike density is at 0·0, 0·25, 0·50 and 0·75 levels compared with that at the point of maximum density indicated as 1·0. Plots start at 5 ms after stimulus presentation. Depth coordinates range from 3 mm above horizontal zero to 2·5 mm below it. It is helpful to read each plot as if one were "looking through" the nucleus from behind along the corresponding plane; imagine observing changes in number and depth distribution of spikes as time elapses after the stimulus.

the *s* neurones, but for only 42 per cent of the *m* neurones. The latter set contains in addition a number of cells which were unresponsive to all forms of natural stimuli applied (11 per cent) and a group which responded only to noxious stimuli (14 per cent).

The population was examined for rostro-caudal, lateromedial, and/or dorso-ventral segregation of all the types of cells encountered; the absolute numbers of cells found within each region and the proportional contribution of each kind of cell to the total found in each region were both considered. The results indicate an aggregation of neurones according to stimulus sensitivities and according to receptive field size as nuclear organizational features in addition to somatotopy. Both hair and touch cells are most numerous ventrally, but hair cells cluster in the medial portion of the nucleus (A10, L5 and L6) whereas touch cells are scattered more diffusely throughout the central portion (A8–A10, L6 and L7). The *s* neurones are concentrated medially (A10, L5) and ventrally, while *m* neurones occur with greatest frequency in lateral, posterior regions (A8 and A9, L7 and L8) and are rather uniformly distributed in depth. In view of the caudal position of *m* neurones, it should be noted that the volume under study does not extend as far posteriorly as the posterior nuclear group of Poggio and Mountcastle[11], in which units possessing similar wide fields were also found. The *m* neurones I describe are within VPL, and are not predominantly pain-sensitive like those described for the posterior nuclear group by Poggio and Mountcastle. Moreover, at least a representative sample of them were found to project to areas within the S1 somatosensory receiving area of the cortex.

The conclusion that touch and hair cells, and *s* and *m* neurones, are segregated within the nucleus is given additional support by comparison of time-depth spike density plots depicting the time course and spatial distribution of evoked activity typical of each subset. These spike density maps should be read like geographical contour maps; the spike discharge density contour lines are the equivalent of altitude or elevation indices, while the stereotaxic plane and the depth axis indicate where the observer is and the time axis indicates when—relative to the time of stimulus application—the discharge is being considered. For example, one might ask: In the L5 plane, at 0·0 depth corresponding to the midpoint of the nucleus, how intense is the discharge at 8·0 ms after stimulation?—and answer this by reference to the density plots. These plots show that the locus of maximum spike density, as well as the overall time course and distribution of discharge, differs for hair and touch cells and for *s* and *m* neurones. The region displaying peak density of activity in touch cells is located 1 mm lateral to that for hair cells (Fig. 1), and peak activity occurs later (by about 2 ms) for touch cells than for hair cells. Most activity in the extreme lateral portion of the volume, occurring still later, is supported by *m* neurones.

Subsets were also compared with respect to several other functional characteristics defined on the basis of their response to electrical stimulation of CFP and with respect to the effects produced by cortical stimulation. Mean first-spike response latency is shorter for hair cells (8·7 ms) than for touch cells (9·2 ms). Hair cells also have a lower threshold (1·03 units) and higher frequency following capability (31 per s) than do touch cells (1·26 units; 21 per s). The *m* set is characterized by longer first-spike latency (12·1 ms), lower mean threshold (1·04 units) and poorer frequency following (10 per s) than the *s* set (8·7 ms; 1·21 units; and 29 per s). Descending cortical influences were found to be exerted on all types of units encountered. The coronal area of cortex (that is, the S1 forepaw focus) was the best site for the evocation of synaptically mediated effects on the thalamic elements studied. Reports by others led to the expectation that thalamic touch cells would project to the coronal cortex while hair cells would project to pericruciate areas[6,8,12]; it was surprising to find both sets trans-synaptically influenced from the coronal cortex in similar numbers. When the spatial distribution of cortically influenced neurones was taken into consideration, however, cortically inhibited cells corresponded in distribution with hair cells and cortically excited cells with touch cells. Thus cortical influences seem to be distributed in accordance with organizational lines established through modality segregation.

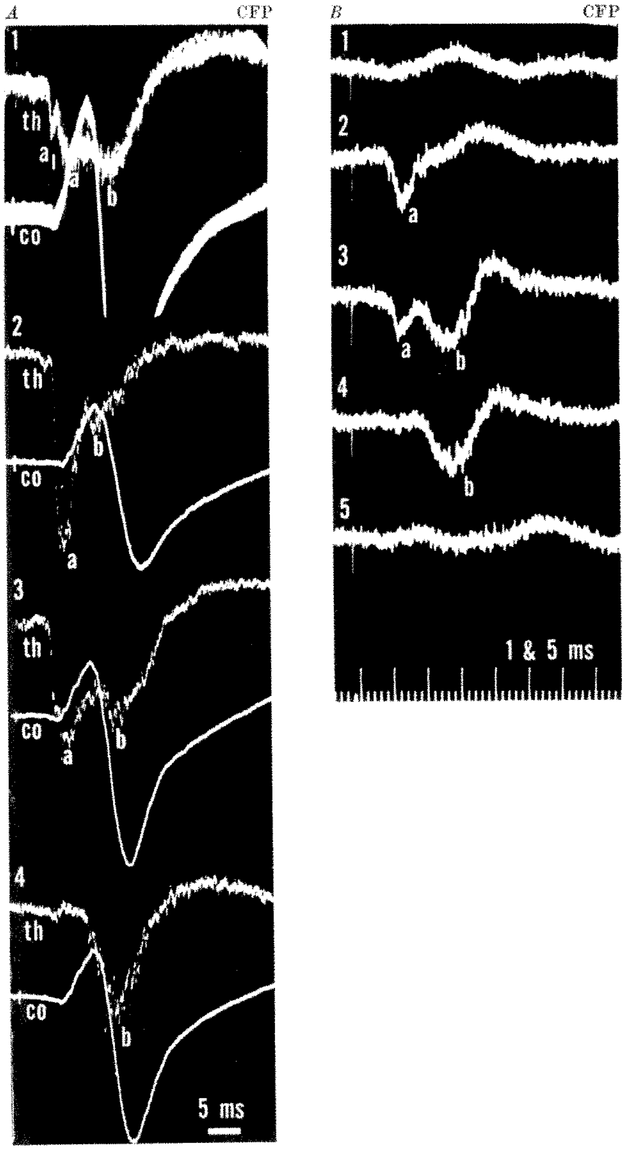


Fig. 2. Gross potential responses to stimulation of contralateral forepaw (0·1 ms, 7 V pulse), recorded through microelectrode with enlarged tip (indifferent lead clipped to scalp). In *A*, the upper trace of each pair is from the thalamus; the lower trace is from the coronal area of the cerebral cortex (S1 forepaw representation at the cortex). The thalamic recordings were taken during penetration at A8, L7. Note three components (a_1, *a* and *b*) of thalamic response which develop differently in depth. The a_1 component is probably pre-synaptic (lemniscal input); the early *a* component is probably identical with Mountcastle and Henneman's "localizing potential"[9], while the later *b* component may represent corticofugal activity or late activity from the periphery. In *B*, taken during penetration at A9, L7, the differential development in depth of *a* and *b* components is again illustrated; here, the *b* component is a positive–negative complex. In *A* and *B*, successive records were taken at 0·5 mm intervals in depth ranging from 1 mm above horizontal zero to 0·5 or 1·0 mm below it. Positivity is downward for thalamic records, upward for cortical records.

Gross potential records from regions within the volume of tissue studied reveal a compound response—an early positive wave, followed by a later positive or positive–negative wave—in response to CFP stimulation (Fig. 2). The position of the maximum amplitude early response establishes the CFP "focus" medially within the volume (in the L5 or L6 plane); the late wave is often more pronounced in lateral areas (L7 or L8). Because many of the units encountered could be influenced from the cortex, one interpretation of the late wave is that it reflects activity in elements which are driven (or inhibited) via a loop through the cortex, after the effective cortical sites are first activated from the periphery. Alternatively, the late wave may reflect a component of unit activity driven through a slowly conducting route from the periphery; the *m* neurones would probably support this activity, and the spinothalamic pathway would be the most likely route over which they would be driven (the "neospinothalamic" component of the spinothalamic system has been shown by anatomists to reach the region corresponding to the maximum concentration of *m* neurones within VPL[1-3,5]). Experiments involving tract sectioning and cortical ablations will be necessary to differentiate between these possibilities.

This work was supported by grants from the US National Institute for General Medical Sciences, the US National Institute for Neurological Diseases and Blindness, the Social Rehabilitation Service and Initiative 171, State of Washington.

FREDRIC A. HARRIS

Department of Physical Medicine and Rehabilitation,
University of Washington School of Medicine,
Seattle, Washington 98105.

Received June 4; revised August 15, 1969.

[1] Anderson, F. D., and Berry, C. M., *J. Comp. Neurol.*, **111**, 195 (1959).
[2] Bowsher, D., *J. Comp. Neurol.*, **117**, 213 (1961).
[3] Getz, B., *Acta Anat.*, **16**, 271 (1952).
[4] Gordon, G., and Jukes, M. G. M., *J. Physiol.*, **173**, 263 (1964).
[5] Mehler, W. R., Feferman, M. E., and Nauta, W. J. H., *Brain*, **83**, 718 (1960).
[6] Morse, R. W., Adkins, R. J., and Towe, A. L., *Exp. Neurol.*, **11**, 419 (1965).
[7] Morse, R. W., and Towe, A. L., *J. Physiol.*, **171**, 231 (1964).
[8] Mountcastle, V. B., Berman, A. L., and Davies, P. W., *Amer. J. Physiol.*, **183**, 646 (1955).
[9] Mountcastle, V. B., and Henneman, E., *J. Neurophysiol.*, **12**, 85 (1949).
[10] Perl, E. R., Whitlock, D. G., and Gentry, J. R., *J. Neurophysiol.*, **25**, 337 (1962).
[11] Poggio, G. F., and Mountcastle, V. B., *J. Neurophysiol.*, **26**, 775 (1963).
[12] Towe, A. L., Patton, H. D., and Kennedy, T. T., *Exp. Neurol.*, **10**, 325 (1964).

Distribution of ^{14}C-Thiosemicarbazide in the Rat Brain: an Attempt to localize Sites of γ-Aminobutyric Acid Production

IT is still not known whether γ-aminobutyric acid (GABA) is a "real" inhibitory substance in the mammalian central nervous system[1,2]. Before the role of GABA in inhibitory mechanisms can be understood, it is important to locate histochemically the sites of GABA metabolism.

Intrinsic GABA cannot be detected histochemically; nor can the enzymes involved in its production and breakdown be reliably located[3]. Moreover, attempts to locate extrinsically injected GABA-^{3}H have been unsuccessful because of impeded transport or enhanced breakdown of the radioactive compound[1]. We therefore attempted to locate one of the most powerful inhibitors of GABA synthesis—the convulsive drug thiosemicarbazide. Like other hydrazides, this drug inhibits the activity of enzymes dependent on vitamin B_6 including that of glutamate decarboxylase (GAD, 4.1.1.15), which is responsible for the formation of GABA[4]. Thus the distribution of thiosemicarbazide, at the height of symptoms induced by inhibition of GAD, might help locate the site of GAD activity, or at least the absence of thiosemicarbazide

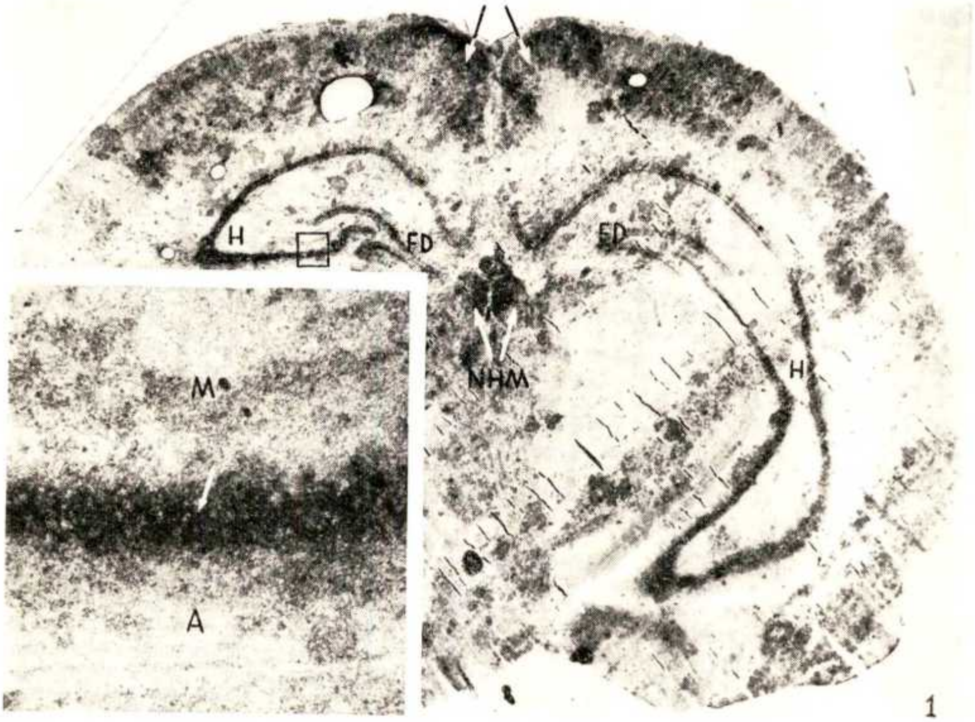
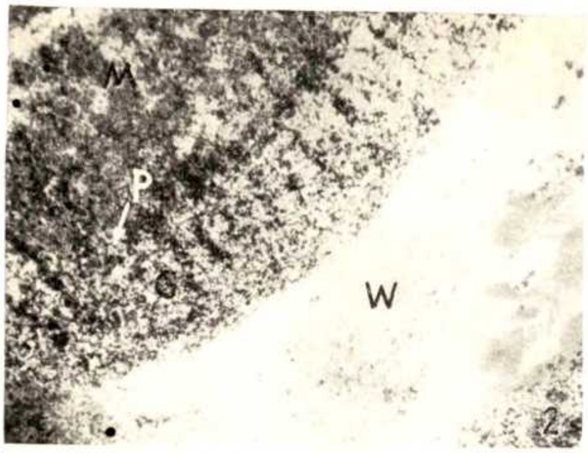


Fig. 1. Coronal section of rat brain, 90 min after two consecutive injections of TSC-^{14}C. Unstained autoradiogram of a cryostat section. H, Hippocampus; FD, fascia dentata; NHM, nucleus habenularis medialis. Arrows indicate cortical areas exerting a relatively strong reaction. Inset, enlarged detail of the hippocampus (the area in the rectangle). Note the heavy reaction in the layer of hippocampal pyramids; cell bodies (arrow) do not exert any reaction. A, Alveus; M, molecular layer.



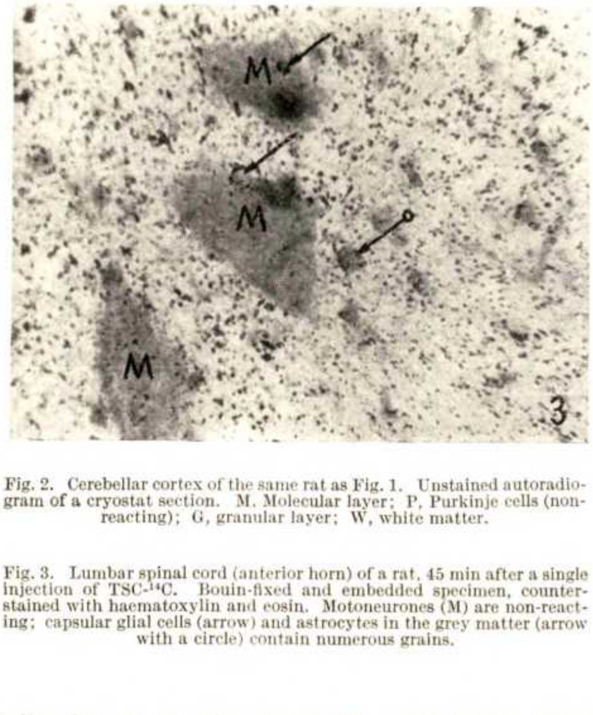


Fig. 2. Cerebellar cortex of the same rat as Fig. 1. Unstained autoradiogram of a cryostat section. M, Molecular layer; P, Purkinje cells (non-reacting); G, granular layer; W, white matter.

Fig. 3. Lumbar spinal cord (anterior horn) of a rat, 45 min after a single injection of TSC-^{14}C. Bouin-fixed and embedded specimen, counterstained with haematoxylin and eosin. Motoneurones (M) are non-reacting; capsular glial cells (arrow) and astrocytes in the grey matter (arrow with a circle) contain numerous grains.

binding by non-reacting structures might indicate those cellular elements that do not exert GAD activity. Such an approach is actually an adaptation of the principle described by Ostrovsky and Barnard[5].

Rats weighing 160 g were injected intraperitoneally with 5–20 mg/kg thiosemicarbazide-^{14}C (specific activity 10 mCi/mmole, obtained from the Central Isotope Institute, Budapest). Characteristic convulsions and lethal "jumps" appeared 45–120 min after injection. The animals were killed by decapitation and samples of the central nervous system (brain, medulla, cerebellum, spinal cord) as well as other tissues were either fixed on Bouin's solution and prepared for autoradiography in the usual way or were not fixed but frozen with dry ice, cut on a cryostat and applied to slides pre-coated with Kodak *AR*-10 stripping film, emulsion side up. The exposure time was 3–8 weeks; autoradiograms were developed in Kodak autoradiographic developer and some were counterstained with haematoxylin and eosin.

Fig. 1 shows a coronal section of the brain of a rat given two intraperitoneal injections of 10 mg/kg TSC-^{14}C, with an interval of 40 min between them, and then killed 90 min after the first injection. The heaviest reaction is confined to the hippocampus and fascia dentata chiefly in the layer of hippocampal pyramids (Fig. 1, inset); silver grains are, however, not found in the nerve cells themselves but are concentrated in the surrounding neuropil. Activity is high also in the nucleus habenularis medialis. There is a moderate reaction in the cortex, especially in the vicinity of the interhemispherical fissure.

Both the molecular layer and the granular layer of the cerebellum contain numerous silver grains (Fig. 2). The localization pattern of the reaction does not, however, conform to any of the neural elements but resembles more closely the glial structure. The reaction is slightly stronger around Purkinje cells, which are themselves devoid of any reaction both in pre-fixed and in non-fixed specimens. On the other hand, in deep cerebellar nuclei (and also in some of the brain stem nuclei, for example the substantia nigra and nuclei pontis) silver grains are located within the cytoplasms of nerve cells. Weak or virtually no activity can be seen in the white matter, although the glial cells in the white matter react if higher doses of TSC are used or if the animals are killed after a shorter time interval.

The nerve cells in the spinal cord do not contain silver grains, but capsular and other glial cells in both the grey and white matter contain numerous grains (Fig. 3). According to Curtis[2], GABA is not involved in spinal inhibitory mechanisms.

The gross distribution of silver grains reduced by TSC-^{14}C is in accord with the distribution of GAD and GABA anticipated on the basis of earlier biochemical and pharmacological studies (cerebellar cortex[2], hippocampus[2], substantia nigra[6]). While the concentration of silver grains in nerve cells (dentate nucleus, substantia nigra) and in the neuropil surrounding them (hippocampus) is consistent with current views, it is striking that Purkinje cells do not exert any reaction. Proteins sensitive to TSC are undoubtedly more widespread than GAD, so the apparent localization of TSC in glial cells of the cerebellar cortex may be partly due to binding of the drug to other B_6-dependent enzymes. The possibility cannot be excluded, however, that, at least in this area, GABA is produced by glial cells, perhaps in order to be taken up by nerve terminals and/or nerve cells in a second step.

BERTALAN CSILLIK
ELIZABETH KNYIHÁR

Department of Anatomy,
University Medical School,
Szeged, Hungary.

Received August 12; revised October 6, 1969.

[1] Roberts, E., in *Structure and Function of Inhibitory Mechanisms*, 401 (Pergamon Press, Oxford, 1968).
[2] Curtis, D. R., in *Structure and Function of Inhibitory Mechanisms*, 429 (Pergamon Press, Oxford, 1968).
[3] Van Gelder, N. M., *J. Neurochem.*, **12**, 231, 239 (1965).
[4] Killam, K. F., Dasgupta, S. R., and Killam, E. K., in *Inhibition in the Nervous System and Gamma-Aminobutyric Acid*, 302 (Pergamon Press, Oxford, 1960).
[5] Ostrovsky, K., and Barnard, E. A., *Exp. Cell Res.*, **25**, 456 (1961).
[6] Albers, R. W., in *Inhibition in the Nervous System and Gamma-Aminobutyric Acid*, 196 (Pergamon Press, Oxford, 1960).

Natural Selection and the Concept of a Protein Space

SALISBURY[1] has argued that there is an apparent contradiction between two fundamental concepts of biology— the belief that the gene is a unique sequence of nucleotides whose function it is to determine the sequence of amino-acids in a protein, and the theory of evolution by natural selection. In brief, he calculated that the number of possible amino-acid sequences is greater by many orders of magnitude than the number of proteins which could have existed on Earth since the origin of life, and hence that functionally effective proteins have a vanishingly small chance of arising by mutation. Natural selection is therefore ineffective because it lacks the essential raw material—favourable mutations.

I should like to look at the problem from a different point of view. I shall assume that mutations, while not random in a chemical sense, are random as far as their chances of improving the function of the corresponding proteins are concerned. I shall also assume that evolution has occurred either by the natural selection of favourable mutations or by the chance fixation by genetic drift of selectively neutral mutations. The justification for making these assumptions is that no sensible alternatives have been suggested and that no evidence exists at the moment to invalidate them. If these assumptions are true, what can we say about the frequency and distribution of amino-acid sequences which are functional, either as enzymes or in some other way?

The model of protein evolution I want to discuss is best understood by analogy with a popular word game. The object of the game is to pass from one word to another of the same length by changing one letter at a time, with the requirement that all the intermediate words are meaningful in the same language. Thus WORD can be converted into GENE in the minimum number of steps, as follows:

WORD WORE GORE GONE GENE

This is an analogue of evolution, in which the words represent proteins; the letters represent amino-acids; the alteration of a single letter corresponds to the simplest evolutionary step, the substitution of one amino-acid for another; and the requirement of meaning corresponds to the requirement that each unit step in evolution should be from one functional protein to another. The reason for the last requirement is as follows: suppose that a protein A B C D . . . exists, and that a protein a b C D . . . would be favoured by selection if it arose. Suppose further that the intermediates a B C D . . . and A b C D . . . are non-functional. These forms would arise by mutation, but would usually be eliminated by selection before a second mutation could occur. The double step from a b C D . . . to A B C D would thus be very unlikely to occur. Such double steps with unfavourable intermediates may occasionally occur, but are probably too rare to be important in evolution.

This is a model of the way in which one gene may change into another. An increase in the number of different genes in a single organism presumably occurs by the duplication of an already existing gene followed by divergence. If so, it remains true that new genes arise as modifications of pre-existing ones.

It follows that if evolution by natural selection is to occur, functional proteins must form a continuous network which can be traversed by unit mutational steps without passing through nonfunctional intermediates. In this respect, functional proteins resemble four-letter words in the English language, rather than eight-letter words, for the latter form a series of small isolated islands in a sea of nonsense sequences. Of course, this is not to deny the existence of isolated island proteins, analogous to the four-letter words ALSO and ALTO.

It is easy to state the condition which must be satisfied if meaningful proteins are to form a network. Let X be a meaningful protein. Let N be the number of proteins which can be derived from X by a unit mutational step, and f the fraction of these which are meaningful, in the sense of being as good as or better than X in some environment. Then, if $fN > 1$, meaningful proteins will form a network, and evolution by natural selection is possible. In estimating N it is necessary to distinguish two classes of mutations: (i) substitutions of single amino-acids, and additions or deletions of small numbers of amino-acids, making only a small change to the protein; and (ii) mutations producing a major change in amino-acid sequence, such as frame shifts and intramolecular inversions.

Mutations of the former type are much more likely to give rise to meaningful proteins than the latter. In the same way, a single random letter substitution in a meaningful word is more likely to give rise to a meaningful word than the simultaneous alteration of all the letters. Although frame shift mutations are known to occur, it is not clear whether they have ever been incorporated in evolution. It is therefore better to take N as the number of possible substitutions of single amino-acids. If all substitutions were possible in a single mutational step, N for a protein of 100 amino-acids would be 1,900. In practice the genetic code limits N to approximately 10^3.

Hence f must be greater than 1/1,000. It does not follow that the fraction of all possible sequences which are meaningful need be as high as 1/1,000. It is probably much lower. There is almost certainly a higher probability that a sequence will be meaningful if it is a neighbour of an existing functional protein than if it is selected at random. In fact, in treating N as the number of amino-acid substitutions rather than as the total number of possible mutational steps, it was in effect assumed that a random sequence has a negligibly small probability of being functional; this assumption will be confirmed if it turns out that frame shifts are rarely or never incorporated in evolution.

Suppose now that we imagine all possible amino-acid sequences to be arranged in a "protein space", so that two sequences are neighbours if one can be converted into another by a single amino-acid substitution. Then the requirement that $fN > 1$ requires that the "density" of functional proteins in certain regions of the space must be quite high—perhaps greater than 1/1,000. This agrees with Salisbury's conclusion that proteins, and hence the genes that determine them, cannot be as unique as all that. As a convinced Darwinist, I published[2] the conclusion that $fN > 1$ when little was known about the frequency of amino-acid substitutions in evolution. Since then evidence has accumulated (for a review, see King and Jukes[3]) that many substitutions are either selectively neutral or at least make comparatively minor changes in the function of proteins.

If $fN > 1$, no quantitative difficulty arises in explaining the evolution of proteins by natural selection. A difficulty nevertheless remains in explaining the origin of life—that is, in explaining the origin of the first functional proteins together with the genetic mechanism for producing them. If it were true that only a minute fraction of possible amino-acid sequences have even the slightest enzymatic activity, it would be difficult to understand how the first proteins arose. I do not want to discuss the problem of the origin of life, but only to point out that it is a quite different problem from that of the mechanism of evolution.

Some questions about molecular evolution can be formulated more clearly in terms of a protein space. For example: (i) Are all existing proteins part of the same continuous network, and if so, have they all been reached from a single starting point? Possible alternatives are that there are two or more distinct networks, or that there is one network with multiple starting points. (ii) How often, if ever, has evolution passed through a non-functional sequence? If so, has this been achieved by the random walk of genes rendered redundant by duplication, or by the chance concurrence of two or more mutations? (iii) What fraction of the functional network has already been explored in evolution? (iv) What fraction of potentially useful proteins are inaccessible?

JOHN MAYNARD SMITH

School of Biological Sciences,
University of Sussex.

Received November 7, 1969.

[1] Salisbury, F. B., *Nature*, **224**, 342 (1969).

[2] Maynard Smith, J., in *The Scientist Speculates* (edit. by Good, I. J.) (Heinemann, London, 1961).

[3] King, J. L., and Jukes, T. H., *Science*, **164**, 788 (1969).

Book Reviews

DETACHMENT AND INVOLVEMENT

Psychiatry in a Changing Society

Edited by S. H. Foulkes and G. Stewart Prince. Pp. xviii + 211. (Tavistock: London, October 1969.) 50*s*.

THIS volume is a byproduct of its senior author's year of office as chairman of the Psychotherapy and Social Psychiatry Section of the Royal Medico-Psychological Association (the organization to which nearly all British psychiatrists belong). As Dr Foulkes explains in his introduction, he took office at a time when there was vigorous discussion about the future of this section: in particular, about the desirability of separating its two principal components.

One school of thought, expressed in a lucid opening chapter by E. H. Hare, argues that the term "social psychiatry" has come to be so vague and all-embracing as to serve little purpose. Hare points out that some writers—notably Maxwell Jones—have used this term to refer to methods of treatment which enlist the contribution of members of a social group; others—notably Sir Aubrey Lewis—have emphasized the need for research designed to identify and measure the influence of social factors on the incidence and outcome of mental disorders.

Foulkes makes it very clear where his own sympathies lie. He vigorously rejects Hare's contention that "between psychotherapy and social research there is a deep, almost a fundamental, distinction". As he puts it: "The philosophy behind this concept of social psychiatry is that of an impartial science, incompatible with participation in or motivation for change". He believes that emotional and rational motivations determine the behaviour of those colleagues who disagree with him, preferring, to what he pejoratively calls objective psychiatry, his own brand of personal psychiatry: "The latter is a dynamic pursuit, entailing involvement and change in one's own person, and threatening values and attitudes. There is resistance against such change. The techniques of segregation, isolation, detachment and non-involvement are clearly preferred".

Many social scientists would contend that only by introducing an element of detachment and non-involvement can they guard against the self-deceptions which so readily accompany subjective impressions. Their doubts on this score can only be intensified by some of the *obiter dicta* in Foulkes's closing chapter, such as: "The concept of scientific detachment is outdated. . . . We must get away from a false impartiality in favour of a conscious participation in the service of humanity".

There is an important difference of opinion here about the proper content and methods of research in social psychiatry; but the argument presented is a rather one-sided one. Most of the book consists of essays by seven guest speakers, none of them psychiatrists, who addressed the section during Foulkes's year as chairman. Their contributions are interesting and diverse; Professor and Mrs Fortes report on the apparent increase in psychoses among the Tallensi people in Northern Ghana, during a period of 30 years; Dr Menzies describes her method of carrying out a study of nurse training in a teaching hospital; Dr M. R. A. Chance gives a detailed account of the application of techniques of ethological observation and analysis in the study of primate behaviour.

Of these papers, that by Menzies corresponds most clearly to Foulkes's concept of personal involvement in the social interactions which are being studied. Norbert Elias, a sociologist who writes on "Sociology and Psychiatry", contrasts the paradigms of "Homo psychiatricus" (who tends to be viewed as if in isolation from his fellows) and of "Homo sociologicus". He urges that more use should be made of the conceptual model which sees men as open systems, in which both events in their private histories (the usual concern of psychiatrists) and influences arising from their social interactions (the usual concern of sociologists) are each given due weight as determinants of behaviour. In his view, only Foulkes's own group analytic approach succeeds in fully exploiting both individual and group factors in therapy.

Paul Halmos, a social psychologist, contributes a refreshingly disquieting essay entitled "Psychology and the Ideology of Progress". This starts by recalling the familiar antithesis between vitalistic and mechanistic psychologies, but soon becomes more particular by focusing on the question: does the infant's experience of "good mothering" consist only of a finite, artificially reproducible number of sensory impressions—or is there more to it than that ? In this chapter, as in the book as a whole, the interest lies in the questions which are raised rather than in any claim to have discovered the answers.

G. M. CARSTAIRS

VIRUSES REVEALED

The Chemistry and Biology of Viruses

By Heinz Fraenkel-Conrat. Pp. x + 294. (Academic Press: New York and London, September 1969.) 84*s*.

THE study of virology as a biological science falls naturally into two parts. First, there is the nature of the complete virus particle, its chemistry and macromolecular structure; and second, there is the manner in which the virus interacts with host cells to give an infected complex. Professor Fraenkel-Conrat's concise and readable book covers both of these aspects, although approximately three-quarters of his book are concerned with the structure of viruses. It is difficult to write a textbook in a rapidly changing field such as virology, but in this case the author and publishers have chosen to produce the book in less than a year, an achievement well in evidence from the many recent references to research literature.

The book has been written to provide an introduction to virology both for students in the biological sciences and for research workers who are seeking to broaden their horizons, and both groups will benefit greatly from the wealth of interesting information presented. The experimental observations are explained and interpreted particularly well, the bench work being clearly related to the more formal model. Thus in a section describing methods of controlled degradation of viruses, it is easy to visualize the process as it would occur; while in other parts of the book the reader is made aware of the possible dangers of interpretation, for example, when the infectivity of viral nucleic acid is considered. Some excellent photographs, diagrams and tables help to convey the data.

The later chapters are concerned with the various modes of virus-cell interaction, and while many facts are described and many references provided for further exploration if desired, the impact, so much in evidence in the earlier section on virus structure, seems to be less. Perhaps this is because some basic concepts of virus infection are not covered adequately—for example, the single-step growth experiment which in 1939 provided the basis for the development of quantitative virology.

Further, there is very little discussion of the genetics of viruses, an omission which is deliberate and explained on the grounds that other books and review articles have covered this topic in detail. In the past three decades, however, studies on viruses have made a considerable contribution to an understanding of biological processes, particularly with regard to the nature and expression of the genetic code. The relevant experiments were possible because of a combination of approaches, that is, details of the virus structure from a physico-chemical viewpoint and of the biology of infection, both molecular and genetic aspects. Accordingly, in an introductory but nevertheless advanced book of this kind, I feel that it would have been advantageous to develop these themes together, even if this necessitated some increase in length.

There is a useful series of questions for each chapter at the end of the book. I recommend the book, particularly for those who require a predominantly experimental introduction to viruses. D. H. Roscoe

IMMUNOLOGICAL DISEASE

Autoimmunization and the Autoimmune Hemolytic Anemias

By Bernard Pirofsky. Pp. xiii + 537. (Williams and Wilkins: Baltimore. Distributed in the UK by Livingstone: Edinburgh, 1969.) 180*s*.

In this book Professor Pirofsky develops his thesis that autoimmune haemolytic anaemias are but one manifestation of a diffuse immunological systemic disease either genetically determined or caused by vertical transmission of virus infection in an immuno-deficient individual. This theme runs throughout the book, though technically it is divided into two parts, the first dealing predominantly with the clinical and haematological aspects of acquired haemolytic anaemias, and the second reviewing the theoretical and immuno-haematological evidence of a positive antiglobulin test and its relation to auto-immunization.

In the excellent historical review, the acquired haemolytic anaemias are examined in relation to the concept of an antibody produced by an individual and specifically directed against his own unmodified erythrocytes. On this criterion many of the antiglobulin positive immunohaemolytic anaemias can be shown to have antigens modified by drugs or cross-reacting antibodies stimulated by various environmental factors such as disease states, drugs and viruses. There is an extensive analysis of the author's series of 234 patients with warm acting erythrocyte antibodies studied from 1958 to 1966. The most striking features of the series are the remarkably high incidence of autoimmune haemolytic anaemia secondary to the leukaemias and the subsequent development of lymphomas in patients initially diagnosed as idiopathic haemolytic anaemia. Because of these features, the possibility exists that the term idiopathic is illusory and a longer follow up is necessary to define the true pattern of the disease.

The chief diagnostic criterion, a positive direct antiglobulin test, was found to be negative initially in thirty cases in the series and only repeated investigations in combination with enzyme tests enabled a firm diagnosis to be made. The therapy of all aspects of the disease, both primary and secondary, is well covered.

The second part of the book reviews the theoretical and immunohaematological implication of the clinical viewpoint and tries to define the terms so that the subject is not bedevilled by the semantics which have previously caused confusion owing to the extended ways in which the term autoimmune has been used. The final form of the book is derived from a preliminary text dominated by a "stream of consciousness" literary style: there are chapters on the self recognition phenomenon and the thirteen mechanisms leading to the state of autoimmunization are outlined in chapter thirteen and elaborated in the two subsequent ones.

One is conscious, in this book, of flights of imagination many of which are difficult to follow, and the repetition of many of the arguments does not add to its clarity. The whole work could be improved by a severe pruning of repetitive material. It is, however, undeniably useful as a complete compilation of every aspect of acquired and autoimmune haemolytic anaemias together with all the drugs and diseases which have been associated with increased haemolysis. M. M. Pickles

FIELD GUIDE FOR THE ARMCHAIR

Flowers of Europe

A Field Guide. By Oleg Polunin. Pp. 662 + 192 pages of illustrations in colour. (Oxford University Press: London and New York, June 1969.) 84*s*.

One botanizing season has already passed in Europe since the publication of this book, so its value as a field guide to European plants has been experienced by those who have used it. There is no doubt that it filled a gap in European botanical literature, for here, at a modest price for its size and content, is a single volume describing and illustrating a good selection of phanerogams. On the whole, it can hardly be recommended to professional taxonomists, because out of a figure far exceeding 15,000 species of seed-bearing plants natively growing in Europe or naturalized there, this work deals with only about 2,800 species. It is intended for, and best suited to, the ordinary traveller who wants to name and know something about the plants he stumbles across while he is on holiday.

The choice of included species must have been difficult and the criteria used in making it have been abundance, attractiveness, individuality and fame. About 1,900 plants are given full treatments—their principal diagnostic characters, a list of the countries in which they have been found, and their uses in healing, as poisons, dyes or food plants. As this is primarily a book for identification, keys to families and genera are given, and species in each genus are grouped under key-like headings to make identification simpler. The author in his preface stresses the importance to the layman of having colour photographs, and includes in his book more than 1,000 colour plates. There are also about 300 line drawings of plants. Throughout the book botanical terminology is kept at a minimum (but, we are told, not at the expense of accuracy), and those terms that have, inevitably, to be used are defined in a 15-page illustrated glossary. There is a very useful bibliography, and a list of popular names in English, French, German and Italian. The splitting of the contents of the book into two indexes, one English and the other Latin, is a tedious and frustrating practice for which I know no justification.

The descriptions of individual species are concise and consist of the principal diagnostic characters by which the plants may be identified: the mass of detail often discovered in a standard work is eliminated. Occasionally, there is an attempt to give the reader a thumb-nail impression of the plant first, before proceeding to the details. There is some inevitable inconsistency in the success of this, because some plants are more conducive to the treatment than others, but on the whole it is well done. Although the book is called a "Field Guide", this is not to be taken too seriously. Only the most stalwart would brave more than a few hundred yards with it in his rucksack (it weighs more than 3 pounds), and the information on uses of plants, although short, is an incidental inclusion that one can forgo in the field. It is an empty boast that the colour plates show the "nuances of posture, colour, form, and habitat" of the plants. There are some very fine photographs of which the one of *Gentiana lutea* on the dust-jacket showing the whole plant set against a mountainous background is probably the best; but many have unreal colours, a few are actually out of focus, a high proportion show inflorescences without leaves, and only about 10 per cent (a liberal estimate) show the plants growing in a recognizable habitat. The line drawings are excellent.

For those who do not need or cannot afford to buy a comprehensive work such as the *Flora Europaea* (which is as yet incomplete), the present work is a most commendable substitute. A. R. PERRY

DIVERSITY OF FLOWERING

The Induction of Flowering

Some Case Histories. Edited by L. T. Evans. Pp. 488. (Macmillan: Melbourne and London, July 1969.) A$20.

ONE of the dominant themes of contemporary molecular biology and biochemistry is the essential unity of living matter, so that we are understandably more impressed by the similarities in cell structure and metabolism of widely different organisms than by their differences, and we feel justified in extrapolating from unicellular algae to beans and apple trees, or even "from peas to people". When we come to consider whole organisms, however, they manifestly are different, both in structure and in physiology, and it becomes more difficult to extract general principles from a mass of particular detail. Since the importance of day length and winter chilling (vernalization) in controlling flowering in many plant species was first clearly established about 50 years ago, an impressive body of observational data has been accumulated for a large number of species. From these detailed facts certain generalizations have emerged, such as the probable universal involvement of phytochrome in photoperiodic control, which constitutes an excellent example of the inductive method in science. Success of this sort encourages us to seek other unifying concepts, such as a universal mechanism of flowering, involving a single "flower hormone".

This book, which comprises a series of case-histories of twenty species of plants which have been extensively used in studies on the physiology of flowering, is a salutary antidote against a too-ready assumption that there is a single, universal mechanism of flowering control in all higher plants. Twenty authors have each contributed a chapter which brings together and summarizes all the available information on the physiology of flowering of a selected species.

The aims of this study are stated to be "to expose the diversity of flowering behaviour rather than to neglect it or confine it within a few generalizations", and there is little doubt that this aim is achieved. One might have hoped, however, for some new insight into the flowering processes through the bringing together of all the information for single species to form an integrated and consistent picture. But in these studies each chapter remains essentially a separate case-history in which the facts are presented with little attempt at interpretation, although there is a valuable final chapter by the editor, Dr L. T. Evans, in which he critically examines some of the major concepts in the physiology of flowering.

The chosen species were selected primarily on the grounds that they had been extensively used for experimental studies and only a few, such as chrysanthemum, strawberry, pea, tomato and hemp, are of economic importance. The book is likely to be of value primarily to specialist research workers in the field of flowering physiology, and will provide a valuable reference work, as I have already found for my own work. It will also be useful to those responsible for the teaching of advance courses in plant physiology and to postgraduate students working in this field. P. F. WAREING

PROPERTIES OF SEAWATER

Marine Chemistry

The Structure of Water and the Chemistry of the Hydrosphere. By R. A. Horne. Pp. xxii + 568. (Wiley (Interscience): New York and London, November 1969.) 190*s*.

IN spite of its obvious and rapidly growing importance, the subject of marine chemistry has been largely neglected by the authors of textbooks. This is a complete contrast to most other branches of marine science, such as physical oceanography and marine geology, for which many excellent texts are available. This book, by R. A. Horne, is therefore welcome, particularly because it deals with the subject from a physico-chemical standpoint, unlike earlier works which have been largely descriptive. The author intends it to be used as general reading by marine scientists, as an advanced text by graduate students, and also as a source of available tabulated data of the properties of seawater.

The book is divided into four parts which it is convenient to discuss separately. Part one deals with the structure of water and the physical chemistry of electrolyte solutions with particular reference to seawater. As befits the author's distinguished research career in this field, this is the best section in the book. His treatment of this difficult and controversial subject is extremely lucid and could be read with profit by all marine chemists.

In comparison with the overall excellence of part one, the remaining three parts which deal chiefly with processes occurring in the sea are poor and ill-balanced except where they abut onto physical chemistry. I was left with the feeling that the author has been very selective in his reading and that he is unaware of much of the recent work on these subjects. It is unfortunate that there is not even a short account of the oceanic water circulation pattern, or of the processes causing mixing, because these to a very large extent control, or modify, the distribution of the chemical parameters in the sea.

Part two is devoted to a discussion of the chemical composition of seawater and the factors influencing it. The dissolved gases and chemical species in seawater are competently discussed (although it seems extraordinary to state (page 18) that the Ca/Cl‰ ratio is quite constant, when it has been known since 1884 that it is not, and to accept uncritically the controversial variation of the Sr/Cl‰ ratio). Some extremely important subjects, however, are treated in an extremely skimpy fashion, for example, the micro-nutrient elements—nitrogen and phosphorus—are dismissed in a mere 130 lines. Some other sections are rather out of date, and tend to be hotchpotches of rather ill-digested material, some of which has little relevance to the subject. This is particularly true of the chapter on the biochemistry of the ocean—thus, I would question the need for Fig. 9.2, showing the α-helix of the milk protein molecule, and Table 9.6, giving the amino-acid compositions of some proteins, not one of which is marine in origin.

The third part is concerned with the chemistry of the marine interfaces, and this includes a section on marine sediments. The latter consists of a number of isolated topics, evidently chosen from the literature in an unselective way, without any attempt to discriminate between important and trivial aspects of marine sedimentary geochemistry. This is revealed particularly by the author's choice of tables. In part four, a number of selected topics are discussed. These include marine corrosion, recovery of raw materials from the sea, and the origin of life in the sea. The book concludes with a very useful 60 page appendix containing tables of physical and physico-chemical properties of seawater.

The book is well printed and bound. It is marred, however, by an excessive number of printing errors, a few examples of which are: the temperature of −607° C in Table 1.1; the reference to a platform (platinum) indicator electrode (page 133); several errors in the radioactive decay series (pages 293–294). About half of the book is composed of tables and figures, several of the latter being of unnecessary crudity, for example, 1.1, 1.21, which are of primary school level. Although most of the writing is clear and unexceptionable, I found the author's numerous philosophical digressions extremely irritating.

In spite of my criticisms, however, this is a book which, because of its physico-chemical approach, should be read by every marine chemist. Unfortunately, it is unlikely to be of much value to marine biologists because the treatment of biochemical processes in the sea is brief and superficial and, most surprisingly, primary productivity does not seem to be mentioned directly at all.

J. P. RILEY

IONIZED ATMOSPHERE

Introduction to Ionospheric Physics

By Henry Rishbeth and Owen K. Garriott. (International Geophysics Series, Vol. 14.) Pp. x + 331. (Academic Press: New York and London, August 1969.) 149*s*.

THE ionosphere, that vast natural plasma which envelopes the Earth, is the concern of workers in a wide range of scientific disciplines. Over the years, contributions have come from astronomers, physicists, chemists, radio engineers, geomagneticians and others, and a comprehensive account of all aspects of current knowledge of the ionosphere would indeed call for a very substantial volume. The authors of this book are well aware of the problems inherent in writing only about selected topics, and in their preface they set out quite clearly what the book is, and is not, intended to do. The stated purpose is "to discuss the physical processes at work in the ionosphere and to relate them to the observed facts of ionospheric behaviour, without too much detail", and the authors add that the volume is "intended to be useful to physicists, especially graduate students, possessing no great knowledge of the upper atmosphere". A reading of the text confirms that these objectives have been achieved in full.

The subject matter has been grouped into eight principal sections—the neutral atmosphere; ionospheric measurements; photochemical processes; transport processes; morphology; some ionospheric phenomena; geomagnetism and the ionosphere; and storms and their ionospheric effects. Useful tables give, at a glance, orders of magnitude of essential numerical parameters or summary statements on such things as electron production and loss processes, photochemical reactions and ionospheric storm phenomena. A carefully chosen representative bibliography of some 700 documented references is also included. The style and general presentation of the material leave little to be desired and the authors have used wise discretion in deciding the detail with which any particular topic should be treated. In some cases, discussion has been limited because of the uncertain character of current knowledge; in other cases, a subject has not been dealt with in detail because to do so would have upset the general balance of the book. The overall result is a really excellent, authoritative introduction to the subject.

Readers will note that the senior author, Dr Henry Rishbeth, is a member of the staff at the Radio and Space Research Station, Slough, and that his co-author, Dr Owen Garriott, is at the Manned Spacecraft Center, Houston. It may not be generally known that Garriott is, in fact, currently training to be an astronaut. Presently, he will thus have the unique distinction of being the first ionospheric worker to make a first hand acquaintance with his subject.

Bearing in mind the objectives which the authors set themselves, this book can be recommended without reservation.

W. J. G. BEYNON

LAWS FOR SYSTEMS

Thermodynamique et Physique Statistique, Premier Cycle

By Bernard Jancovici. Pp. xii + 184. (Ediscience: Paris, 1969.) n.p.

THIS book is designed to provide a simple introduction to thermodynamics and statistical physics. It proceeds from a standpoint of microphysics, and the principles of thermodynamics are presented as consequences of mechanical laws for systems composed of very large numbers of particles. By presenting thermodynamics in this way, the author deliberately avoids a number of sophistries involved in the classical expositions of this subject. In particular, he eliminates the problem of interpreting the entropy concept in terms of purely macroscopic operations.

The book opens with a provisional definition of temperature, based on elementary kinetic theory. This definition is later supplanted by a more generally applicable one, but its introduction enables the author to provide a description of the macroscopic states of matter (phase diagram, van der Waals's equation and the like) at an early stage of the book.

In chapter two, the internal energy of a system is defined in microscopic mechanistic terms. This definition leads directly to the first law. In the following chapter, the entropy of a system is defined as the logarithm of the number of quantum states in an appropriate energy shell; and the temperature is defined as the reciprocal of the partial derivative of entropy with respect to internal energy. These definitions are then used to formulate the Zeroth, second and third laws of thermodynamics. Chapter four is devoted to various applications, at the macroscopic level, of the laws formulated in earlier chapters.

Thus, in the first four chapters, microscopic principles are used for the purposes of defining the thermodynamical variables and formulating the laws of thermodynamics. On the other hand, the fifth (and final) chapter provides an introduction to statistical mechanics proper. This chapter opens with a simplified derivation of Boltzmann's distribution law for a system (possibly macroscopic) in diathermic contact with a thermal reservoir. This law is then applied to relate a number of macroscopic phenomena to the atomic constitutions of the relevant systems (for example, paramagnetism of solids, specific heats of crystals).

The exposition is very clear throughout. I consider that this book should serve as a useful introduction both to thermodynamics and to statistical physics.

G. L. SEWELL

DEFORMATION OF SOLIDS

Elasticity, Fracture and Flow with Engineering and Geological Applications

By J. C. Jaeger. (Methuen's Monographs on Physical Subjects.) Pp. viii + 268. (Methuen: London, November 1969.) 30*s*.

PROFESSOR JAEGER's monograph is a third edition which incorporates without significant change the whole of the first (1956) edition under the same title. It also includes an extended version of the material on rock mechanics that was added in the second (1962) edition, and a substantial new section on applications of the ideas developed earlier to a study of the fundamentals of structural geology.

The reader is guided step by step from the classical account of deformation and flow with which engineering students will be familiar, including finite strains and yield criteria, through a discussion of such complications as plastic flow, firmo-viscosity, brittle fracture and earthquake waves, until he can appreciate finally the more subtle refinements that the geologist must make allowance for. These include stress patterns near boreholes, tunnels and cracks, the dependence of elastic moduli on strain, strain rate and temperature (attributed largely to the presence of pores in rocks), ductility and strength, planes of weakness, liquid flow through porous media, the difference between laboratory tests on sound specimens and field tests on rock formations with breaks and other imperfections, and progressive finite deformation over geological periods of time (to be explored with veins, fossils and inhomogeneities as the only visible indicators). Wherever possible, both theoretical and observational considerations are brought together on each topic.

The argument is developed throughout with full mathematical detail, a high standard of accuracy and freedom from misprints, always in a clear, easily readable style. A glossary of the chief symbols used and separate author and subject indexes are appended. References to original papers and other works are included in the text.

This book can be recommended as giving a sound and clear account of deformation and flow in theory and in practice, admirably suitable for the geologist and the engineer and worthy of the attention of students of other disciplines seeking a balanced introduction to the general deformation of solids.

J. G. Oldroyd

SPECTRAL ANALYSIS

Rotational Structure in the Spectra of Diatomic Molecules

By István Kovács. Pp. 320. (Hilger: London, November 1969.) 104*s*.

The name of Professor Kovács is well known to spectroscopists who analyse the rotational fine structure of the electronic spectra of diatomic molecules. In this book, all his own work on multiplet formulae, line intensities and on spectroscopic perturbations is included, together with similar equations derived by other workers in the most fundamental area of spectroscopy.

The first chapter deals with the solution of the wave equation for a diatomic molecule and includes many of the small interactions which are frequently neglected in approximate treatments. The rest of the book consists of applications of perturbation calculations to various types of interaction for all the commonly observed species of spectroscopic state and for many which are rarely seen or as yet unknown. Multiplet term formulae are derived for states with all multiplicities from singlet to septet. Each formula is tested by application to observed spectra and in most cases the agreement with theory is impressive. The intensity distribution formulae, which are presented very fully, should be the most useful part of the book. In particular, this section will be welcomed by astrophysicists, who are increasingly interested in the spectra of diatomic molecules.

A regrettable omission in a book published in 1969 is any account of nuclear hyperfine structure, which can alter the appearance of rotational structure and lead to incorrect analysis as in LaO. Again, a difficulty in this type of work is that many of the interactions are introduced as configuration interaction with other states. Such interactions are limited to one or two states, whereas, in reality, a whole spectrum of energy levels will interact. To include more than a few interacting states would make formulae which are already formidable into something quite unmanageable, but it does mean that if the formulae are ever found to fail an excuse in terms of configuration interaction can usually be made.

W. G. Richards

Obituaries

Professor E. H. F. Baldwin

Ernest Baldwin, who died suddenly on December 7, 1969, will be remembered not only as one of the chief protagonists of comparative biochemistry but for his outstanding contributions to teaching. Educated at the Crypt Grammar School, Gloucester, he went up to St John's College, Cambridge, as an exhibitioner in 1928. He took a first in both parts of the Natural Sciences Tripos, including Part II Biochemistry. In 1936 he became a research fellow of his college and in 1943 a lecturer on the staff of the Biochemical Department under Sir Frederick Gowland Hopkins. Greatly influenced by Hopkins's wide ranging approach to biochemistry, Baldwin applied his zoological knowledge to extending the comparative aspects of the subject and in particular nitrogen metabolism. One of the earliest results of this was his little book *Comparative Biochemistry* (1937), which is still recognized as a classic introduction to the subject.

During this time Baldwin was also acquiring a wide reputation as a teacher. His sense of the modern and interesting and his quietly dramatic presentation appealed strongly to students, and the theatre was always full. During the war years much of the teaching burden in biochemistry fell on him, so that he had to cover a wide field. One product of this was *Dynamic Aspects of Biochemistry* (1947), a book that had a worldwide impact on the teaching of the subject. The presentation, based on his inspiring lectures at Cambridge, was entirely new and it was received with unusual warmth. Its sales have by now exceeded 80,000 copies in five editions and five reprints; and it has been translated into seven languages (German, Italian, Spanish, Russian, Serbo-Croat, Japanese and Malay), thus winning acclaim for British biochemical teaching. A considerable part of its success lay in its undoubted literary merit, and indeed it gained for Baldwin the European Cortina-Ulisse prize in 1952.

In 1950 Baldwin was called to the chair of biochemistry at University College, London. His appointment belied the received idea that university teachers are elevated to chairs solely on the basis of their research contributions, for there is no doubt that Baldwin's great merits as a teacher weighed heavily in his selection. University College, under F. G. Young, had taken the first steps towards the establishment of a BSc special degree in biochemistry. It needed a professor with a high reputation as a teacher to carry through the negotiations with the university to a satisfactory conclusion and to establish the new course on a sound footing.

Baldwin remained at UCL for the next twenty years. During that time, in spite of frequent ill-health, he added much to our knowledge of the comparative biochemistry of nitrogen metabolism and the relation between ureotelic metabolism and water shortage, building up an internationally known school of research workers in such subjects. Curiously, this endeavour was always valued more highly in the United States and on the Continent than in Britain. In it Baldwin was ahead of his more conservative contemporaries, and it is only of very recent years that the movement to cross boundaries has made much progress here. Baldwin's influence at UCL was particularly important, for he saw biochemistry as a "biological" subject and his views were thus a valuable counter to the prevailing London conception of biochemistry as a branch of chemistry.

Bearded and amiable, quizzical and fastidious, the figure of Ernest Baldwin will be sadly missed, not only in the neighbourhood of Gower Street but also in his old haunts at Cambridge. It was from St John's that he went forth for his early work with friends at the French marine biological stations such as Roscoff and Tamaris; and the Biochemical Institute in Tennis Court Road saw some of his most notable achievements. There he was a particularly active organizer of the International Congress of Biochemistry in 1949, editing for it in collaboration the memorial volume *Hopkins and Biochemistry*. Baldwin was indeed a most worthy disciple of Frederick Gowland Hopkins. A good friend, a kindly director, a fine scientist and a gifted writer has been lost at too early an age to all of us.

Correspondence

Goldfinger no Longer

SIR,—You say that "it is arguable that a research council should divest itself of supporting the routine data collection that comprises much of the work of the institutions. This is not research and only occasionally aids research" (*Nature*, **225**, 2; 1970). I doubt whether these propositions are arguable in respect of any major field of science but they are quite untenable in regard to environmental research.

Penetrating research on the natural environment is impossible without programmes of field data collection. In answer to your suggestion that work of this kind should not be done in research institutions, I say that it would be disastrous if it were to be done anywhere else.

If your propositions were to be accepted, it would mean that the NERC would be able to support research on the input components of the natural system (in terms of physiology and biochemistry, for example) but would find it difficult to support analyses of the outputs in terms of field observations; or, at least, you would not support those field studies which deal with extensive time-series or large areas. The point which you have missed is that some kinds of research depend on the availability of collections of observations in the field, often on a scale which can be achieved only by establishing a systematic programme of sampling. The resolution, accuracy and scale of such observations must be determined by the objectives of the research programme. Thus they are as integral to research as a physiologist's laboratory equipment and measurements, for example.

Environmental research cannot exist without the description, analysis and interpretation of events and processes in nature. This is becoming even more essential with the increasing threat of pollution. The detection and measurement of the effects of pollution in the field will always be difficult, and often impossible, without a thorough understanding of the base-lines of natural variation. It would be extremely dangerous to predict the consequences of pollution, or to devise precautions against it, without a sound knowledge of natural ecosystems. Any attempt to monitor pollutants will be pointless unless we also monitor the communities and ecosystems. Essential laboratory studies of topics such as toxicity will be wasted unless they can be related to ecological field studies. Thus fundamental research on natural variations lies at the core of the investigation of pollution as it does of almost every branch of environmental science. The scale of natural variation is such that it demands the analysis of field observations taken frequently over a very long period of years and, often, from a very wide area.

In the past, much so-called ecology has dealt with single species, limited regions or restricted periods, rather than with communities and ecosystems. Of the many reasons for this, the most important are the severe practical as well as intellectual difficulties in analysing nature; especially, perhaps, in studies of the oceans. However, the revolution in electronics is now beginning to remove these impediments by providing the sensors with which to make the observations, the data loggers on which to record them and the computers with which to analyse them.

My excuse for making so many self-evident points is that your propositions, if unchallenged, would deny environmental science the principal opportunity for advance in this last third of the twentieth century. This is the establishment of research programmes based on the systematic collection and analysis of field data—linked, of course, to the essential experimental and theoretical studies.

In the environmental sciences, at least, applied science is no more than fundamental research on those things which matter to society. Any attempt to define the limits or to isolate either kind of research is bad for science and wasteful for society. I believe that your propositions are both.

Yours faithfully,

R. S. GLOVER

Scottish Marine Biological Association,
Oceanographic Laboratory,
78 Craighall Road,
Edinburgh EH6 4RQ.

University News

Dr Francis J. Smith has been appointed director of the Computer Laboratory at the **Queen's University of Belfast.**

Dr J. N. Hawthorne, University of Birmingham, has been appointed professor of biochemistry in the **University of Calgary.**

Air Marshal Sir Kenneth Porter, Royal Air Force Maintenance Command, has been appointed director of technical education projects at **University College, Cardiff.**

Professor Maurice D. Kilbridge has been appointed dean of the **Harvard Graduate School of Design.**

Dr James F. Tait, Worcester Foundation for Experimental Biology, has been appointed to the Joel Chair of Physics as Applied to Medicine tenable at the **Middlesex Hospital Medical School.** The title of Professor of Urology has been conferred on **Mr J. P. Blandy** in respect of his post at the **London Hospital Medical College.**

Professor N. Millott, Bedford College, has been appointed director of the newly instituted University Marine Biological Station, Millport, and professor of zoology in the **University of London.**

The **University of Nottingham** has appointed three special professors in the Department of Physiology and Environmental Studies, School of Agriculture: **Dr L. E. Mount,** ARC, Babraham (Environmental Physiology); **Mr K. J. Hill,** Unilever Research Laboratory (Animal Physiology); **Dr W. R. Butt,** United Birmingham Hospitals (Clinical Endocrinology).

Dr Hans Meidner has been appointed to the newly established chair of plant biology in the **University of Stirling.**

Professor Charles L. Miller has been appointed associate dean of the School of Engineering, **Massachusetts Institute of Technology,** and **Professor Peter S. Eagleson** has been named head of the Department of Civil Engineering within the school.

Appointments

Professor Herwig F. Schopper has been appointed leader of the Nuclear Physics Division at **CERN.**

Dr Theresa Clay has been appointed deputy keeper in the Department of Entomology, **British Museum (Natural History).**

Mr G. R. Walter de Silva of Ceylon has been appointed by the **International Atomic Energy Agency** to be regional officer for Asia and the Far East.

Dr J. R. F. Martineau, formerly secretary of the Defence Research Board, has been appointed to take charge of the International Relations Office of the **National Research Council of Canada.**

Dr P. S. Wellington has been appointed director of the **National Institute of Agricultural Botany** in succession to **Mr F. R. Horne.**

Dr Elliot S. Pierce has been appointed director of the Division of Nuclear Education and Training, **US Atomic Energy Commission,** in succession to **Dr Russell S. Poor.**

The **Ministry of Technology** has appointed four new regional directors, following reorganization of its regional offices: **Mr Ian Gray** (director for Wales); **Mr L. Ian Macbeth** (director for the South Western region); **Mr Ronald Wood** (director for the Northern region); **Mr Ernest Atherton** (director for the North Western region).

Mr Oran W. Nicks has been appointed acting associate administrator for advanced research and technology of the **National Aeronautics and Space Administration,** in succession to Mr Bruce T. Lundin.

Professor Keith Legg, Loughborough University of Technology, has been appointed consultant adviser to the Centre for Educational Research and Innovation of the **Organization for Economic Cooperation and Development.**

Announcements

The **Messel Medal** of the Society of Chemical Industry has been awarded to **Sir Eric Rideal.**

Dr Jay W. Forrester, Massachusetts Institute of Technology, has been awarded the **Valdemar Poulsen Gold Medal of the Danish Academy of Sciences** for his outstanding contribution to the development of the digital computer.

5,000 Swiss francs will be awarded by the **International Health Foundation** for the best paper submitted to the Foundation on any aspect of osteoporosis. Papers, which should not exceed 2,500 words and should contain original ideas, must be submitted before May 1. Further details can be obtained from the International Health Foundation, 14 rue de Hollande, 1204 Geneva, Switzerland.

The trustees of the **Lady Tata Memorial Trust** invite applications for fellowships and scholarships for research on any aspect of malignant disease which is relevant to the problems of leukaemia. Further details can be obtained from the secretary of the (European) Scientific Advisory Committee, Lady Tata Memorial Trust, Chester Beatty Research Institute, Fulham Road, London SW3.

The **Institute of Contemporary Arts** invites contributions to an exhibition of Unlikely Photography, which will consist of three sections: photographs of things that cannot be illustrated; photographic misrepresentations; three-dimensional photography. Further details can be obtained from the Institute of Contemporary Arts, Nash House, 12 Carlton House Terrace, London SW1.

A bequest by the late Mrs K. E. Maltwood has enabled the **Royal Society of Arts** to establish a fund to assist projects of archaeological research in Somerset which are of outstanding national importance. The society now invites applications for grants from the fund for work to be carried out in 1970. Further details can be obtained from J. S. Skidmore, assistant secretary, Royal Society of Arts, John Adam Street, Adelphi, London WC2.

International Meetings

March 19, **Automation in Production, Sampling and Testing of Silicate Materials,** London (J. M. Rowell, Society of Chemical Industry, 14 Belgrave Square, London SW1).

March 22–25, **American Association of Physical Anthropologists,** Washington, DC (Dr J. L. Angel, Division of Anthropology, Smithsonian Institution, US National Museum, Washington, DC 20560, USA).

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Wanted: From July 1970 to April 1971. Apartment or house near University of Copenhagen for chemist, wife and two small children. Please contact Dr N. W. Alcock, School of Molecular Sciences, University of Warwick, Coventry, Warwickshire CV4 7AL.

Wanted: 4-bedroom furnished house, preferably centrally heated, convenient to Hammersmith Hospital, for 1 year from July 20, 1970. Can offer similar accommodation in Syracuse, New York. Please contact Professor D. A. Nelson, Department of Clinical Pathology, Upstate Medical Center, Syracuse, New York 13210.

Wanted: For 3 months from April 1, 1970, furnished 3-bedroomed house in London. Rent up to £35–£40 per week, or exchange for furnished 3-bedroomed house in beautiful suburb, 5 miles from Rutgers University, 15 miles from Princeton University and 35 miles from New York City. Please contact Dr Bernard Beer, 6 Renfro Road, Somerset, New Jersey 08873.

Wanted: 4-bedroom house, preferably centrally heated, in or near Oxford, for 1 year starting September 1, 1970. Please contact Dr W. M. Thurlbeck, 602 Victoria Avenue, Westmount 217, Montreal, Quebec, Canada.

Vacant: 5-bedroom house in best part of Montreal, 10 minutes from McGill University and Université de Montreal, for 12–14 months starting summer 1970. Please contact Dr W. M. Thurlbeck, 602 Victoria Avenue, Westmount 217, Montreal, Quebec, Canada.

Vacant: From July 1970 to April 1971, comfortable terrace house, central heating, 3 bedrooms. Leamington Spa, convenient for University of Warwick. Please contact Dr N. W. Alcock, School of Molecular Sciences, University of Warwick, Coventry, Warwickshire CV4 7AL.

British Diary

Monday, February 9

A Volunteer at Masada (5.30 p.m.) Miss Katherine Blair, Royal Institution, at 21 Albemarle Street, London W1.

Colour Television Receiver Design and Maintenance (6 p.m.) Mr G. D. Barnes, Institution of Electronic and Radio Engineers; and the Institution of Electrical Engineers, at the University of Strathclyde, Glasgow.

Polymers in the Year 2000 (7.30 p.m.) Professor C. E. H. Bawn, CBE, FRS, Institution of the Rubber Industry, at the Donnan Laboratories, The University, Liverpool.

Techniques of Mask-Making (2.30 p.m. colloquium) Institution of Electrical Engineers, at Savoy Place, London WC2.

Tuesday, February 10

Dynamic Information Displays (6.30 p.m.) Mr D. W. G. Byatt, Institution of Electronic and Radio Engineers, at the University of Colchester, Essex.

Future Trends in Analytical Chemistry (6.30 p.m.) Dr J. B. Headridge, Society for Analytical Chemistry; and the Royal Institute of Chemistry, in the School of Chemistry, Newcastle University.

Making the Best of a Bad Job—What Engineering Is (5.30 p.m.) Professor E. R. Laithwaite, Royal Institution, at 21 Albemarle Street, London W1. (Lecture for Sixth Form Pupils from Schools in London and Home Counties. To be repeated on February 11, 17 and 18.)

Mathematician's Utopia (1.20 p.m.) Mr M. J. Brown, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

Primers for Metal (forum) Oil and Colour Chemists' Association, at the Griffin Hotel, Boar Lane, Leeds 1.

Recent Work on Dithizone and Its Selenium Analogue (5.15 p.m.) Professor H. M. N. H. Irving, Society for Analytical Chemistry; and Leeds University Union Chemical Society, in the Chemistry Department, University of Leeds.

Scientific Knowledge and the Press (7.30 for 8 p.m.) John Davy and Professor J. M. Ziman, British Society for Social Responsibility in Science, at the Blackhorse, Rathbone Place, W1.

The Ageing Process in the Human Brain (5.30 p.m.) Dr J. A. N. Corsellis, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1.

The Tunnelling of Shipworms, Mr P. A. Board; **Cell Specialization and Chemosensitivity in Fish Skin,** Dr Mary Whitear; **After the Torrey Canyon** (film) (5 p.m.) Zoological Society of London, at the Zoological Gardens, Regent's Park, London NW1.

Wednesday, February 11

Analytical Cosmochemistry (or Migrants from Outer Space Enter the Chemical Laboratory) (6.30 p.m.) Dr A. A. Smales, Society for Analytical Chemistry, in the Mechanical Engineering Department, Imperial College, London SW7.

Annual General Meeting; The Modern Manual Instrument; and **Exhibition** (2 p.m.) Photoelectric Spectrometry Group, at the School of Pharmacy, Brunswick Square, London WC1.

Computer Applications in Shipping (5.30 p.m.) Mr H. Phillips, Institution of Electrical Engineers; the Institute of Measurement and Control; and the Automatic Control Group of the I.Mech.E., at Savoy Place, London WC2.

Gnotobiotic Animals (Germ and Pathogen-Free Animals) (6.15 p.m.) Professor A. O. Betts, Society of Chemical Industry, Food Group, at 14 Belgrave Square, London SW1.

Nuclear Power Up-Dated (7 p.m.) Dr H. Kronberger, FRS, Institution of Electrical Engineers, at Culham Laboratory, Harwell.

Plasticizers—Compatibility and Performance (7.30 p.m.) Mr J. L. Fowler, Institution of the Rubber Industry, at the Polygon Hotel, Southampton.

Problems Arising from the Water-Cooling of Engine Components (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Telecommunication Switching—a Review of Technological Progress (5.30 p.m.) Mr J. R. Pollard, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Communication of Scientific Information through Experimental Demonstrations (1 p.m.) Mr F. C. Brown, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

The Functions of the Ministry of Technology in the Field of Industry (7.15 p.m.) Mr K. W. Doble, Plastics Institute, at The University of Wales Institute of Science and Technology, Cathays Park, Cardiff.

Thursday, February 12

Biochemical Aspects of the Control of Energy Production in the Liver of the Normal Rat and After Physical Injury (5 p.m.) Mr C. J. Threlfall, University of London, at the Middlesex Hospital Medical School, Mortimer Street, London W1.

Botany or Molecular Phytobiology? (8 p.m.) Professor J. Friend, in the University Lecture Theatre, University of Hull.

Fluorescence Spectroscopy (2 p.m.) Society for Analytical Chemistry, in the Special Lecture Theatre of Liverpool Regional College of Technology, Byrom Street, Liverpool.

Mechanization of the Assembly Process in Small and Medium Batch Production (4.30 p.m.) Mr J. R. Ashley, Royal Society, at 6 Carlton House Terrace, London SW1.

New Resin Systems for Use in Coil-Coating Applications (6.30 p.m.) Mr L. A. Tysall, Oil and Colour Chemists' Association, in the New Engineering Block, University College London, Gower Street, London WC1.

Non-Empirical, Synthetic Truths in Mathematics (5.30 p.m.) Professor Paul Lorenzen, University of London, at the London School of Economics and Political Science, Houghton Street, London WC2.

Performance Requirements and Test Methods for High-Voltage AC Motor Insulation (5.30 p.m.) Mr K. K. Schwarz, Institution of Electrical Engineers, at Savoy Place, London WC2.

Photovoltaic and Thermoelectric Conversion—Recent Developments in Materials and Devices (10 a.m. colloquium) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Corrosion of Metals in Aqueous Conditions (7 p.m.) Dr P. T. Gilbert, Liverpool Metallurgical Society, in the Department of Metallurgy and Materials Science, The University, Liverpool 3.

The Effects and Control of Vibration in Machinery (all-day discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

The Engineering Implications of Nucleonic Measurement (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

The North Sea Continental Shelf Cases (5 p.m.) Mr E. D. Brown, University of London, in the Weldon Theatre, University College London, Gower Street, London WC1.

Vascular Endothelium and Its Relation to Blood-Flow (5.30 p.m.) Dr Helen Payling Wright, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Eleventh of fifteen lectures on "The Scientific Basis of Medicine".)

Friday, February 13

Does the Paint Industry Deserve a Research Association? (6.30 p.m.) Dr G. de Winter Anderson, Oil and Colour Chemists' Association, at the Manchester Literary and Philosophical Society, 36 George Street, Manchester 1.

Non-Empirical, Synthetic Truths in Physics (5.30 p.m.) Professor Paul Lorenzen, University of London, at the London School of Economics and Political Science, Houghton Street, London WC2.

Photochemistry of Paranitrosodimethylaniline (1 p.m.) Dr T. S. Godfrey, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

The Ebb and Flow of Atomic Chaos—The Physicist as King Canute (9 p.m.) Professor E. L. Hahn, Royal Institution, at 21 Albemarle Street, London W1.

Monday, February 16

Contention or Polling Techniques for Data-Computer Systems (5.30 p.m. discussion) Institution of Electronic and Radio Engineers, Joint IEE/IERE Computer Group, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Insect Hormones and Insect Control (5 p.m.) Dr K. C. Highnam, Society of Chemical Industry, at 14 Belgrave Square, London SW1.

Recent Advances in Medicine, Surgery and Therapy (6 p.m.) Sir Brian Windeyer, Royal Society of Arts, at John Adam Street, London WC2. (First of three Cantor Lectures. Further lectures on February 23 and March 2.)

The Treatment and Disposal of Industrial Effluent (all-day discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

HOW TO BUY NATURE

Volumes start in January, April, July and October, but subscriptions may begin with any issue.

The direct postal price per subscription for one copy of NATURE each week is:

12 MONTHS (52 issues and 4 indexes)

Great Britain and Eire.................................**£14.0.0** each
United States of America........(by air freight)............ **$48** each
Canada...........................(by air freight).......... **$52** each
Elsewhere overseas by surface mail....................**£14.0.0** each
(Charge for delivery by air mail on application)
*Shorter periods *pro rata*. (Minimum three months.)

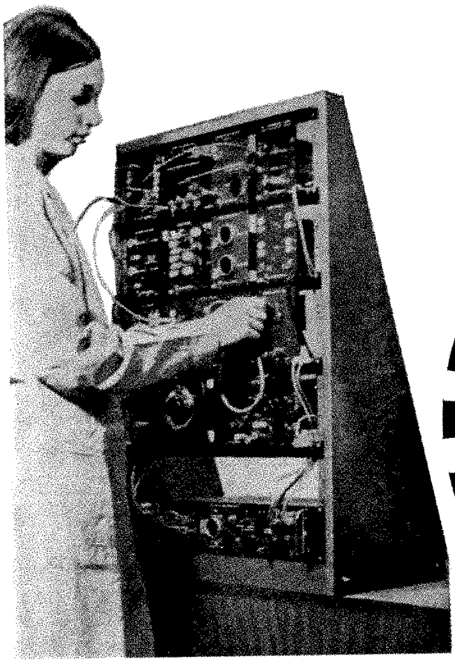
Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
4 LITTLE ESSEX STREET, LONDON WC2
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2
Subscription Department
MACMILLAN (JOURNALS) LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 5431
Advertisements only should be addressed to
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2
Telephones 01-242 6264/01-405 4743
Telegrams: Textualist London WC2
Registered as a newspaper at the Post Office

Copyright © Macmillan (Journals) Limited, February 7, 1970

combined behavioural and data acquisition systems

These systems can be operated with equal ease by technicians or research staff. They are research tools for people whose interests are not primarily electrical or electronic. Inexperienced staff can construct a system to reduce qualitative statements into quantitative information.

The apparatus was primarily designed for the Life Sciences but its versatility is such that many applications related to other sciences come to mind viz. complex organic syntheses.



PROGRAMMING, CONTROL AND PRESENTATION SYSTEMS CAN BE DIVIDED INTO THREE CATEGORIES

1. Systems where it is desirable to operate on a single problem for a reasonable period of time and thereafter disperse the equipment throughout individual laboratories for reassembly into smaller self-contained systems for use by separate investigators. The dispersible systems requirements are fully met by the front-patch equipment.

2. Fixed, single-purpose systems in which the requirements are well known and in which extremely infrequent changes are contemplated.

3. A central programming system used by many individuals for different programmes at separate times.

From an enormous list there is a choice of equipment to suit every investigation

Power supplies, print-out and read-out modules, time generators, digital and analogue logic units, statistical generators, interface devices and other interesting units are available. Send for further details now.

B&T

Baird & Tatlock (London) Ltd.
P.O. Box 1, Romford RM1 1HA

SEND ME

further details of Massey Dickinson Apparatus

NAME ....

POSITION ....

COMPANY ....

....

....

N

BT.215

Classified Advertisements

All copy is subject to the approval of the Publishers, who retain the right to refuse or withdraw advertisements at their discretion. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes. Semi-displayed £6 per single column inch. Minimum £3, each additional 1/12th of an inch 10s. Full page £158, Half page across £80. Colour (orange) £20 extra. 2/6 is charged for the re-direction of replies to advertisements with a box number.

ADVERTISEMENTS SHOULD BE ADDRESSED TO: T. G. Scott and Son, Limited, 1 Clement's Inn, Strand, London, W.C.2. Telephone: 01-405 4743. Telegrams: Textualist, London, W.C.2.

APPOINTMENTS VACANT

INSTITUTE OF OPHTHALMOLOGY

(University of London)
Judd Street, London, W.C.1

DEPARTMENT OF PATHOLOGY

TECHNICIAN OR JUNIOR TECHNICIAN required for the routine clinical laboratory. I.M.L.T. Intermediate or equivalent qualifications preferred, but not essential. Good facilities for training for Final examinations.

Please apply in writing, stating age, sex, qualifications, experience and give the names of two referees to The Director at the above address.

(480)

INSTITUTE OF OPHTHALMOLOGY

(University of London)
Judd Street, London, W.C.1

DEPARTMENT OF PATHOLOGY

CHEMICAL PATHOLOGY

SENIOR I/II TECHNICIAN, with experience in automated techniques, required in Chemical Pathology.

The work would entail (a) research on the biochemistry of vascular diseases, and (b) routine investigations in an expanding chemical pathology service to Moorfields Eye Hospital.

Please apply in writing, stating age, sex, qualifications, experience and give the names of two referees, to The Director at the above address.

(481)

UNIVERSITY OF ZAMBIA

Applications are invited for (a) SENIOR LECTURESHIP or (b) LECTURESHIP in PHYSICS. The present research interests are in the fields of defects in solids and applications of radioisotopes ; preference will be given to candidates with experience in one of these fields, or for Senior Lecturer, in geophysics. Experience in teaching elementary Materials Science advantageous. Salary scales (including contract addition for non-Zambians) (a) K5,500 to K6,300 per annum ; (b) K3,500 to K5,300 per annum (K1 = 11s. 8d. sterling). Salary supplemented in range £200 to £250 per annum (sterling) in appropriate case under British Expatriates Supplementation Scheme. Family passages ; various allowances ; biennial overseas leave ; superannuation and medical aid schemes.

Detailed applications (six copies), naming three referees, by March 3, 1970, to Inter-University Council, 90/91 Tottenham Court Road, London, WIP ODT, from whom particulars are available.

(474)

UNIVERSITY OF ALBERTA

DEPARTMENT OF CIVIL ENGINEERING

Applications are invited for two positions at the rank of Assistant or Associate Professor. Rank and salary will depend on qualifications.

One position is in STRUCTURAL ENGINEERING. The second position is in GEOTECHNICAL ENGINEERING, specifically the application of numerical methods to field problems. Appointments effective July 1970 if feasible.

Submit applications with curriculum vitae and names of referees to Chairman, Department of Civil Engineering, University of Alberta, Edmonton, Alberta, Canada. Replies confidential.

(210)

WINDWARD ISLANDS BANANA RESEARCH SCHEME

FRUIT QUALITY RESEARCH OFFICER

Applications are invited for the post of Fruit Quality Research Officer. The successful candidate will be part of a research team investigating banana quality in a comprehensive manner, conducting experiments, observations, surveys and analyses pertaining to the improvement of quality of Windward Islands' banana. His main duties will be agronomy, field handling and packaging of bananas.

Minimum qualifications are a degree in agronomy, agriculture, plant physiology or agricultural biochemistry, together with some experience in field experimentation and/or statistics. An interest in practical mechanics and simple construction would be most useful.

The initial salary scale is $EC7,440 (£1,550) by $EC432 (£90) to $EC10,032 (£2,090) by $EC432 (£90) to $EC12,624 (£2,630) and conditions of service are based on those of the University of the West Indies.

Applicants, giving the candidate's background, together with the names of two referees, should be sent to the Director of Research, Windward Islands' Banana Research Scheme, from whom further details may be obtained at the following address:

P.O. Box 115,
Castries,
St. Lucia,
West Indies.

(489)

UNIVERSITY OF WALES

UNIVERSITY COLLEGE OF SWANSEA

SECOND CHAIR OF GEOGRAPHY

Applications are invited for the Second Chair of Geography in the department of Geography.

Further particulars, quoting reference No. B6, may be obtained from the Registrar, University College of Swansea, Singleton Park, Swansea, Glam. SA2 8PP, by February 21, 1970.

ANEURIN DAVIES.
Registrar.

(383)

UNIVERSITY OF MALAWI

Applications are invited for the following posts tenable from September, 1970. Although the staff appointed will be attached to Soche Hill College they will also be required to teach at Chancellor College. In all posts teaching experience in secondary schools or teacher training colleges is desirable:

(a) BIOLOGY. Lecturer—degree in Biology (Botany and Zoology) with a special interest, preferably in Ecology required. Research opportunities in terrestrial and aquatic ecology are available.

(b) CHEMISTRY. Lecturer—degree in Chemistry required with a subsidiary qualification in Physics.

Salary scales (including expatriate addition): Senior Lecturer, £2,500 to £2,800 per annum ; Lecturer, £1,340 to £2,250 per annum plus University addition of £360 per annum OR salary supplemented by £275 per annum (normally tax free) in appropriate case under British Expatriates Supplementation Scheme. Gratuity ; Superannuation Scheme transferable with F.S.S.U., family passages ; various allowances ; biennial overseas leave.

Detailed applications (six copies), naming three referees, by February 27, 1970, to Inter-University Council, 90/91 Tottenham Court Road, London, WIP ODT, from whom particulars are available.

(469)

AGRICULTURAL RESEARCH COUNCIL requires an ABSTRACTOR at its Weed Research Organization, near Oxford. The duties include abstracting and dealing with scientific inquiries in the field of weed control. A degree in agriculture or a biological science is desirable ; an ability to write clear, concise English is vital and a knowledge of either Dutch or a Scandinavian language and a willingness to acquire proficiency in the alternative language is essential. Salary according to age and experience in scales either (i) £872 (age 21) to £1,208 (age 26) (maximum starting pay) rising to £1,454 or (ii) £1,590 rising to £2,006.—Application forms and further details from Secretary, Weed Research Organization, Yarnton, Oxford OX5 1PF, quoting 2/70. Completed applications to be returned by February 20, 1970.

(471)

UNIVERSITY OF BRITISH COLUMBIA

INSTITUTE OF OCEANOGRAPHY

Applications are invited for a position in zoological oceanography in this Institute in the Faculty of Graduate Studies. There are twelve faculty members and thirty-five graduate students in various aspects of oceanography. Qualifications required are appropriate to the duties, which are to carry out research, to supervise graduate students and to present lectures at upper and graduate levels. The preferred field of research is in marine ecology with emphasis on zooplankton crops.

The position will be available in August, 1970 ; rank and salary will be appropriate to the appointee's qualifications and experience. Base salaries, on a twelve month basis, are at present: Assistant, $9,500 ; Associate, $11,700 ; and Full Professor, $15,800 plus 10 per cent superannuation and other benefits equivalent to about 5 per cent of salary. Appointments are generally made jointly in Oceanography and the appropriate department ; appointees are encouraged to take part in departmental activities.

Applications should include a curriculum vitae (academic training, professional experience and publications), the names of three referees, and an indication of the type of research projects which are proposed. They should be sent to: Director, Institute of Oceanography, University of British Columbia, Vancouver 8, B.C., Canada, to whom inquiries should be directed.

(314)

UNIVERSITY OF EAST AFRICA

THE UNIVERSITY COLLEGE, DAR ES SALAAM

DEPARTMENT OF STATISTICS

Applications are invited for the following appointments in the Department of Statistics of the Faculty of Arts and Social Science:

1. PROFESSOR/READER IN STATISTICS. Special interests of the Department include: econometrics, computer methods, experimental design. Experience of educational administration would be an added recommendation.

2. SENIOR LECTURER/LECTURER. Two vacancies available. Preferably with at least several years of professional experience on: Mathematical Statistics, Economic Statistics (Econometrics) or theory of decision.

Salary Scales: Professor, £EA3,150 per annum; Reader, £EA2,540 to £EA2,825 per annum; Senior Lecturer, £EA1,950 to £EA2,615 per annum ; Lecturer, £EA1,350 to £EA2,230 per annum (£EA1 = £1 3s. 4d. sterling.) Salary supplemented in range £612 to £1,068 per annum (sterling) and education allowances and childrens' holiday visit passages payable in appropriate cases under British Expatriates Supplementation Scheme. F.S.S.U. Family passages ; biennial overseas leave.

Detailed applications (six copies), naming three referees, by February 26, 1970, to Inter-University Council, 90/91 Tottenham Court Road, London, WIP ODT, from whom particulars are available.

(472)

UNIVERSITY OF SYDNEY

LECTURER IN MECHANICAL ENGINEERING

Applications are invited for the above-mentioned post. The appointee will assist in planning and teaching of undergraduate and graduate courses relating to dynamics of linear and non-linear mechanisms and control systems and pursue original research in one of these or a closely allied field. Salary range: $A5,400 to $A7,300 per annum.

Further information is available from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London, W.C.1. Applications close on March 2, 1970.

(512)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF CHEMICAL ENGINEERING

Applications are invited preferably from persons with industrial experience for the post of ASSISTANT LECTURER or LECTURER in Chemical Engineering. Salary in the range £1,240 to £1,470 (Assistant Lecturer) or £1,585 to £2,850 (Lecturer).

Further particulars and forms of application, returnable not later than March 2, from the Staff Appointments Officer.

(476)

COUNSELLOR (SCIENTIFIC)

British Embassy—Moscow

£4170—£4745

This appointment is made in connection with the promotion of co-operation between the UK and the USSR under the Anglo-Soviet Technological and Cultural Agreements. The Scientific Counsellor is responsible to HM Ambassador advising him and Embassy staff on scientific and technological affairs and also reporting home on trends and developments in this area recommending policies for their support. To do this he will need to establish personal contacts with Soviet scientific organisations.

Candidates should normally have a first or second class honours degree in science or engineering. Preference will be given to candidates with experience of dealing with industry and government departments and in the conduct and management of research. A good knowledge of Russian or an ability to become fluent is essential.

Salary will be in the above range (graded Senior Principal Scientific Officer) together with Diplomatic Service Allowances. The vacancy occurs in September 1970 and is for a period of 2 years.

Fuller details and an application form may be obtained by writing to ***Miss B. C. Taylor, Department of Education and Science, Curzon Street, London W1Y 8AA, (01-493 7070 ext. 311).*** *Closing date 20th February 1970.*

(550)

UNIVERSITY OF BELFAST

DEPARTMENT OF MECHANICAL ENGINEERING

The Senate of The Queen's University of Belfast invites applications for the following posts from October 1, 1970, or other date, as a result of reorganisation and expansion in the Department of Mechanical Engineering: (a) Senior Lectureship in Industrial Engineering to initiate undergraduate and postgraduate studies in this field ; (b) Three Lectureships in Mechanical Engineering. Candidates should preferably have interests in Thermodynamics or Fluid Dynamics or Dynamics and Control.

The salary range for a Senior Lectureship is £2,800 to £3,670 and for a Lectureship £1,585 to £2,850. Both scales carry contributory pension rights under the F.S.S.U. Initial placing on the salary scales will depend on qualifications and experience. Applications should be received by February 28, 1970.

Further particulars may be obtained from The Secretary, The Queen's University of Belfast, Belfast, BT7 1NN, Northern Ireland. (547)

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF THEORETICAL MECHANICS

Applications are invited for an **ASSISTANT LECTURESHIP** or **LECTURESHIP** within this Department. Candidates should be qualified in any branch of **STATISTICS, OPERATIONAL RESEARCH, APPLIED MATHEMATICS** or **NUMERICAL ANALYSIS**. The duties of the successful candidate will include responsibility for the teaching of statistics to students of engineering and mathematics-with-engineering. The appointment will be effective from September 1, 1970

Salary within the £1,240 to £1,470 (Assistant Lectureship), or £1,585 to £2,850 (Lectureship).

Further particulars and forms of application, returnable not later than March 2, 1970, from the Staff Appointments Officer, The University of Nottingham, University Park. (561)

RADIOBIOLOGIST

A graduate research worker with interest or experience in mammalian radiobiology and its application to radiotherapy is required for a post in the Churchill Hospital Research Institute. The successful applicant will join an interdisciplinary group studying various aspects of tissue and tumour growth and the effects of ionizing radiation and drugs upon it. Salary according to age, qualifications and experience.

Replies to Dr. R. J. Berry, M.R.C. External Scientific Staff, Radiobiology Laboratory, Churchill Hospital, Oxford. (441)

AREA LABORATORY

KING EDWARD VII HOSPITAL WINDSOR

Wanted immediately—SENIOR GRADE BIOCHEMIST in the Department of Chemical Pathology, with salary according to National scales.

This well equipped Department serves 1,000 beds, of which 600 are acute, at the District Hospital Centres at Windsor and Ascot, together with the Maidenhead Hospitals, and a large volume of work is undertaken for General Practitioners. Opportunities exist for useful research and working for the M.C.B. Applications to the Senior Pathologist, Area Laboratory. (544)

UNIVERSITY OF BRISTOL. DEPARTment of Zoology. Applications are invited for the post of Research Assistant to undertake physiological investigations on animals in transition from water to land. Applicants should be interested in problems of respiratory adaptations and experience in respiratory work would be an advantage. Graduates and postdoctorals will be considered. Salary will depend upon qualifications and experience and will be in the range of £800 to £1,470 per annum.—Applications, giving the names of two referees, should be sent to Professor G. M. Hughes, Department of Zoology, The University, Woodland Road, Bristol, BS8 1UG, by February 18, 1970. (501)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF ELECTRICAL ENGINEERING

EXPERIMENTAL OFFICER

Applications are invited from suitably qualified candidates for this post. The successful applicant will be required to take responsibility for the design, construction and preparation of electronic measuring equipment for research purposes.

This is a senior appointment and candidates will be expected to have a university degree or professional qualification, together with appropriate experience.

Salary scale: £1,240 to £2,045 per annum plus F.S.S.U.

Applications, giving details of age, qualifications and the names of two referees, should be sent to the Deputy Secretary, The University, Southampton, SO9 5NH by February 28, 1970. Quoting Reference Na. (560)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

(UNIVERSITY OF READING)

ASSISTANT EXPERIMENTAL OFFICER/EXPERIMENTAL OFFICER with an interest in biophysics required to assist in the running of an analytical ultracentrifuge. The work would involve participation in several research projects, one of which would be concerned with the characterisation and interaction of milk proteins. Salary scales: A.E.O., up to £940 (age 22) to £1,208 (age 26) to £1,454; E.O., £1,590 to £2,006. Qualifications: Degree, Dip.Tech., H.N.C. or equivalent. Under 22, minimum qualification is two G.C.E. " A " levels in scientific subjects.

Apply to Secretary, N.I.R.D., Shinfield, Reading, quoting two referees and reference 70/3. (485)

MARINE LABORATORY, ABERDEEN

DEPARTMENT OF AGRICULTURE AND FISHERIES FOR SCOTLAND

MARINE BIOLOGIST in the **ORGANIC ENVIRONMENT SECTION** (graded Senior Scientific Officer/Scientific Officer) to work on the feeding and metabolism of invertebrates as part of a general study of marine food chains and especially in relation to problems of pollution. A knowledge of biochemistry and of experimental techniques in this field is most desirable.

Qualifications: 1st or 2nd class honours degree in zoology or equivalent and for SSO at least three years post-graduate experience.

Age: SO under 29, SSO normally 26–31.

Salary: SO £1,071–£1,827, SSO £2,021–£2,491.

Prospects of permanent pensionable employment.

Application forms from the Secretary, Department of Agriculture and Fisheries, Room 172(C), St Andrew's House, Edinburgh, EH1 3DA
Closing date 27 February 1970.

(467)

Searle

SEARLE SCIENTIFIC SERVICES MEDICAL RESEARCH

This new department in High Wycombe requires laboratory technicians and junior laboratory technicians to work in the hormone laboratory. The work performed in this laboratory is in the field of medical research and involves the determination of steroid hormones and protein hormones using the latest techniques available.

Qualifications: For the technician posts applicants should possess I.M.L.T., H.N.C. or equivalent. For the junior posts O or A levels in Science or Biology are needed. Experience in laboratory work would be an added advantage but is not essential. A Hospital background is preferred to industrial experience.

Working conditions are excellent and include:

CANTEEN RECREATION CLUB PENSION FUND

Please apply to:

The Personnel Officer,
G. D. Searle & Co. Ltd.,
Lane End Road, High Wycombe, Bucks.
Telephone: High Wycombe 21124.

(549)

NEW ZEALAND

University of Canterbury • Christchurch

Applications are invited for the following positions:

ASSISTANT LECTURER IN GEOLOGY:

The appointee's duties will be to assist in the teaching of one or more of these subjects: engineering geology, engineering geophysics, applied geology.

Applications close on March 14, 1970.

Salaries are under review. **Existing** salaries for Assistant Lecturers are $NZ2,500 by $NZ100 to $NZ2,900 per annum.

Particulars, including information on travel and removal allowances, study leave, housing and superannuation may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London, W.C.1. (509)

QUEEN ELIZABETH COLLEGE (UNIVERSITY OF LONDON) CAMPDEN HILL ROAD, W.8

Applications are invited for the post of SENIOR LECTURER in the Department of BIOCHEMISTRY. The successful candidate will be required to take an active part in teaching Biochemistry to Honours B.Sc. standard and to pursue an independent line of research as well as to share in the administration of the Department. Salary range £2,770 to £3,760 plus £80 London Allowance.

Further particulars from the College Secretary, to whom applications should be sent by February 28. (517)

THE UNIVERSITY OF ASTON IN BIRMINGHAM

DEPARTMENT OF BIOLOGICAL SCIENCES

READER/SENIOR LECTURER IN BIOCHEMICAL ENDOCRINOLOGY

Applications are invited from suitably qualified biochemists preferably with a medical qualification, to be responsible for the co-ordination and furtherance of hormonal and intestinal physiology research under the general direction of the Head of the Department. The successful applicant will also be expected to lecture in animal biochemistry and general physiology.

SALARY: £2,930 by £115 to £3,390 by £140 to £3,670 per annum.

Further details and application forms may be obtained from the Staff Officer (Ref. No. 734/6), the University of Aston in Birmingham, Gosta Green, Birmingham 4, to whom applications should be forwarded within 14 days of the appearance of this advertisement. (542)

UNIVERSITY OF LONDON

BRITISH POSTGRADUATE MEDICAL FEDERATION, INSTITUTE OF LARYNGOLOGY AND OTOLOGY
330/336 Gray's Inn Road, W.C.1

BIO-ACOUSTICAL INVESTIGATION

There is a vacancy for a graduate research assistant which could be filled by a candidate preferably experienced in Experimental Psychology, Experimental Physiology or other appropriate field. Salary £1,435 to £1,665.

Application to: The Secretary-Administrator, Institute of Laryngology and Otology, 330/336 Gray's Inn Road, W.C.1. Closing date February 14 1970. (304)

NATURE

MACMILLAN (JOURNALS) LTD

No. 5233 SATURDAY FEBRUARY 14 1970 Vol. 225

CONTENTS

Repentance Postponed on Polytechnics—What if they Forget to Pay the Piper ?—100 Years Ago 573

Old World—Imperial College—Computers—Pollution—Countryside—Aviation—Science Research Council—Education—Conservation—Ionosphere—Social Medicine—Conservation—Parliament in Britain—Miscellaneous Intelligence . 575

New World—Marking Time for 1971—Desert Research—Earthquakes—Sun. 582

News and Views—One Way for Neutron Stars to Pulse—What Sigma Factor Does—Polymerases—Invertebrates—Bacteria—Virology—Cancer—Ichthyology—Archaeology—Pulsars—Spectroscopy—Atomic Beams—Geophysics—Composite Materials 585

Problems of Expanding Populations by D. Wolfers 593

RNA Polymerase σ-Factor and the Selection of Initiation Site by M. Sugiura, T. Okamoto and M. Takanami 598

Inactivation of Prostaglandins by the Lungs by Priscilla J. Piper, J. R. Vane and J. H. Wyllie 600

Novel Correlations between Global Features of the Earth's Gravitational and Magnetic Fields by R. Hide and S. R. C. Malin 605

Abundance of Iron in the Solar Photosphere by John E. Ross 610

Possible Mechanism for the Pulsar Radio Emission by M. M. Komesaroff . 612

Observations of Atmospheric Turbulence with a Radio Telescope at 5 GHz by R. A. Hinder 614

Letters to the Editor (see overleaf) 618

Book Reviews by F. F. Roxborough, F. A. B. Ward, A. R. Robbins, J. R. L. Allen, John R. V. Brooks, A. F. G. Wyatt, M. H. Choudhury, P. L. Cloot and R. T. Williams 665

Obituaries—Professor Max Born 669

Correspondence—X-Ray Stars and Infrared Excess by Robert F. O'Connell and Satya Dev Verma; Diffusion in Embryogenesis by Elizabeth M. Deuchar . . 671

Nature in North America: The *Nature* office in Washington DC has opened and contributors in the United States and Canada are invited to submit manuscripts for publication either to the address given below or to the present London office. The intention is that each office will be able to answer inquiries about all manuscripts and other matters. This is one of several steps by which it is hoped to provide a better service for readers and contributors.

London Office
4 Little Essex Street,
London WC2.
01-826-6633

Washington Office
711 National Press Building,
Washington DC 20004

(Continued overleaf)

And that's exactly what Pye Unicam have done with the world-famous Unicam SP90 Atomic Absorption Spectrophotometer. It's now the Unicam SP90 Series 2, but it's not just the name that has changed. Absorbance or energy scales can now be presented in linear form on the integral meter or on a pen recorder, with continuous scale expansion of up to 10x for absorbance. Results can also now be shown on a digital display and printout accessory. The sensitivity specification has been increased as a result of improved hollow cathode lamps and excellent optical quality, whilst still preserving the outstanding detection limits. And, of course, there are all the first-class features and accessories associated with the original SP90 spectrophotometer–like flame emission facilities, a wide choice of flame and burner systems (including nitrous oxide with a new burner head), a three-lamp turret and automatic sampling. For more information and full technical details on this new instrument and the range of hollow cathode lamps, write to Pye Unicam Ltd.

PYE UNICAM

1966 1969
THE QUEEN'S AWARD TO INDUSTRY

Precision Instruments

Pye Unicam Ltd
York Street Cambridge, CB1 2PX, England
Telephone (0223) 58866 Telex 81215

LETTERS TO THE EDITOR

PHYSICAL SCIENCES

Page

Radio astronomy—Level populations of excited states of hydrogen—BROCKLEHURST (London) 618

Neutron stars—Long period modulation of oscillations—RUDERMAN (Columbia) 619

Quasi-stellar objects—Conclusion of Plagemann *et al.* verified—DEEMING (Texas) 620

NGC 1068—Explanation of optical polarization in the nucleus—NANDY (Royal Observatory, Edinburgh) and WOLSTENCROFT (Hawaii) 621

Cassiopeia A—Absorption spectrum and the inter-arm medium—DE JAGER (Jodrell Bank) 622

Moon—Thermal history—GILVARRY (General Dynamics) 623

Mineralogy—Silicon tetrahedral sheet in stilpnomelane—EGGLETON (Australian National) 625

Crystallography—Structures of alginic acids—ATKINS, MACKIE and SMOLKO (Leeds) 626

Creep—Stored energy in graphite deformed by creep—GREEN and ZUKAS (California) 628

Carbon films—Catalytic oxidation of SO_2 and electrical resistance—McLINTOCK and ORR (Dundee) 629

Lubrication—How iodine might act as a boundary lubricant—FUREY (Virginia Polytechnic Institute) 630

Organic chemistry—Charge transfer complexes of tropylium and N-heterocyclic cations with aromatic amines—BEAUMONT and DAVIS (Leicester) 632

Reaction mechanisms—Effects of non-equilibrium *p*H conditions in the radiolysis of aqueous systems—FIELDEN, STEVENS (Institute of Cancer Research), PHILLIPS, SCHOLES (Newcastle upon Tyne) and WILLSON (Mount Vernon Hospital) 632

Textiles—Radiation grafting of polysulphides and wool—SAMWAYS and HASTINGS (New South Wales) 634

Piezoelectricity—Resonances in crystals of many L-amino-acids—VASILESCU, CORNILLON and MALLET (Nice) 636

Communication—An experiment—DAVIES, McKENZIE and TURNER (Cambridge) 636

BIOLOGICAL SCIENCES

Prostaglandins—An experiment—PICKLES (Cardiff) 636

Visual pigment—Amended values of molar absorptivity and quantum yield—HELLER (California) 636

—Linkage of retinal to opsin and absence of phospholipids—HALL and BACHARACH (California) 637

Ion transport—Depletion of intracellular Ca^{2+} and the retention of K^+—VAN ROSSUM (Pennsylvania) 638

Collagen models—New conformational data from NMR study of deuterated molecule—CHAPMAN, CAMPBELL and McLAUCHLAN (Oxford) 639

γ-Globulins—Anti-Rh activity of human γG4 subclass—FRAME, MOLLISON (London) and TERRY (US National Institutes of Health) 641

Page

Evolutionary biochemistry—Identity of human and chimpanzee fibrinopeptides—DOOLITTLE and MROSS (California) 643

Isoenzymes—Separation by zonal centrifugation—SELF and WEITZMAN (Leicester) 644

Antigenic expression—Characteristics of lymphoma cells growing *in vitro*—CIKES (Karolinska Institutet) 645

Transplantation antigens—Occurrence in central nervous system—GERVAIS (London) 647

Enzyme induction—Neural regulation of hepatic tyrosine aminotransferase—BLACK (US National Institutes of Health) 648

Actinomycin-D—Effects on memory at different times after training—SQUIRE and BARONDES (Yeshiva) 649

Brain RNA—Synthesis in different areas after imprinting stimulus—ROSE (Open University), BATESON, HORN and HORN (Cambridge) 650

Psychopharmacology—*p*-Methoxyamphetamine not excreted in amphetamine induced psychosis—ANGRIST, SCHWIETZER, FRIEDHOFF and GERSHON (New York) 651

L-Asparaginase—Inhibition of DNA synthesis in rat liver—SEEBER and WESER (Tübingen) 652

Anaesthetics—Biochemistry of halothane induced malignant hyperpyrexia in pigs—BERMAN, HARRISON, BULL and KENCH (CSIR and Cape Town) 653

Macrophages—Antimacrophage serum promotes development to giant cells—PTAK, PORWIT-BOBR and CHLAP (Cracow) 655

Regulation of mitosis—Effects of antibodies against kidney chalone—CHOPRA and SIMNETT (Newcastle upon Tyne) 657

Biochemical analysis—Absolute and unambiguous method for complex extracts—MAJER (Birmingham) and BOULTON (Saskatchewan) 658

Pterosaurs—Take-off and landing by *Pteranodon* were easy after all—BRAMWELL and WHITFIELD (Reading) 660

Millipedes—Walls of egg capsules in two species do not contain antimicrobial or antifungal substances—EISNER, ZAHLER, CARREL, BROWN (Cornell) and LONES (US National Institutes of Health) 661

Mutualism—Sterol metabolism as basis for mutualistic symbiosis—KOK, NORRIS and CHU (Wisconsin) 661

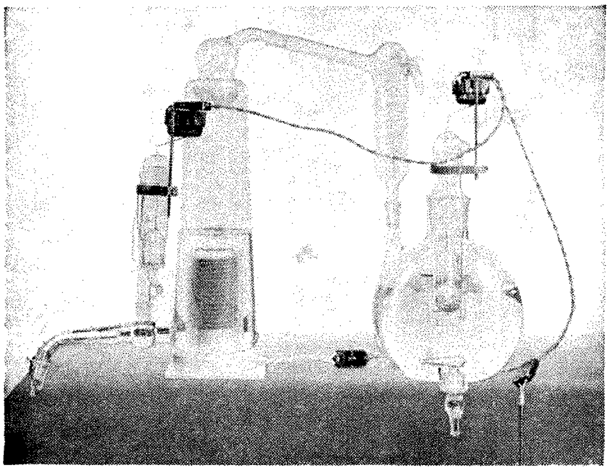
Entomology—Hereditary sex ratio, favouring females, in the butterfly *Acraea encedon*—OWEN (Sierra Leone) 662

Ipomoea seeds—Identity of *Kaladana*—ABOU-CHAAR (Beirut) 663

Horticulture—Strawberry pips help calculate potential yield—ABBOTT and WEBB (Bristol) 663

Soil—Non-biological reduction of nitrite—BULLA, GILMOUR and BOLLEN (Oregon State) 664

The titles on this page are prepared by the staff of *Nature* on the basis of the titles submitted by authors which appear in full on the pages indicated



This unique water still is in use in a number of research establishments and hospitals where water of the highest purity is essential.

PRICE, complete with heater and all electrical equipment 230 volt (a.c. only)—**£45-0-0**

Obtainable only from

L. V. D. SCORAH, M.Sc.

(Manufacturer of the "SCORAH" Blowpipe)

44 NORTHFIELD ROAD, KINGS NORTON,
BIRMINGHAM, 30. 021-458 1885

The SCORAH Automatic Electric all glass WATER STILL (patented)

Automatic Can be left on without attention. Heater current cuts off if water supply is interrupted, or inadequate or forgotten. When receiver (5 litres capacity) is full, current cuts off. When distilled water is drawn from receiver, the still resumes operation until receiver is refilled.

Purity Electrostatically charged condenser droplets can pick up fumes, dust and air-borne organisms from the laboratory atmosphere. The design of the receiver vessel ensures absence of such contamination.

Efficiency Heater of Scorah design and manufacture, consumption 1,700 watts, output 2.4 litres per hour (the full theoretical yield). Cost 3½d. per gallon at 1d. per unit.

Safety The heating element is out of contact with the feed water, thus eliminating danger from "live" water supply or electrolytic effects or "burn out".

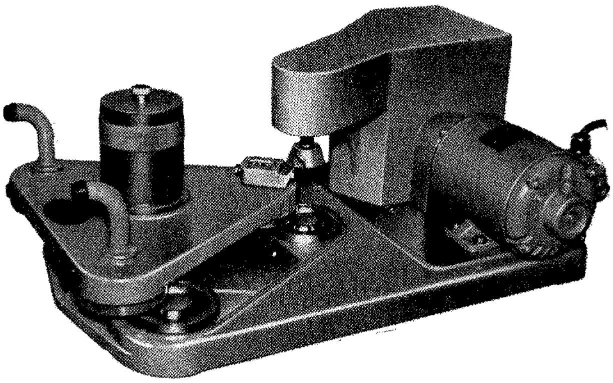
Assembly No retort stands or clamps are required. The 2½-in. diameter ball joint gives a strainless set-up. Outlet cap on boiler for quick periodical drain-off of sediment. The still may be installed on a shelf or platform about 1 ft. above bench level near a sink and enclosed in a transparent plastic cover to keep out dust.

Phoenix heat-resisting glass. Interchangeable ground glass joints.

CUTROCK

CROFT PARALLEL GRINDING MACHINE (MOTOR DRIVEN)

For precision grinding of thin sections and fossils



The travelling carriage of the machine moves in a circular track over the grinding plate controlled by steel balls running in precision machined tracks. Mounted in the centre of the carriage is a metric micrometer which houses the specimen holder. When the carriage is in the grinding position as illustrated, the micrometer can be adjusted until the specimen is in contact with the grinding plate, at this point the reading should be taken and then the amount to be ground off should be set on the micrometer. The specimen holder will lift by this amount and as the machine grinds the holder will return to its setting position, which once reached will stop any further grinding of the specimen even if the machine continues to run.

Grinding plates can be supplied in zinc, copper, cast iron, glass or lead.

CUTROCK ENGINEERING CO., LTD.

Telephone: 01-346–5978/9 35 BALLARDS LANE LONDON N3

NATURE Volume 225 FEBRUARY 14, 1970

Repentance Postponed on Polytechnics

One of the conventions of British public life is that a reshuffling of ministerial posts in the government can be decently regarded as an opportunity for making small adjustments of policy. A new minister, like a new broom, can for a time act a little more freely than his predecessor, and can in the process jettison or at least modify policies which may have become less popular with the passage of time. All this is widely accepted, which is why it is a great misfortune that Mr Gerald Fowler, the new minister of state at the Department of Education and Science, has this week nailed his colours to the rickety mast of the policy on polytechnics and their place in higher education which has been evolved or at least accreted in the past few years. At the same time, in a recent article in the *Guardian*, Mr Fowler went a long way to harden the gulf which now divides the British system of higher education into two discordant parts. The way things are going, there is a serious danger of permanent damage to the system as a whole.

A convenient text for this assertion is Mr Fowler's definition of the distinction between universities and polytechnics. He says the two kinds of institutions will be "sharply differentiated", and that polytechnics will be recognized by the diversity of their student body as well as by the diversity of the courses which they offer. Mr Fowler goes on to express his hopes that the thirty polytechnics—fourteen have so far been established—will "undertake sponsored research projects" for industrial companies, although he does not think that research will ever play a very large part in the work of a polytechnic. Rather, he says, the need is to work out ways of providing a more educative and humane foundation for vocational courses of the kind which have been the traditional bread and butter of the technical colleges. Although Mr Fowler insists that he does not want "to see a gap develop" between the polytechnics and the universities, his vision of collaboration between them seems to consist of the exchange of staff or of facilities where one institution is able usefully to complement the work of the other—a valuable enough task in all conscience, but one that is hardly likely to flourish except in exceptional circumstances.

The trouble with all this is not merely that there will inevitably grow up what Mr Fowler calls a gap between the universities and the polytechnics, but that the doctrine of the binary system will project into higher education some of the inconsistencies which have beggared British secondary education in the past few decades. How, for example, will students choose between universities and polytechnics? Will they always have a free choice, and what are the circumstances in which young men and women able to find a place for themselves at a university will voluntarily settle for a polytechnic? With the best will in the world, it is exceedingly hard to see how the innate attractions of the universities, with their cosy staff to student ratios, their facilities and their opportunities for research, will fail to skim the cream off the available supply of talent. The result, for a time at least, may well be that the student population in the polytechnics is not nearly as diverse as Mr Fowler hopes but, rather, that it will consist of those bright young men and women who have tried but failed to win a university place for themselves. It is in the circumstances a little too pious of Mr Fowler to insist that neither he nor the government will regard the polytechnics as second-class institutions—what matters, alas, is not even the truth but what the customers think. Under the present arrangements, there is a serious danger that the polytechnics will fail to attract the students and the staff which are essential to Mr Fowler's idyll.

If the new minister has not seized the chance to turn his back on these and other defects of the new proposals, what can be done? It may not be entirely outrageous to suggest that it would be possible for the universities and the polytechnics themselves to take the initiative. The objects should be plain—the integration of the divided system. Ultimately, this implies that there should be "comprehensive universities" along the lines which seem to attract the present government, but they should be built around the present universities and not separately from them. There is no reason why groups of institutions should not already make a start. The way in which the University of Manchester and the one-time Technical College (now the University of Manchester Institute of Science and Technology) have made a durable marriage is a good precedent which at the same time reveals how flexibility within the university system is limited not so much by the conventions of the governing bodies as by the rigidity of the academic departments. In the long run, the best way of pulling Mr Fowler's chestnuts from the fire will be some enterprising marriage or at least liaison between a traditional university and a polytechnic. The sooner, for everybody's sake, the better. •

What if they Forget to Pay the Piper?

THE newest budget from the United States Administration may be an intellectual masterpiece by combining acumen with information, but it is also a sombre document, full of evidence that people are now accustomed to living in a world in which expenditure on science and technology stagnates. It is true that nobody will miss the full flower of Plowshare, and that there may even be a kind of logic in the decision of the Atomic Energy Commission to finance the 200 GeV accelerator in Illinois at the expense of older and presumably duller projects, but the question now urgently arises whether it is really wise that the amount of money spent on uncommitted science and technology, and especially on basic research, should remain the same in the seventies as it became in the sixties. On the face of things, there is good reason for fearing that such a state of affairs will bring only trouble. More distantly, there is a serious risk that the continuing drain on the ebullience of the scientific community now in prospect will eventually bring about a condition in which industrialized society as a whole, not just the American, will languish.

This is why it is first important to count the blessings which the budget for 1971 contains. To begin with, there is the problem. For one thing, if Mr Nixon's people have their way with Congress, the National Science Foundation will have more to spend. That is as it should be, even if the increase is accompanied by a decrease in the amount of money for basic research provided by the other agencies—the Pentagon's Project Themis has now sunk without trace, and there will be very little surprise that it has now become necessary for NASA to give up its traditional but diminishing request for money with which to support university research. Putting the responsibility for the support of basic research with the National Science Foundation is the right strategy, and doubtless it is no accident that the NSF has already begun to respond to the commendation by the National Science Board (the NSF's board of trustees) of a pattern of research financing in which good institutions are picked out for particular rewards. Yet there is a long way to go before the NSF can properly acknowledge that a large part of its responsibility is the support not merely of scientific research but of the institutions in which it is carried out. Before that can happen, Congress will have to acknowledge more openly that public money can be legitimately spent thus. A more radical change of pattern will only be possible when the NSF has more power to its elbow and more money in its purse.

In the meantime, the signs and symptoms of penury are plain for all to see. One important change has been the way in which many of the agencies have been forced to spend a greater proportion of their funds in their own laboratories. Among the obvious dangers of such a course are those of creating institutions which are less able to change with the times that the changing needs of scientific research require. American institutions may have been especially able to change their ways in the past few years, but there are limits to what is possible in the face of adversity. A second and more serious problem is the way the new pressures may shift the initiative in research from the universities to the institutions. This is not so much a matter of scale as of temperament—a great many potential researchers may persuade themselves that life in a research institute may be preferable to life in a university when research is not considered to be an integral part of university life. But there is also the problem of scale. Nobody would find it easy to show in precisely what ways the generous spending on research and development in the decades since the Second World War has contributed to the present economic strength of the United States, but it would be a great surprise if the correlation was negative or even small.

But even economic strength is only a small term in the equation. The much more serious danger is that too much parsimony for too long may rob research of its capacity to sustain itself. The steam may condense in the cylinders. It may be too gloomy by half to fear that the comparatively gentle restrictions of the new budget could bring about such a tragedy. Yet it is also well known that modern science is substantially an invention of the past few decades. The budget men should sometimes ask themselves how much restraint for how long will be necessary to make it disappear all over again.

---

100 Years Ago

THE 25-inch Equatorial Telescope, commenced several years ago by T. Cooke and Sons, of York, for R. S. Newall, Esq., of Gateshead, is now so far completed that it has been removed from the works at York into its observatory in Mr. Newall's grounds, at Fern Deal.

The completion of a telescope with an object glass of 25 inches aperture, marks an epoch in astronomy, and its completion in England again places us in the front rank in the matter of the optical art, as we were in Dolland's time.

The history of the progress of the manufacture of telescopes since the time referred to, shows very clearly the long-lasting effects of bad legislation; for it is not too much to say that the duty on glass entirely stifled, if indeed it did not kill, the optical art in England. Hence we depended for many years upon France and Germany for our telescopes to such an extent indeed that the largest object-glasses at Greenwich, Oxford, and Cambridge are all of foreign make.

From Nature, **1**, 409, *February* 17, 1870. *The instrument, described as a "monster among telescopes", sported a cigar-shaped tube* 32 *feet in length and weighed nearly* 9 *tons.*

OLD WORLD

IMPERIAL COLLEGE

Architecture of Discontent

After six years of negotiations, Imperial College, London, has unilaterally called off its plans for a merger with the School of the Architectural Association. Lord Penney, rector of Imperial College, has said on behalf of the governors that the basis for a merger no longer exists but Mr John Lloyd, the principal of the school, claims that the governors acted with "a total lack of good faith" and says that he cannot approach Imperial College again. Talks had continued until the last moment, and the break seems to have been a complete surprise; the University Grants Committee had approved the merger, which was to be dependent on the raising of £500,000 by the Architectural Association for new buildings. The lease on the association's present accommodation expires in 1976.

Two explanations for the sudden decision are being aired. The first, and most widely favoured, turns on the question of student power. The school of the Architectural Association (AA) has a tradition of liberal student representation, and was founded by students in 1847. Further, it appoints staff only for short periods and does not provide the life tenure that a university would give. Both of these points go against the grain at Imperial College, where the students are already seeking more involvement in administration. The second view is that the decision was forced by an anti-interdisciplinary lobby, and the idea of a conflict between architectural and engineering interests was even raised in a letter to the *Times* (February 9) by Professor Colin Buchanan of the department of civil engineering at Imperial College.

The AA itself apparently gives priority to the first explanation, and its council resolved on Monday night that there was, in its opinion, "a fundamental conflict of educational objectives". So, too, do the students at Imperial College, according to the union president, Mr Piers Corbyn. On Tuesday more than five hundred of them, accompanied by members of the staff and some students from the AA, left a wreath at Lord Penney's home and a message in his office.

One of the AA's main objectives at the moment is to guarantee the future of its students, many of whom take five-year courses. Because its lease ends in 1976 it cannot yet offer a complete course to prospective students for this autumn. Since the school is privately owned and cannot afford to continue beyond then without outside help, it seems that its future probably lies within the state system. Discussions on Monday with Mr Gerald Fowler, Minister of State at the Department of Education and Science, were said to be encouraging, and the immediate aim has become the investigation of "every possible alternative" now that the Imperial College plans have fallen through. Meanwhile the AA's president, Miss Jane Drew, who is on a fund-raising tour in the United States, has an even more urgent task ahead of her.

In the midst of the debacle the governing body of Imperial College has published its report for 1968–69, which was completed too soon to say anything about the architectural crisis beyond noting simply that there was agreement in principle ever the merger. But other problems are revealed. The appeal for £2 million, launched last January to bring in funds for building and research (see *Nature*, **221**, 118; 1969), has made "steady, if disappointing progress", reaching £400,000 by July 31, but with a better response from city financiers than from industry. Nearly all the money that has come in so far has been spent on providing or converting student houses. In spite of a forecast deficit, however, the college managed to reach a surplus of £62,691 by the end of the year, by enforcing a 6 per cent cut in expenditure. Present estimates for 1969–70 suggest a likely deficit of £50,000, mainly because of salary increases, and more cuts may be needed next year.

During the session there were 3,792 full time students, some 1,200 short of the figure that the University Grants Committee thinks the college can take when its site is fully developed. How this growth is to be directed over the coming decade is now occupying a steering committee under the chairmanship of the pro rector, Lord Jackson. The committee is asking the college's departments how they intend to expand, and hopes to report during the first half of 1970 on what might be done.

COMPUTERS

ICL at the Select Committee

The spectre of IBM haunted the room at Westminster where the Select Committee on Science and Technology was taking evidence from International Computers Ltd last week, and it will no doubt loom over the rest of the hearings on the British computer industry. The managing director of ICL, Mr Arthur Humphreys, put his company's plight in a nutshell: "There is no problem in the computer business which would not be solved by the demise of IBM". But IBM cannot be exorcised by wishful thinking, and the subcommittee appointed by the Select Committee to carry out the investigation under the chairmanship of Mr Airey Neave wanted to know if anything more concrete could be done. Mr Humphreys and his colleagues made plain their feeling that the British Government could do more to help. For example, American computers have been ordered for the Army Pay Corps and for the Home Office crime-fighting system, and this was affecting the credibility of the ICL System-4 in the market place. It is proving difficult to sell System-4—teething troubles have not helped—and most of the company's business will continue to come from the 1900 series. Another problem is the rapid turnover of senior staff, and with the forward-looking orders going to the United States there is not enough of the advanced work which encourages people to stay.

Little progress is being made in the talks on collaboration with Europe as a way of spreading the competition with IBM, Mr Eric Moonman of the subcommittee pointed out, and he wondered whether the Industrial Reorganization Corporation or the government could do anything to help. This was clearly a sensitive point, chiefly because the Aigrain proposal to cooperate on a powerful computer was the only proposal set up by industry rather than by civil servants, and pride was forcing the industry to make another effort to get the project moving before calling for help.

Mr Basil de Ferranti of ICL thought that there was "an enormous potential for success" in European collaboration, but failure would be damaging.

The subcommittee was critical of the confidential memorandum submitted by ICL, and questioned the basis for the market forecasts which it contained and why it made no mention of markets outside the United States and Europe—such as Japan, Mr Moonman said. Mr Humphreys had a sad tale of how the Japanese market is denied to ICL by the fearsome trinity of intense competition, high import duties and the quota system. Another difficult market is eastern Europe, where the orders rather awkwardly tend to be for equipment which is outside the embargo rules. Mr Humphreys thought that ICL was being used to get the embargo system lifted.

The success of IBM was attributed by Mr Humphreys to the ready-made market which they had from their punched card business, and to the purchasing policy of the US Government. IBM has had orders for large batches of particular computer models from the US Government, but ICL had never been asked for more than four of a particular type of computer at once. This was the secret of IBM's success, and not their research expenditure, which was not extraordinary.

The ICL representatives went away impressed by the subcommittee's knowledge of the computer industry—the computer appreciation course at Imperial College which the members attended obviously paid off—but few ideas were forthcoming from the ICL side of what measures the government could reasonably take to help.

COMPUTERS

How IBM Forged Ahead

SOME essential reading for Mr Airey Neave's subcommittee (see page 575) has just been published by the OECD in the form of a survey of the computer industry in OECD countries—the latest in the remorseless series on "Gaps in Technology" which examine the disparity between the United States and the rest (available from HMSO, 21*s*). What it boils down to, of course, is why IBM is so far ahead. In most European countries, according to the report, IBM was responsible for between 60 and 70 per cent of the value of computers installed in 1967 (an exception was Britain, where ICL brought IBM's share down to 39 per cent), and in the United States IBM has a similar dominant position over other US manufacturers.

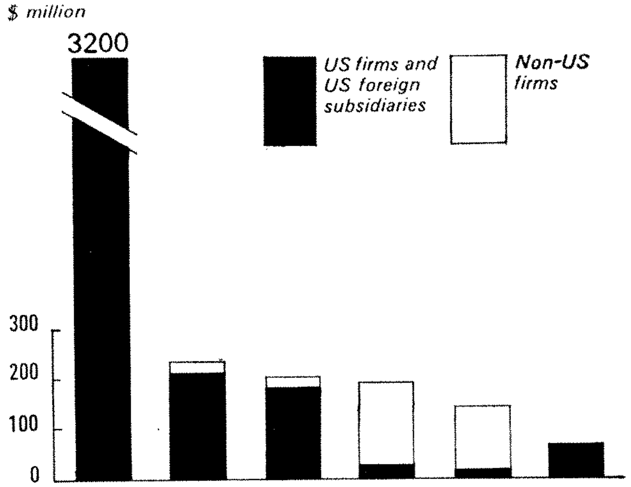
The investigation of the British computer industry now taking place makes the OECD more topical than it deserves to be—the report was prepared for the meeting of science ministers of OECD countries in March 1968. This means that the statistics which are the chief virtue of the report are out of date, which is a pity because the group of experts responsible obviously went to great trouble to make accurate statistics out of meagre data supplied by member countries, and one of their grumbles is that governments ought to have a better idea of what is going on in their countries.

What the report shows is that the pre-eminence of the US computer industry was not due to a deliberate policy on the part of the government, a lesson which the subcommittee will no doubt take to heart. Rather it has been the existence of the large government market, and the large amount of government financing of R and D work compared with other countries which has stimulated the industry. But IBM seems to have had less than its fair share, and one definite policy which the report mentions is that companies other than IBM are preferred for government contracts so as to stimulate smaller companies. The IBM share of the Department of Defense market, for example, is less than its share of the US computer market as a whole, and when IBM computers are used they tend to be leased (in common with the computers of other large companies such as NCR and GE) rather than bought. Yet these factors seem not to have harmed IBM, and the moral which the report points to is that successful companies are those with products which suit the business market rather than the scientific or military market.

This is where Britain missed the bus, and the report criticizes the failure to sell the LEO I computer which remained one of the first machines designed for business uses until five years after it was completed. This was the computer designed for the Lyons food chain by the Lyons Electronic Office in cooperation with Cambridge University. The report also raps the UKAEA and the Treasury for their unencouraging attitude, but praises the NRDC for ordering some of Ferranti's early computers.

It is clear that the strong connexions in the office equipment field which predated the entry of IBM into the computer business are not the whole explanation of IBM's success—the ill-fated French firm Bull had a marketing organization which was equivalent to that of IBM. Neither was IBM first into the market, and here it was beaten by Remington Rand with Univac I in 1951. Rather the report attributes the success of IBM to three factors: (1) an appreciation of what the customers wanted, in particular the importance of software; (2) the ability to stimulate the market by developing new products and helping customers; and (3) good internal organization.

What can be done in the face of US competition? One approach is to concentrate on particular aspects of the computer business, and the report mentions process control by computer, and the manufacture of



The world computer industry in 1966. Estimated shipments in the main computer producing countries, from *The OECD Observer*.

terminal equipment for time-sharing systems as areas which are wide open. Although "the example of the United States also shows that a clearly delineated policy is not a prerequisite to successful support on the part of the government", the report says that governments can help to produce the conditions for a flourishing industry. For example, governments can help by encouraging education in the computer sciences, by supporting new and fast growing areas of the industry, and by improving the communications network (chiefly the telephone system) so that the development of time-sharing computers will not be hampered by expensive and unreliable communications.

POLLUTION

Sewage Works are Inadequate

Environmental pollution is fast becoming the talking point of the British Establishment. Only five days before the Duke of Edinburgh spoke at the European Conservation Conference in Strasbourg, the House of Lords debated the pollution of British rivers. Lord Kennet, Parliamentary Secretary to the Ministry of Housing and Local Government, gave a comprehensive survey of the inadequacies of sewage treatment in Britain and outlined government thinking on new measures of control, although, in the absence of a comprehensive survey of the situation, few concrete proposals emerged.

Three out of every five local authority sewage works are producing effluent which is below standard and this, said Lord Kennet, is only part of the story. We do not know how much effluent is being poured directly into British rivers by industry, nor just how dirty our rivers are. Before the government can impose new regulations for sewage disposal, he said, a complete survey of British rivers must be carried out, and this is at present being undertaken by all the river authorities. Preliminary results of the survey, which should be available in June, will give some indication of changes in the condition of the rivers since 1958, but until then, "action taken before ignorance is dispelled will be capricious action".

Some hints of the government's intentions on new regulations were, however, given by Lord Kennet. Penalties for discharging insufficiently treated sewage need to be brought to a more realistic level, he said, because many firms find it cheaper to risk being fined than to spend the substantial sums needed to meet the standards demanded by the river authorities. The government is also looking into the suggestion that pollution of rivers should be an absolute offence, whether caused knowingly or not.

Lord Molson, who opened the debate, accused the government of soft pedalling on river pollution. In 1966, borough treasurers stated that 60 per cent of the effluent being poured into rivers failed to comply with standards laid down by river authorities, but these standards are difficult to enforce. First, sewage authorities may appeal to the Minister of Housing and Local Government that standards are unreasonable, and Lord Molson accused the government of showing a bias towards the sewage authorities. A circular sent by the Minister of Housing to the river authorities in 1968 pointed out that standards laid down for industrial effluents must be justified, whether they are unusually stringent or not. Second, river authorities have no right of inspection of sewage works and, third, Lord Molson said that the fines for river pollution are "hopelessly inadequate".

Lord Molson said that the cost of sewage treatment, which is running at about £100 million a year, should be increased to about £300 million, and he indicated that this could be done by transferring money from housing, roads and so on. Such a step would go far towards meeting water requirements for the end of the century and would be more desirable than submerging additional areas of the Peak District to provide pure water.

One reason why sewage works are outdated, and river pollution is not being tackled fast enough, was suggested by Lord Nugent, chairman of the Thames Conservancy. There are no votes in sewage, he said, and local authorities are reluctant to spend ratepayers' money on sewage works when there are more attractive projects to be undertaken.

COUNTRYSIDE

Cars Curbed in Peak District

by our Planning Correspondent

For the first time in Britain, there is to be an attempt this summer to control weekend traffic in a beauty spot in a national park. Along a four mile stretch of road in the Goyt Valley in the Peak District National Park, motorists, other than those with permits, will be banned from using their cars at summer weekends and on public holidays from July to September this year and from Whitsuntide to September in 1971. There will, instead, be four new car parks at strategic points for up to 300 cars, marshalling arrangements and a mini-bus service to take people to picnic sites and nature trails. Walkers, cyclists, pony trekkers and people who need access to the land in the area will be allowed through the traffic free zone.

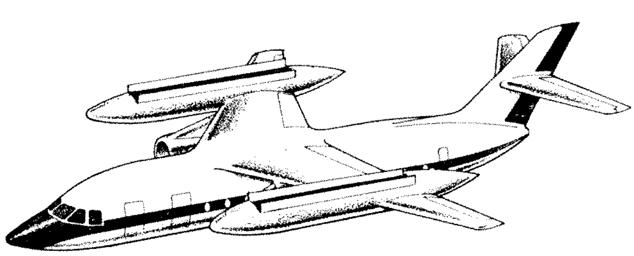
The experiment, which will last for two years and which has yet to be finally approved, is being organized by the Countryside Commission in cooperation with the Peak Park Planning Board using new powers now possible under the Road Traffic Regulation Act of 1967 and the Countryside Act of 1968. The scheme will cost the commission about £17,500 over the two years, but it is hoped that the motorist, by accepting some restrictions on the free use of his car in the Goyt Valley, will be able to enjoy the tranquillity of the countryside which he is at present destroying. The experiment, although on a small scale, is important because it could be the forerunner of similar schemes in parts of other national parks in Britain which are under heavy pressure from motor cars at certain times of the year. Parts of the Lake District National Park, for example, badly need some sort of traffic management in the summer months. But, according to the Friends of the Lake District, it is not just the weekend traffic that is threatening the beauty of the Lake District and the enjoyment of the majority of its visitors. In their latest newsletter the Friends describe the threats of new road works, increasing numbers of caravans, powerboats on the lakes, abandoned motor cars, and the removal of water-worn rock from attractive limestone areas in and around the Lake District. The Friends are particularly opposed to the route and scale of the proposed new road to by-pass

Ambleside in the heart of the national park. Apart from the incongrousness of such a road which would be dual carriageway in parts, the Friends feel that it may become unnecessary when heavy goods traffic and motorists bound for Scotland start to use the new M6 motorway extension which will by-pass the Lake District.

AVIATION

Taking Off for the Eighties

By the end of the year the Ministry of Technology should be in a position to decide what encouragement to give to the proposed types of vertical take-off and landing (VTOL) aircraft designed for inter-city links on a European scale for the 1980s. About £50,000 has already been spent by the ministry on studies of various aspects of future VTOL civil operations and rather more is likely to be spent in the current year. A lot more than the flying craft is involved in adopting this system of getting from A to B. Where are the vertical landing craft to land in city centres ? Will the noise they make doing it be acceptable in a built-up area ? Will the air traffic control features be compatible with control procedures for the conventional aircraft which they will supplement rather than replace ? Will the passenger demand for faster inter-city travel be sufficient to justify not only the high cost of development but the higher fares predicted for the service ? Definition on all these points is being sought in parallel with ministry assessment of three design submissions of craft from industry. Hawker Siddeley Aviation, Westland Helicopters, Ltd, and the British Aircraft Corporation were invited to present their ideas, and reports several inches thick which were delivered to the ministry at the turn of the year are still under study.



Hawker Siddeley Aviation fan-lift VTOL configuration. Eight lift fans are carried in each wing pod for vertical flight.

At the completion of this process it would be expected that one or more of the more promising design proposals would be subsidized by MinTech to the point of detailed feasibility. A decision on whether to go ahead on the system as part of a total travel network for Europe for the 1980s is not required till 1971. Possibly the most critical factor is the actual supply of suitable sites in city centres. A metropolitan VTOL port requires a minimum area 1,000 metres in diameter which is free of noise-sensitive institutions such as schools and has good ground transport connexions. Such sites do not hang about on the market long. London is a particular problem. Surrey Docks has just joined the two other possible candidates—Kings Cross goods yard and Nine Elms (already earmarked for the new Covent Garden). But will any of these still be available when MinTech comes to take decisions in maybe 18 months time ? It is pointed out that it is no part of MinTech's task to implement a VTOL inter-city service and that no commitments have yet been made.

The aircraft proposals divide into two categories—rotorcraft and fan-lift aeroplanes. The Westland proposal employs the same power units for lift and forward flight, namely rotors that are turned through 90° by a tilting wing. One of the Hawker Siddeley proposals also employs rotors—the unconventional controlled circulation rotor originally developed by the National Gas Turbine Establishment, Pyestock. Two of these would be used for the lift phase only; they are folded and stowed for forward flight and this is the most problematic aspect of the design. This configuration would be expected to be faster than the tilt rotor craft and to have a more modern image.

Hawker Siddeley has studied fan-lift for 10 years and makes use of it in what appears to be the most attractive of the proposals put forward. The fan-lift engine is a specialized form of jet engine in which the axis is at right angles to the direction of flight and so provides thrust for vertical ascent. As with the company's other submission, separate power plants are employed for forward and vertical flight. Such an airliner might be faster than VTOL, has a very modern image but would have high maintenance cost and fuel consumption.

BAC has limited its proposals to various ways of shortening the take-off of its conventional aircraft, so that its submission is a STOL rather than a VTOL proposal. The airliners in view are all in the 100-passenger bracket.

At present, largely through Hawker Siddeley's development of the military VTOL, the Harrier, and Rolls-Royce work on specialized "lift" engines, Britain is considered to have a respectable world lead in VTOL. But there is much activity in both France and Germany, and there would seem an excellent argument for joint European development here. The weakest aspect of MinTech's sensible "phased approach" strategy is that little headway has been made in discussion with our European neighbours.

Meantime, as the results of the half dozen or so background studies come in, a valuable body of facts previously unavailable is being built up. They will be of interest much beyond the immediate VTOL terms of reference, particularly those carried out at Southampton and Leeds Universities on traffic patterns and passenger attitudes. Altogether 30,000 individual journeys have been analysed by Southampton and 10,000 questionnaires collected by Leeds. Neither report has yet been published.

SCIENCE RESEARCH COUNCIL

Equality for Engineering

By creating separate engineering and science boards the Science Research Council had set engineering on a manifestly equal footing with science, Professor Sir Brian Flowers told the Institution of Electrical Engineers in London last week. Moreover, by placing the interdisciplinary sciences of metallurgy, computing science, polymer science and materials under the aegis of the Engineering Board, the Science Research Council had clearly indicated which way it wanted university research to develop.

As an example of the SRC's policy towards the growing number of interdisciplinary activities, Professor Flowers explained how control engineering had been concentrated into three main centres, at Cambridge, Imperial College and the Manchester Institute of Technology, each with extensive computing facilities. The SRC had invested more than half a million pounds in the Cambridge control group alone, where industrial applications have included a model of a hot steel strip rolling mill and the control of once through boilers required in the nuclear power field. Computing science is one of the major emerging disciplines where the SRC is spending more than £2 million, with major groups centred in Edinburgh, Manchester and Cambridge.

The SRC was also increasing its interest in social problems, Professor Flowers said. On the study of transport systems, he hinted that with the findings of the SRC review panel at present under discussion it was likely that the SRC would be able to double its present commitment of £280,000 in this field. The SRC would also participate in defining the role of the new transport and environment centre, recommended in Lady Sharp's recent report *The Men for the Job* and now accepted by the government. The deliberate exploitation of spin-off was another important factor in SRC policy, Sir Brian said, but despite the growing preoccupation with applied science the SRC had no intention of neglecting the pure sciences. He also hoped that pure mathematics would be resuscitated under the new Science Board.

Lord Bowden, replying to Professor Flowers, said that he greatly welcomed the SRC's new posture on engineering. He could never understand the logic of the Haldane Report of 1918, for so long the bible of the research councils, in which the control of research was vested in those who had no interest in the outcome. "It's like entrusting the direction of Covent Garden to someone primarily interested in acoustics", he said.

He thought the idea of the University Grants Committee and the SRC working under different philosophies was a constructive one, with the UGC trying to spread grants evenly and SRC being selective according to merit. But pure science was still getting too much of the cake. He suggested that astronomy should be financed by the Arts Council as it satisfied the required test of giving considerable intellectual satisfaction to a tiny fraction of the community.

EDUCATION

Royal Society's Committee

The Royal Society has underlined its interest in education with two new initiatives. It has appointed a full-time education officer and set up a standing committee on education under the chairmanship of Professor C. C. Butler. Professor Butler, who was joint chairman of the working party of the Schools Council and the Standing Committee on University Entrance which put forward the controversial proposals for "Q" and "F" level examinations, has said that the new committee would be concerned with all levels of education. It will help to coordinate the work of the various joint committees of the Royal Society concerned with education, and will also look at specific problems itself.

Such a committee seems to be a logical development of the Royal Society's greatly expanded activities in education during the past few years. It already has joint committees with the Royal Institute of Chemistry, the Institute of Biology and the Institute of Physics, which are concerned with education in chemistry, biology and physics respectively. A joint committee of the Royal Society, the Council of Engineering Institutions and the Council of Science and Technology Institutes recently reported on the shortage of science and mathematics teachers in schools. Professor Butler said that the committee, which has met only once, has no specific problems under investigation as yet.

CONSERVATION

Environmental Revolution on Film

European Conservation Year has had a good week. While royalty and politicians were conferring in Strasbourg, Shell-Mex and BP Ltd was unveiling its contribution towards the education of the general public, a thirty minute colour film called *Environment in the Balance*, which had its premiere on February 11. This is the second film in a series on changing Britain and seeks to put the problems of today's environment in the context of Britain's history. The film modestly refrains from mentioning the battle against oil pollution. The figure, taken from the film, shows China clay spoil heaps in Cornwall.

IONOSPHERE

Omega off the Ground

from our Soviet Correspondent

On February 5, a new joint Franco-Soviet experiment was launched, designed to investigate near-space and the Earth's magnetic field, using a combination of drifting balloons and ground-level observations. This experiment, the first stage of the much publicized "Omega" project, is based on ground stations near Karpogor (Arkhangelsk province) in north Russia and the French base on the Kerguelen islands in the sub-antarctic Indian Ocean. Taking part in the experiments are specialists from the Soviet Institute of Terrestrial Magnetism, Ionosphere and Radio-waves, Institute of Terrestrial Physics, Polar Geophysical Institute, Leningrad and Gor'kii Universities and the French Centre National d'Études Spatiales, University of Toulouse (cosmic radiation centre), Centre National d'Études de Telecommunications, Institut d'Astrophysiques (Paris) and the Institut de Géophysique (Paris).

The programme of experiments calls for the launching of 37 high-altitude balloons, (27 French and 10 Russian) carrying loads of recording equipment of from 100 kg to 10 tonnes, over a period of 2 months. The polythene balloons themselves each weigh 250 kg.

The two stations lie at the ends of the same line of magnetic force, and by making simultaneous observations from both, it is hoped to investigate the variations of the radiation pattern in near space and the course of the entry of charged particles into the atmosphere. In particular, data are expected on the effect of the solar wind, and consequent magnetic storms, on the ionization of the upper atmosphere and the disruption of telegraphic and radio-telephone communications. It is hoped that these experiments will lead to practical improvements in spacecraft safety, and greater reliability of radio communications and navigation.

SOCIAL MEDICINE

Abortion Act in Action

THE Abortion Act is working better than either its opponents or its advocates hoped, according to a preliminary survey by P. Diggory, J. Peel and M. Potts (*Lancet*, i, 287; 1970). From the data for the first year and a half of its operation and estimates of the pattern of abortion over the past ten years, they conclude that the total of 65,000 legal abortions in England and Wales represents the continuation of a trend that has existed for at least a decade and that there has been no net increase in the number of abortions. There has instead been a switch from criminal abortions to therapeutic abortions under the National Health Service.

The act seems to have worked particularly well in ensuring minimum delays between seeking abortion and its performance; some 85 per cent of all terminations were carried out before the thirteenth week of pregnancy. Dr Peel believes that the proposed amendments to the act, which insist on the supervision by a doctor of consultant status, would inevitably increase the delays.

The abortion rate in Britain has been rising steadily from 6,000 in the first quarter since the act was implemented in April 1968 to 16,000 in the last quarter of 1969, but the rate of increase now seems to be levelling off. An encouraging sign is that the proportion of terminations carried out under the National Health Service has increased from 60 per cent of the total in the first quarter to 65 per cent in the final quarter. The regional discrepancies in the availability of National Health abortions also seem to be narrowing. In Sheffield, for instance, the abortion rate in the first half of the second year was 80 per cent greater than in the first year. In Liverpool the increase was 70 per cent, and even in Birmingham, where many doctors are opposed to abortion, there was an increase of 50 per cent. By far the greater proportion of all the private abortions, however, are in the north-west London area.

The chief contention, that there has been no net increase in abortions, is difficult to support statistically because there are no reliable figures for the number of criminal abortions performed before the act was passed. To support their argument, the authors show that the estimated increase of 6,000 in the total number of National Health Service and private abortions during 1967 was accompanied by a decrease of 2,000 in the total number of admissions for spontaneous and criminal abortions. On the assumption that only a quarter to a fifth of criminal abortions are admitted to hospital, it seems likely that most of what would have been criminal abortions were transferred to the private sector. Evidence for the rising trend in abortions in the years before the act comes from the increase in the number of National Health Service terminations from 2,000 in 1961 to 10,000 in 1967 and from Dr Diggory's estimates that the number of private therapeutic abortions has risen from 12,000 to 18,000 during the same period.

The act has been remarkably successful in reducing the death rate from abortion. The latest figures from the Ministry of Health show that in England and Wales the number of deaths from abortion, which has been between 45 and 50 per year for the past twenty or so years, dropped to 22 during 1969. It is estimated that, of these, three were due to private legal abortions, ten to National Health abortions and the remaining nine to criminal abortions.

Although the number of deaths from abortion is quite low, there is an urgent need for more research into the best techniques for abortion and into possible long term complications of the operation. But the chief point to emerge from the report is the need to improve contraceptive services. The survey has shown that "many women who have knowledge of and access to contraception fail to use it and yet are dismayed by the resultant pregnancy. . . . Diggory's series of 1,000 abortions included 18 women doctors, 99 nurses, 84 students and 29 teachers". Furthermore, the provisions of the 1967 Family Planning Act for contraceptive advice to be given to the unmarried have been fully implemented by only 39 out of 250 local authorities.

CONSERVATION

Environmental Postgraduates

HERIOT-WATT University has made a positive response to the growing interest in the environment. It has set up a one year postgraduate course leading to an MSc degree in environmental conservation. The course, which will be under the control of the joint department of architecture of Heriot-Watt and Edinburgh School of Art, aims to provide a study of the techniques involved in physical preservation, together with an interdisciplinary approach to the planned integration of existing buildings, sites and town areas, with new developments. Mr Peter Whitson, the director of the course, said that it will concentrate on "the conservation of areas, keeping what is good in the traditional, but relating it to the modern demands of traffic management, local government, law, finance and sociological implications".

Subjects studied will include the theory and practice of environmental conservation, together with its history and planning, and students will also look at the technology of conservation. The course will start next October, and it will take students with good honours degrees, preferably in architecture, town planning, landscape, building and social studies.

Parliament in Britain

Student Costs

Mr Gerald Fowler, Minister of State, Department of Education and Science, said that the cost per student in British universities was £1,408 in 1968–69, compared with £1,317 in 1964–65. The equivalent figures for students in colleges of education are £885 and £821 respectively. Mr Fowler pointed out that much of the capital expenditure included in these calculations is intended to provide for future expansion, not for present numbers. (Written answers, February 5.)

Ice Pollution

The British Antarctic Survey, a component body of the Natural Environment Research Council, will undertake a study of the Antarctic ice sheet later this year. Announcing this, Mr Fowler said that ice will be sampled at various depths, to provide a history of atmospheric precipitation over the last thousand years. The study should also indicate the extent of global pollution by long-lived substances. Results are not expected until 1971. (Written answers, February 3.)

Carbon Fibres and Lasers

Mr John Morris, Minister of Defence for Equipment, said that the possible use of carbon fibres in fighting vehicles is being investigated by government research establishments and by industry. It seems, however, that except for specialized applications, carbon fibre material offers no advantage over conventional materials, and their use is therefore not justified. Mr Frank Hooley, who had asked what studies the government is making on carbon fibres for defence purposes, also asked the Secretary of State for Defence to publish a White Paper on the military applications of laser technology. Mr Morris said, however, that, although defence research takes full account of laser technology, it would be inappropriate to publish the details. (Written answer, February 4.)

Computers

More than 5,200 computers are in use in the UK, and Britain ranks roughly on a par with the Federal Republic of Germany and Japan in computer usage. This estimate was given by Lord Delacourt-Smith, Minister of State, Ministry of Technology, and he added that manufacture of computers in the UK has increased by 24 per cent since 1966, while computer exports have increased by 20 per cent. In the year ended September 30, 1969, the total value of computers made in Britain was £143 million, and total exports were more than £55 million. (Oral answers, Lords, February 3.)

University Places

Mr Gerald Fowler said that he could not agree with the suggestion put by Mr Christopher Ward that thirty new universities must be built in addition to the expansion of existing institutions, in order to accommodate students in 1980. He said that there is considerable room for expansion in many existing institutions of higher education, and no decisions have yet been taken about providing new institutions. Mr Fowler strongly denied any intention to push up entry standards for higher education as the demand for places increases. (Oral answers, February 5.)

Miscellaneous Intelligence

Some 2,200 works on cancer are published yearly in 30 languages, according to the *Annual of Czechoslovak Medical Literature*, which makes no apology for contributing to the proliferative growth. The annual was compiled "in the memorable year 1968 which marked" —how could anyone forget it?—"the fiftieth anniversary of the independent state of Czechs and Slovaks. This year intensified our belief in the value of the individuality of small nations among other nations, well grounded in every branch of culture and science. We must, and wish to, compensate for our inadequate material forces by our spiritual strength".

Glomar Challenger, the research vessel sponsored by the JOIDES consortium of American oceanographic institutions, has steamed nearly 50,000 miles since the project began in August 1968 and has recovered four miles of oceanic sediment from 149 holes drilled at 84 sites in the Gulf of Mexico, Caribbean, Atlantic and Pacific. Core samples are available for study by any qualified scientists in the world.

The consumption of spaghetti immediately after hard physical exercise increases muscular efficiency threefold. This remarkable finding is reported in the organ of the health service of the Italian army, *Giornale di Medicina Militare*, **3**, 273; 1969.

The hazards of independent discovery are not confined to the scientific world. This month the Amsterdam publishing company of Elsevier has launched a new journal called the *International Journal of Bio-Medical Computing*, while from across the street the North-Holland Publishing Company has simultaneously issued the first number of a journal entitled *Computer Programs in Biomedicine*. The most pressing task of the new journals will doubtless be to compute each other's circulation.

The world that faced with equanimity the late Dr Philip Blaiberg's book *Looking at My Heart* is to be burst upon in April by Dr Christian Barnard's side of the story, which will tell of his "deeply religious conviction that it is his destiny to save human life". To help convey the message Dr Barnard has enlisted as co-author Mr Curtis Bill Pepper, whose previous works include *The Pope's Back Yard* and *An Artist and the Pope*.

The Wattle Research Institute of the University of Natal, South Africa, has clearly established that the Brown Mirid (formerly known as the Brown Jassid) wreaks more havoc in wattle than does the Large Green Wattle Cicadellid (formerly known as the Large Green Wattle Jassid). Wattle is also prone to attacks from the ballooning Wattle Bagworm, the Wattle Emperor Moth, the Large Wattle Chafer, the Wattle Looper and the Small Wattle Lappet Moth. What'll whittle the wattle away next?

It is little wonder that the Society of Cosmetic Chemists of Great Britain is buoyed up with joyous expectation because at its last meeting "the speaker finished by giving hope for the future indicating that the study of coalescence rates for the different droplets by measuring change in particle size distributed in time offered promising scope for further research".

•

NEW WORLD

Marking Time for 1971

THE United States budget for the fiscal year beginning this July is a spartan document, and there is nothing in it to suggest that the Federal Government is about to lift research and development off the plateau on which they have languished for the past several years. In the year ahead, the gains are neatly balanced by the losses, but the continuing pressure of the purse seems also to be forcing many of the Federal agencies to spend more of what they can spare for research and development on their own domestic activities. In the circumstances, the universities are probably lucky to have fared no worse than is promised for 1971. Another consolation, for the philosophically minded, at least, is that the latest budget provides an even better picture than its predecessors of how the Federal Government is proposing to manage its affairs.

In the coming year, combined federal expenditure on research and development is reckoned actually to fall from $15,192 million in the current fiscal year to $14,981 million in 1971. A small increase of $124 million in the cost of research supported by the government will be more than offset by a decrease in the cost of federally supported development, chiefly because of the money that will be saved by the attenuation of the NASA programme of manned space flight. Spending on academic research will, however, increase by about 1·6 per cent to a total of $1,475 million in 1971. The small decreases in the expenditure of the mission oriented agencies will be more than offset by increases in the amounts of money to be spent by agencies such as the National Science Foundation—at least if Congress eventually lets the Administration have its way. (See Table 1.)

Table 1. FEDERAL SUPPORT FOR ACADEMIC RESEARCH AND DEVELOPMENT (MILLIONS OF DOLLARS)

	1969 (actual)	1970 (estimated)	1971 (estimated)
Health, Education and Welfare	604	641	653
National Science Foundation	219	213	230
Defense	243	225	224
AEC	101	100	97
NASA	144	131	105
Agriculture	61	69	80
Other	56	70	88
	1,426	1,449	1,475

Military research and development is, however, unlikely to find sufficiently powerful friends to reverse the decline expected in the coming year, from $7,910 million in the current year to $7,763 million in 1971. The chief causes are the declining expenditure on development programmes for the F111 aircraft (in both its versions), the Poseidon missile and the newer versions of the Minuteman. On the other hand, more money is being spent on the new F15 fighter, antiballistic missiles and a variety of tactical aircraft projects. Laboratories such as those at the Wright–Patterson Air Force Base will grow. The Department of Defense will, however, save a substantial amount of money—the cancellation of the Air Force plan for a Manned Orbiting Laboratory has made possible a reduction of expenditure to an estimated $480 million in the coming fiscal year for military research and development in "astronautics and related equipment". This chimes in well with the general trend of expenditure on military defence, which is expected actually to decrease to nearly $6,000 million in the coming year, and which accounts for only 36·7 per cent of the budget for the coming year, compared with nearly 50 per cent in 1960.

The continuing decline of NASA expenditure is one of the most conspicuous features of the budget for 1971, as is the way in which NASA has given up its hopes for a continuing programme of journeys to the Moon. Between the current fiscal year and 1971, the expenditure of NASA as a whole will fall from $3,110 million to $2,606 million. Most of the reduction will be accounted for by the stretching out of the programme of landings on the Moon, but NASA is also hoping to save a great deal by the attenuation of the biological research programmes (down to $12·9 million in 1971).

The programme of fiscal and astronomical research is, however, sustained. In 1971, NASA plans to spend $127·6 million on physics and astronomy and $165·1 million on exploration of the planets. This represents a reduction of less than $2 million on the year. By contrast, expenditure on the support of the university research by means of training grants for graduate studies is being brought entirely to a halt.

The budget statement says that a great deal of hope rests on the development of rockets which can shuttle to and from orbits above the Earth—during 1971, the basic work on the design of such a vehicle should be complete. There is also continuing interest in the nuclear rocket motor from which it is hoped to attain about 75 thousand pounds of thrust for long distance journeys. In the distribution of research money, the NASA laboratories at most places except Cambridge, Massachusetts, where the Electronics Research Center has recently been closed, will share in what seems to be a general 2 per cent increase in expenditure.

But medical research under the aegis of the Department of Health, Education and Welfare is one of the few fields of scientific research which will benefit substantially from the budget—it is expected that the total expenditure in 1971 will be increased by close on $200 million to a total of $1,459 million in 1971. The total amount of Federal support for health research amounts to $1,660 million, provided by thirteen agencies of which the Department of Health, Education and Welfare accounts for 73 per cent. The budget says that expenditure is being channelled deliberately towards fields such as "research on viruses implicated as the cause of cancer", heart disease, dental caries, contraceptives, drug abuse and alcoholism. The other agencies of the Federal Government are also supposed to contribute—NASA is helping with "the control of noise pollution from aircraft and the use of aerial and satellite mapping of sources of pollution, erosion and other ecological problems".

The National Institutes of Health do well out of the 1971 budget. The total expenditure will amount to $1·526 million in 1971, an increase of 4 per cent, and the money available for grants and fellowships will increase from $706 million in the current year to $723 million in 1971. There will, however, be a small reduction in the cost of support for medical education, from $469 million to $454 million. The National Cancer Institute (which will receive an extra 16 per cent in 1971) is said now to have the "aim of developing a vaccine for preventing virus induced cancers". Most of the extra money will be spent internally.

The National Institute of Dental Research also has 12 per cent extra to spend ($34 million) as does the National Heart and Lung Institute ($171·7 million). By contrast, the National Institute of Allergy and Infectious Disease is on a declining budget—$99·2 million in 1971.

The National Science Foundation, the perennial Cinderella, may have better luck this year than in the past. The Administration is asking for an extra $73 million, although it seems clear that most of this is destined for extra work in the environmental sciences. The National Science Foundation should have rather more than $511 million to spend in the coming year but the increase of its expenditure in research will be from $317 million in 1970 to $384 million in 1971. The budget statement says that the increase is deliberately intended to give "support to research in areas where new scientific understanding is needed to aid in the solution of pressing problems affecting mankind" and it goes on to mention the environment, oceanography, ecological systems "and other problems of our society".

Specifically, the Foundation will spend more on its Arctic Research Program, its Earthquake Engineering Program and what is called the International Decade of Ocean Exploration. The Foundation is also planning to spend more on the International Biological Program.

The extra funds in the budget for the support of research should, the calculation goes, make it possible to award 4,305 grants in the coming year—the greatest number in the Foundation's history. But the Foundation is also planning to increase its investment in national research centres—the improvement of the 1,000 foot radio telescope at Arecibo will cost $3·8 million, for example—and it is clearly willing to go some way to meet the case made by the National Science Board for a new basis for supporting academic science by spending $55 million on institutional support.

The Atomic Energy Commission has managed to keep its research and development at a more or less constant level in spite of the decline which there has been in its total budget over the years. Roughly 18 per cent of its total budget on research and development will be spent in 1971. The Nuclear Weapons Program still remains a large part of the AEC's programme. In 1971, the Commission's expenditure on development of civil nuclear power stations will decline from $221 million to $149 million, chiefly a recognition of the extent to which the electrical companies are taking over this work. Basic research will cost the Commission $488 million—a decline of $14 million a year. What seems to have happened is that the AEC has been able to shoulder the continuing cost of the 200 GeV accelerator by shutting down similar facilities elsewhere. The most spectacular change, however, in what the Commission proposes is a reduction in the cost of the Plowshare Program from $14 million to $8 million—evidently the first results of the evaluation programmes leave a lot to be desired.

DESERT RESEARCH

National Science Foundation Grant

The International Biological Program has stimulated the National Science Foundation to give $654,000 to the Utah State University at Logan for a programme of research on desert ecology. The intention is that the Utah centre should serve as a place for a large group of biologists and others drawn from universities in the Western states. To begin with, the money will be spent on field studies in a valley on the border between Utah and Idaho, on a site near Tucson, Arizona, and another in New Mexico. The objective of the research programme includes a study of the relationship between human beings and desert conditions, and it is intended that other sites will be chosen for the experimental work once the programme is under way. With conservation very much in the air, the National Science Foundation says that there is a danger that deserts can be "easily destroyed", partly by the rapid growth of population, so that there is an urgent need of research programmes.

The immediate programme will consist of a co-ordinated network of field studies linked with computer modulators of them and followed by specific attempts to check the prediction of the computer models against real life. There will be particular interest in the rates of growth of desert plants and of the relationships of water and nutrients to the plants. There will be a special investigation of photosynthesis in desert conditions especially in its relationship to flowering, and there will also be a systematic study of the life span of desert animals.

The validation studies which form a part of the programme are in many ways its most novel feature. A part of the work involved will be a careful inventory of the biological environment at each of the three sites to be investigated so as to provide a starting point for later studies. One project will involve the fixing of small radio transmitters to various wild mammals and two radio towers will be built to collect information from this equipment.

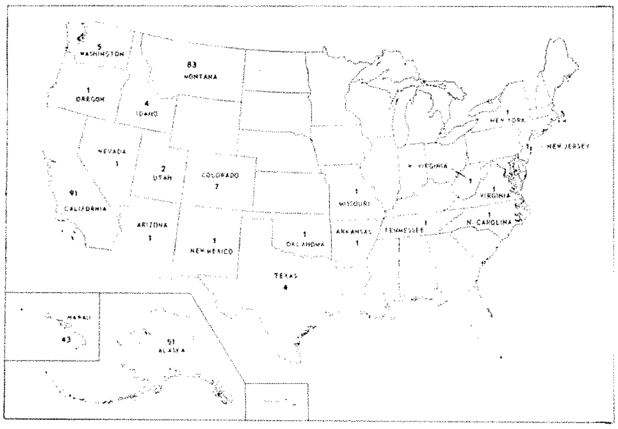
EARTHQUAKES

The American Toll

from our Geomagnetism Correspondent

During the past hundred years, earthquakes in the United States have killed more than 1,500 people and caused damage costing more than $1,300 million. North America continues to be earthquake prone; but the National Earthquake Information Center at Rockville, Maryland, reports that 1969 was the fourth successive year without fatalities. The most recent deaths (seven) resulted from a tremor in Seattle, Washington, in 1965.

Last year 303 epicentres of earthquakes strong enough to be felt or cause damage were detected in



Earthquake map of the United States for 1969.

twenty-two states, although seismographs recorded many thousands of minor shocks. Top of the league table was California (ninety-one), followed by Montana (eighty-three) and Alaska (fifty-one). It is unusual for Montana to feature so high—the last really serious tremor there occurred in 1959 at Hebgen Lake when twenty-eight people died. Normally Alaska and California fight for top place, although because of the sparse population in Alaska it is probable that many earthquakes are not recorded. The most severe tremor last year caused six million dollars' worth of damage in Santa Rosa, California, where two shocks occurred within 85 minutes, injuring several people. Both of these earthquakes were felt along the west coast from Clear Lake to San José and shook large buildings in San Francisco.

Elsewhere in the world, the worst earthquake killed 136 Peruvians in October. Reports of 3,000 deaths by an earthquake in China last July have not been confirmed. Other fatal earthquakes in 1969 included those in the Celebes Islands (sixty-four killed), Iran (fifty), Ethiopia (twenty-four), Yugoslavia (twenty), Morocco-Portugal (thirteen), South Africa (eleven), Turkey (eleven), Egypt (two) and Albania (one).

SUN

America in Eclipse

by our Astronomy Correspondent

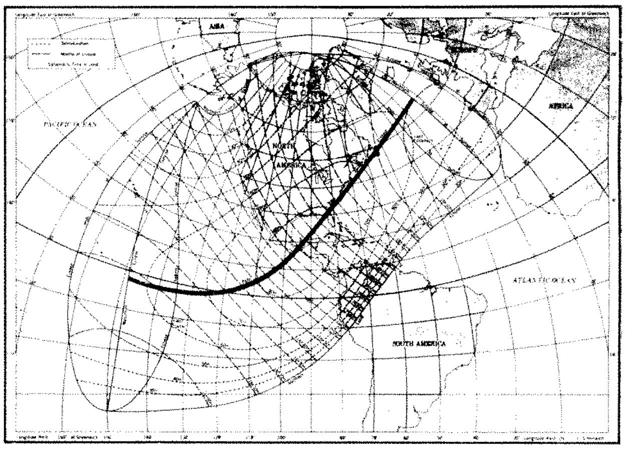
THE next astronomy spectacular is the total eclipse of the Sun on Saturday, March 7. As the accompanying eclipse map from *The American Ephemeris and Nautical Almanac* shows, the partial eclipse will be visible over all of central and north America with the exception of parts of Alaska and Greenland, but the total eclipse is limited to a roughly 100-mile wide band which sweeps across Mexico, Georgia and the Carolinas, and grazes the east coast of the United States and Canada.

Readers planning to be on the east coast next month would do well to arrange their itinerary around the eclipse, the first since 1963 to touch the continental United States. All the details are in the circular published by the US Naval Observatory—the eclipse observer's bible—in this case circular No. 125. In the United States the best locations are in Florida, the Carolinas and Virginia, where the climatological data show that there is a 40 per cent chance of seeing the eclipse. Farther north the conditions deteriorate, and Nantucket Island, which is on the path, is not a recommended site. In Nova Scotia and Newfoundland the chances that March 7 will be a clear day are small: Shearwater, NS, is probably best with a 26 per cent probability of a clear day, but at Gander Airport, just off the path of totality, the probability is down to 19 per cent.

	Time of mid-eclipse	Percentage of totality
Albany, NY	13.43 EST	92
Boston, Mass.	13.46 EST	96
Chicago, Ill.	12.25 CST	72
Columbus, Ohio	13.30 EST	83
Dallas, Tex.	12.00 CST	71
Denver, Col.	10.57 MST	46
Halifax, N.S.	14.55 AST	100
Kansas City, Mo.	12.12 CST	64
Los Angeles, Cal.	9.26 PST	36
New York City	13.42 EST	96
San Francisco, Cal.	9.26 PST	27
Tucson, Ariz.	10.34 MST	48
Washington, DC	13.37 EST	95

But devotees will be going to Mexico—not to Minatitlan where the eclipse path crosses the north coast and which has a weather record inferior to the southern United States, but to Oaxaca de Juarez where cloudiness is low because of the elevation of more than 5,000 feet, or to Salina Cruz on the south coast. The magazine *Sky and Telescope* has prepared a guide to eclipse sites in Mexico together with useful hints (stock up on supplies, including canned and prepared foods, and especially bottled water and drinks; your physician may suggest additions to your medical kit), as well as a list of sites which have been set aside for observers in the United States.

The table lists the times of mid-eclipse and the percentage of totality for several locations in north America.



NEWS AND VIEWS

One Way for Neutron Stars to Pulse

ALMOST everybody agrees that the mechanics that keep pulsars ticking away must be the rotation of neutron stars—the range of periodicities which have been recorded sees to that—but the question of why neutron stars should emit radiation in such quantities is still as wide open as ever. With well over forty pulsars now listed, there is an embarrassment of observational detail to explain. Two pulsars have aroused most interest, NP 0532 in the Crab Nebula supernova remnant and PSR 0833–45 which is believed to be associated with the suspected supernova remnant Vela X. Both are famous for their rapid periodicities of 30 and 10 pulses per second respectively and for the surprising irregularities which they have shown in their pulsations, and it is through the irregularities that the Crab and Vela pulsars are believed to hold the key to the structure of neutron stars. But an understanding of the emission mechanism is more likely to come from a distillation of the features of the radio signals from all forty or so pulsars.

There is a lot to account for, and the value of the article by M. M. Komesaroff (of CSIRO) on page 612 is that it is a hypothesis against which future observations will be measured. The basis is the oblique rotator model which has it that the magnetic axis of the spinning neutron star is at a large angle of tens of degrees to the rotation axis. Komesaroff gives reasons why the radiation should be emitted in a hollow conical beam which is coaxial with the axis of the dipole magnetic field. The spin of the neutron star sweeps the narrow beam around, and depending on how the beam crosses the observer the double-pulse structure of some pulsars and the single-pulse structure of others can be synthesized.

Any theory, of course, has to account for the intensity of the radio emission, and this involves large numbers of particles radiating in phase. According to Komesaroff, most of the radiation comes from particles within a few radii of the surface moving along lines of magnetic field near the magnetic pole. The radiation, however, is not the ubiquitous synchrotron radiation of particles gyrating around field lines, but rather arises simply from the acceleration of particles which describe a curved path, in this case relativistic particles in a field of something like 10^{12} Gauss.

As such, the model of Komesaroff is a crystallization of two ideas—the notion that a narrow beam could be obtained if the radiation comes from near the magnetic pole (Radhakrishnan and Cooke) and the notion that the radiation could be from the curved paths of particles along field lines rather than from synchrotron radiation (Radhakrishnan).

Despite the impressive list of features of the radio pulses which Komesaroff has accounted for, nobody is going to say that this is the answer. For one thing, the model does not account for everything, although this is hardly surprising. For another, not enough is said in the article about the polarization of the signals which most people suspect must hold the key to the emission mechanism. But what Komesaroff has succeeded in doing is to set up a feasible Aunt Sally against which the measurements can be matched, and the outcome should be a better realization of what properties the ultimate model should have.

What Sigma Factor Does

UNTIL last year it was far from clear how the enzyme RNA polymerase recognizes the sites on DNA where transcription is to be initiated. But then Burgess *et al.* reported fractionation on phosphocellulose columns of the RNA polymerase from *E. coli* into a "core enzyme" and a "sigma factor" (*Nature*, **221**, 43; 1969). The former comprises protein of four subunits with a combined molecular weight of about 400,000, and the latter a single polypeptide chain of molecular weight about 95,000. The core enzyme retains the ability to catalyse the synthesis of RNA chains under direction from a DNA template *in vitro*, quite efficiently when alien calf thymus DNA is provided as template but with a great reduction in activity when the DNA comes from its usual source, *E. coli* or phage T4. But in the latter case the addition of sigma factor restores the synthetic activity of the core enzyme, and later work (see, for example, *Nature*, **222**, 537; 1969) showed that the factor acts on the initiation of RNA synthesis. It associates with the core enzyme to yield a "complete enzyme" complex, which then binds to the DNA template; once the synthesis of RNA by the core enzyme is under way, the sigma factor is released from the transcription complex, and can be used again to initiate transcription by another molecule of core enzyme.

An obvious possible use of this mechanism is for selecting the genes at which transcription is to take place. Indeed, Bautz *et al.* have shown (*Nature*, **223**, 1022; 1969) that the sigma factor may play such a part when *E. coli* cells are infected by phage T4. An analysis of the RNA synthesized *in vitro* on T4 templates showed that addition of the sigma factor specifically stimulates the core enzyme into transcribing those genes of the phage which are usually expressed *in vivo* during the first minute after infection. In the absence of factor, RNA chains are initiated at random sites along the whole T4 DNA molecule.

On page 598 of this issue of *Nature*, Sugiura, Okamoto and Takanami report the latest development in the story of how the sigma factor exerts its selective control over transcription. They used the double stranded form of bacteriophage *fd* to direct the synthesis of RNA *in vitro* by core enzyme, both in the absence and presence of sigma factor. Phage *fd* consists of a single strand of DNA which is converted to a duplex replicating form (RF–DNA) when infecting its hosts.

Sugiura *et al.* found significant differences in the nature of the RNA species synthesized when the core enzyme is complemented by the addition of sigma factor to the incubation medium. RNA chains in *E. coli* begin predominantly with purine nucleoside triphosphate, largely comprising the species pppGpPyp. . . and pppApPyp. . . When the core enzyme alone was used to achieve synthesis, less than half the termini of the RNA species synthesized were in this category. Addition of sigma factor stimulated RNA synthesis, so that more than 70 per cent of chains began with the expected sequences. Sedimentation analysis of the RNA products showed that species of a definite size (about 26S) tend to be synthesized by complete enzyme, but there is a much greater spread of size distribution when sigma factor is absent. The RNA synthesized by the complete enzyme can hybridize only with denatured RF–DNA, that is, it is complementary to only the minus strand. By contrast, the RNA synthesized by core enzyme can hybridize both with denatured RF–DNA and with the single-stranded DNA from mature phages. About twice the amount of hybridization takes place with the denatured DNA species as with the mature phages.

It seems, therefore, that in the absence of the sigma factor the core enzyme is free to initiate RNA synthesis at random points along either strand of the DNA template; this results in the production of species with unusual 5′ terminal sequences, of a more dispersed size distribution, and which can hybridize with either strand of the phage DNA. The effect of the sigma factor is to restrict the catalytic activities of RNA polymerase, so that it transcribes only the minus strand of the template from certain starting points. These presumably correspond to the sites at which transcription is initiated *in vivo*.

POLYMERASES

Multifarious Molecules

from our Molecular Biology Correspondent

THE DNA-dependent polymerases are highly specialized enzymes, with various functional modes. RNA polymerase must be able first to recognize and attach to the initiation site on the DNA template, then to take up the appropriate nucleoside triphosphate, to initiate the chain, to catalyse chain propagation and finally to liberate the RNA when synthesis is complete. The initiation and termination steps are now known to involve protein factors, the first of which is in effect a subunit of the enzyme, as isolated.

The uncoupling of various of the functional modes of RNA polymerase by chemical modification is described in an interesting study by Ishihara and Hurwitz (*J. Biol. Chem.*, **244**, 6680; 1969). They establish that any of the four common nucleoside triphosphates will bind to the polymerase in the absence of the template, though the number of binding sites is still uncertain: Ishihara and Hurwitz obtain conflicting results by two different experimental procedures. When DNA is present, there is strong preference for the attachment of CTP or GTP, which accords with the known composition of the initiation sites and means that the DNA is able to control the affinity for different substrates. In addition, in the presence of DNA the bound triphosphate will exchange against labelled pyrophosphate, which may well reflect the formation of a phosphodiester bond, and is probably therefore a measure of the polymerase activity of the enzyme.

Chemical modification produces striking results. Treatment with specific reagents for thiol groups leads to loss of activity, but the ability to bind the triphosphates in the absence of DNA was scarcely impaired. The capacity to interact with the template was entirely lost. The contrary is found when the enzyme is exposed to reagents specific for amino groups: the binding of RNA is unaffected, but there is no longer any interaction with the triphosphates. Finally, after treatment with a reagent largely specific for histidine, or photo-oxidation in circumstances most likely to lead to destruction of histidine, the enzyme is again inactivated, this time, however, with retention of the ability to bind both DNA and substrates; the exchange reaction against pyrophosphate is completely inhibited, however, and this may cautiously be interpreted to signify failure of the polymerization function. Only with thiol reagents is there any detectable physical change: a fragment sedimenting at 2S is released, which it would be agreeable to be able to equate with the σ (initiation) factor; this is still uncertain, however, and indeed the σ factor has been reported to sediment at 4–5S (though it may itself, of course, be prone to dissociation by thiol reagents). Differential sensitivity of the several functions to thermal denaturation has also been observed. A different effect again is produced by the antibiotic, rifampicin, which blocks initiation of the RNA chain, but fails to interrupt propagation if initiation has already been allowed to happen (Maitra and Barash, *Proc. US Nat. Acad. Sci.*, **64**, 779; 1969). There seems good reason to hope that the operation of this key enzyme will soon be understood in some detail.

DNA polymerase (or, as it should perhaps be called in the prevailing atmosphere of retrenchment, repair enzyme) is also a multifarious protein. It is reported to consist of a single chain with the unusually high molecular weight of 109,000, and it catalyses DNA synthesis, pyrophosphate exchange against the bound triphosphates, and two modes of exonucleolytic degradation, from the 3′ and 5′ ends. Klenow and Overgaard-Hansen (*FEBS Lett.*, **6**, 25; 1970) have treated the enzyme with a protease, subtilisin. When DNA is absent, this causes loss of exonuclease, but not of polymerase activity. When DNA is present, both activities remain substantially intact, but fractionation by gel filtration reveals the presence of two fragments, one possessed only of polymerase, the other only of nuclease activity. The molecular weights, as estimated from the elution positions, are respectively some 70,000 and 35,000. The inference is that DNA polymerase consists of two parts, joined perhaps by only a single exposed loop of polypeptide chain, that can be cleaved without other damage to either half of the molecule. This evidently is a case in which two different functional subunits are tethered together. The advantage to the cell of this arrangement is not at this stage clear.

INVERTEBRATES

Worms on Ice

ALTHOUGH ice worms (*Mesenchytraeus solifugus*) have been reported from Alaska, Yukon Territory, British Columbia and Washington State, few have been seen alive in their natural habitat by zoologists. Dr M. Tynen of the University of Alberta has recently found some in the ice of the Cliffe Glacier in Central Vancouver Island and on the Snow Dome of Mount Olympus, Washington State.

Ice worms in the snow of the Cliffe Glacier, Vancouver Island.

During the day, ice worms, which are black and about three quarters of an inch long, were found clumped together 6–12 inches deep under the drainage furrows of the névé. Although they were in hard crystalline ice, which could only be removed with the aid of an ice axe, the worms seemed to move with considerable ease. Tynen thinks that they may have crawled along the crystal boundaries. At dusk, the worms rose to the surface and started to disperse from the drainage furrows into the mounds between. They all disappeared, however, as the Sun rose. This nocturnal activity has been noted before.

The worms probably feed on the "redsnow" algae (*Chlamydomonas nivalis*) which collect in the drainage furrows together with windblown dust and other debris. The furrows seem to be self perpetuating: they tend to collect dust which imparts a darker colour to their surface, and this in turn results in increased ablation. The growth of the algae may be enhanced by mineral nutrients present in the dust, made mobile by rapidly melting ice. The drainage furrows could also trap windblown organic particles, such as pollen grains, which would be another possible source of food for the worms.

If the worms feed on algae and pollen grains, there is, of course, the question of how they digest cellulose. Many of those found had large ciliates in the fore-part of the gut, and these may have been symbionts that digest cellulose. Their occurrence, however, is by no means universal in ice worms, and they have not been noted before. Possibly, the mineral particles which are ingested, and can be seen in serial sections of the worm, act with the movements of the gut to triturate the food and break down cellulose.

The Cliffe Glacier, like all those in which ice worms have been recorded, is temperate, so that during the summer its temperature is never below 0° C. In winter the surface may be covered with 20–30 feet of insulating snow, so that the level where the worms live probably maintains a temperature at or very near the freezing point of water. Worms held in the hand with a piece of ice became very agitated as the ice melted, but Tynen thinks that this may have been a response as much to the change in consistency as to any increase in temperature.

Samples of the ice worm population of the Cliffe Glacier collected at the end of July contained a large proportion of immature individuals. It is not possible, however, to do more than speculate as to the pattern of the annual life cycle, for samples were not collected at other times of the year. There seems to be no reason why the worms could not feed throughout the winter on the resting stages of the snow algae, trapped with them under the winter snowfall. The immature individuals observed in July would then be mature by the following spring.

BACTERIA

The Stuff of Cell Walls

from our Microbiology Correspondent

MICROBIOLOGISTS are finding that changes in the chemical and physical environments can induce profound changes in microbial cell walls. Schleifer, for example, has noticed that changes in the nature and amount of the amino-acid complement of culture media can affect the type of substitution on the L-lysyl residues and the extent of interpeptide linkage in the walls of staphylococci (*J. Gen. Microbiol.*, **57**, xiv; 1969). Such modifications may be crucial in determining the susceptibility or resistance of bacteria to lytic enzymes.

Ellwood and Tempest did pioneering work when they described modifications in the anionic polysaccharide content of the walls of *Bacillus* which are dependent on magnesium and phosphate. Recently they obtained similar results with Gram positive cocci (*J. Gen. Microbiol.*, **57**, xv; 1969). And Garrett found that when *Bacillus subtilis* is starved of magnesium, not only is teichoic acid synthesized in the cell wall, but the synthesis of peptidoglycan is inhibited (*Biochem. J.*, **115**, 419; 1969). The likely explanation for this is that the protoplasmic membrane loses some of its structural and functional integrity when magnesium is lacking, and that the activity of membrane-bound peptidoglycan synthetases is precluded.

Peptidoglycan, which comprises about 5–10 per cent of the wall of Gram negative bacteria, and up to 80 per cent of the wall of Gram positive organisms, has been thought to be chiefly responsible for the mechanical strength and morphology of the cells. Rogers and his colleagues, for example, found an increase in the degree of cross-linking in peptidoglycan when certain mutants of *Bacillus subtilis* were converted from the spherical to the rod form by supplementing the medium with certain amino-acids (*Nature*, **219**, 285; 1968).

Two configurational models have been constructed for the peptidoglycan of *Staphylococcus aureus* in which the polysaccharide chains are aligned head to tail and are hydrogen bonded together (Kelemen and Rogers, *J. Gen. Microbiol.*, **57**, xiii; 1969). An α-helical model accorded little hydrogen bonding, but a β-configuration in the form of a pleated sheet possessed *trans* carboxyl groups which accommodated a regular net-like organization of the peptidoglycan in which 60 per cent of the amino groups participated in hydrogen bonds.

Although acknowledging that peptidoglycan is essential in determining cell morphology, Braun and Shwarz (*J. Gen. Microbiol.*, **57**, iii; 1969) argue that this function is a reflexion of its association with other cell wall components. They have analysed the interaction between peptidoglycan and lipoprotein in *Escherichia coli* and proposed a model in which about 10^5 lipoprotein molecules are distributed regularly over the surface of the peptidoglycan. One lipoprotein moiety appears to be associated with every tenth repeating unit of the peptidoglycan and is linked by an N-terminal lysine to the carboxyl group of diaminopimelic acid residues. Under the microscope, cell walls treated with trypsin were seen to be split into two layers. This, coupled with a rapid decrease in the optical density of the treated material, suggested to Braun and Shwarz that lipoprotein, too, plays a significant part in stabilizing the cell wall.

VIROLOGY

Fusion Within and Without

from our Cell Biology Correspondent

In the past five years the use of inactivated para-influenza viruses to effect the fusion of somatic cells into viable hybrids has emerged as a powerful biological tool. The technique has already transformed somatic cell genetics from the emeritus professor's pipe dream to a rapidly expanding experimental discipline; it is also increasingly being applied in virology and viral oncology, for example in promoting infection of refractory cells with such viruses as polio and polyoma, and no doubt there are many more applications to come. Most of the credit for this innovation belongs, of course, to Henry Harris and his collaborators at Oxford; it was they who realized (*Nature*, **205**, 640; 1965) the potential for cell biology of Okada's discovery (*Exp. Cell Res.*, **26**, 98; 1962) that high multiplicities of Sendai virus induce cell fusion. But the way in which these viruses induce fusion remains obscure for perhaps the good reason that, for most people who use the technique, all that counts is that it works; how it works is of secondary importance. The mechanism of fusion is not, however, without its intrinsic interest and Bratt and Gallaher (*Proc. US Nat. Acad. Sci.*, **64**, 537; 1969) suggest that viral lipids or possibly lipoproteins are the fusion factor, at least in Newcastle disease virus (NDV).

While investigating the infection of secondary cultures of chick embryo cells with NDV they noticed that different strains of the virus vary in their ability to induce cell fusion; the strains seemed to fall into two classes, those which induce fusion directly and those which induce fusion only after the cells are infected and viral materials have been synthesized. Bratt and Gallaher call the two mechanisms fusion from without and fusion from within respectively. Fusion from without is a property of NDV–HP and NDV–EH strains; the fusion induced is as great 2·5 hours as 7 hours after virus has been added; it is independent of the *p*H of the cell's medium; it is greatest at the highest multiplicities of infection; and, most important of all, the virus does not have to be infectious to induce fusion.

Fusion from within by NDV–AV, on the other hand, requires at least 6 hours, is dependent on *p*H—there is most fusion at high *p*H—and the virus must be infectious. Inhibition of protein synthesis by cycloheximide either before or after infection has no effect on fusion from without by NDV–HP; fusion from within is inhibited by the drug if it is added early, but susceptibility decreases as addition is progressively delayed after infection. Both fusion processes are, however, completely suppressed by pretreatment of the virus with antiviral antibody. When antibody is added after infection, fusion from without becomes completely insensitive to inhibition between 20 and 40 minutes after infection even though fusion does not reach its maximum until 2 to 3 hours after infection. Fusion from within, on the other hand, remains completely susceptible to inhibition by antibody for 5·5 hours after infection.

Clearly Bratt and Gallaher have established that the two types of fusion are quite distinct. Fusion from without seems to result from the direct interaction of cell and virus, and the fact that it occurs at 38° C and 43° C but not at 23° C brings to mind two other temperature dependent interactions of cells with NDV virus-penetration and cell interference. Fusion from within, on the other hand, seems dependent on viral protein synthesis but not DNA-dependent RNA synthesis. The two types of fusion seem to be strain specific and, because the capacity to induce fusion is restricted to viruses with lipoprotein envelopes and because agents which alter these lipids are known to diminish fusion from without, Bratt and Gallaher suggest that either viral lipids or lipoproteins are the fusion factor. The fact that fusion from either within or without is strain specific and the lipid composition of NDV envelopes is also strain specific, of course, supports this notion.

CANCER

Oncogeny and Ontogeny

from a Correspondent

The considerable problem of defining the chemical events involved in carcinogenesis was tackled at the second meeting of the annual winter symposium of the Department of Biochemistry of the University of Miami and the Papanicolaou Cancer Research Institute in Miami on January 22 and 23 (see *Nature*, **225**, 498; 1970, for report of first meeting). One approach to this problem is to use a minimal deviation hepatic tumour, the Morris hepatoma, which in its well differentiated form differs from other experimental rodent tumours by its low glycolytic capability, high respiration and use of fatty acids as metabolic fuel. Many years of study of this hepatoma has led Professor Van R. Potter (Madison) to the concept that "oncogeny is blocked ontogeny".

Long term evolutionary changes in gene expression as seen in the amino-acid sequences of the same protein of many species have a counterpart in the relatively rapid change from the normal to the cancerous state. Dr S. Weinhouse (Philadelphia) concluded, after study of several glycolytic enzymes, that certain types of Morris hepatoma tumours produce a switch in genome readout involving the suppression of enzymes concerned with hepatic function and the unmasking of other enzymes normally suppressed in their tissues of origin. Dr M. Farber (Pittsburgh) spoke of a chemically induced "hyperplastic nodule" that is homogeneous, not normal, but not yet cancer, and which seems to be a "precursor"; from this relatively homogeneous cell population a heterogeneous mixed population of cancer cells evolves. These "precursor" cells have abnormal DNA and carbohydrate metabolism.

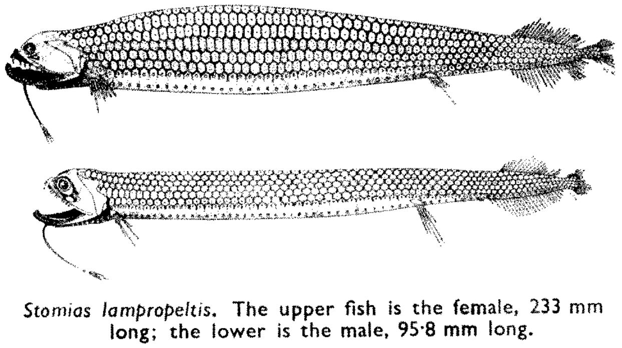
The homology and variations of the gamma globulins with respect to both primary structure, discussed by Professor R. R. Porter (Oxford), and tertiary structure, discussed by Dr F. W. Putnam (Bloomington), reflect the "fast evolution" of response to antigens with variations in structure, both primary and tertiary, which play a part in determining the antibodies formed, as Professor M. Sela (Rehovot) pointed out. The results of researches with substances of known structure, leading to exact chemical data, throw no more light on the origin of cancer than less exact studies. But Dr M. Potter (Bethesda), making use of myeloma protein synthesis, found that the precursors of neoplastic cells actively formed antibodies when they underwent neoplastic transformation.

Dr H. Busch (Houston) reported further evidence of a "cancer operon" as evidenced by the nature of the RNA found in the nucleolus of cancer cells. Finally, a way of studying the genome which controls the events leading to the replication of DNA, which precedes mitosis in cancer, was described by Dr R. Beserga (Temple, Philadelphia). He has used salivary glands taken from rats and then treated with the catecholamine isopreterenol.

ICHTHYOLOGY

New Name in Deep Waters

The fish in the illustration has been raised recently from subspecies to full species by Dr Robert H. Gibbs jun. of the Smithsonian Institution. This creature, which inhabits the deep waters, between 200 and 1,000 metres down, in the eastern tropical Pacific, was first included in the species *Stomias colubrinus* by Brauer in 1906, and in 1964 Blache decided that it was a subspecies and called it *Stomias colubrinus orientalis*. Gibbs, however, scorns this distinction, based on a statistical test of differences from specimens of *S. colubrinus* living in the south-eastern Atlantic. He is not prepared to accept differences between two nominal subspecies if they are so slight as to be unrecognizable from an inspection of specimens. But he has looked again at the two populations and found a much more impressive difference than Blache's variations in rays and photophores. The teeth and their arrangement are sufficiently different, according to Gibbs, to distinguish the eastern population as a separate species, *Stomias lampropeltis*, from the Atlantic population, which remains *Stomias colubrinus* (*Smithsonian Contributions to Zoology*, No. 31; 1969).



Stomias lampropeltis. The upper fish is the female, 233 mm long; the lower is the male, 95·8 mm long.

This piece of taxonomy is but one aspect of a study of the ten species and subspecies of *Stomias*, during which Gibbs was the first person to describe sexual dimorphism in this genus. The sexes differ principally in that males are smaller than females and tend to have larger eyes.

ARCHAEOLOGY

Signs of Early Man

The village of Swanscombe, on the banks of the River Thames in Kent, may in a year or two yield the first intact Lower Palaeolithic camp site to be found in Britain. Dr J. A. Waechter told members of the Royal Anthropological Institute on February that the first two seasons of excavations sponsored by the institute have proved more promising than pessimists expected, and have yielded what seems to be a refuse dump. If this is so it can only be a matter of time and careful excavation before a camp is found nearby.

Such a find would thoroughly vindicate everyone who has supported the project of excavating this site. It is of course best known for having yielded in 1935, 1936 and 1955, three bones which fitted together to make up the back of the skull of Swanscombe man, dating from the second interglacial period (200,000 to 350,000 years ago). Because the front of this skull was never found it has not been possible to say more than that this was a being very similar to *Homo sapiens*. Waechter feels that at least one of these men must have drowned himself in the lake that used to stand nearby, and have been preserved intact in the sediments. This would be a great find indeed.

When the present investigations began in 1968 the site, previously much dug for chalk and gravel but now protected by the Nature Conservancy, had been more or less cleared of the upper layers of gravel. These contained the typical flint hand axes of the Acheulian culture (which lasted from about 200,000 to 100,000 years ago). But the lower layers of loam and gravel, which had remained untouched since they were once covered by the still water of the lake, held promise of yielding material dating from the earlier Clactonian culture (probably 250,000 years old). This promise was fulfilled in 1968 and 1969. Throughout the lower loam Waechter and his team found Clactonian flints, which are more primitive than their Acheulian counterparts, some in mint condition. There were also considerable quantities of antlers, one complete set attached to the skull of a red deer. Other finds in the loam included elephant bones, the tooth of a rhinoceros and a complete skull of a bear. The gravel below yielded very little bone but many more implements than the loam.

The bone, which has proved difficult to handle because of its great fragility, is at present being identified, and will later be carefully examined for any evidence of human influence on it. One hopeful sign is an antler which seems to have been worked into a point, probably for use as a tool. Working on the assumption that he has found a refuse pit, Waechter enlisted a local band of small boys to emulate their Palaeolithic forebears and see how far they could effortlessly throw objects with the dimensions of his finds. Having concluded that about thirty yards was probably the limit for easy throwing of refuse to the dump, he said, he has marked with a twig the place where he thinks the Swanscombe camp site is to be found. Another two or three seasons should show whether he is right.

PULSARS

Crab Pulsar and its Planet

from our Cosmology Correspondent

With the growing evidence that the entire source of energy for the Crab Nebula lies in the rotating neutron star believed to be associated with the pulsar NP 0532, several attempts have been made to determine the mechanism linking the two physically separated parts of this system, namely the small central star and the large expanding shell of luminous matter. These have been hampered by incomplete understanding of the processes producing supernovae and neutron stars, but A. Cavaliere and F. Pacini have now been able to describe in reasonably accurate terms the evolution of a supernova remnant which does contain a central rotating dense star. The star is taken to be coupled to the remnant largely through the electromagnetic forces involved, because gravitational radiation and mass loss subsequent to the original explosion seem likely to produce only small effects. Cavaliere and Pacini find (*Astrophys. J. Lett.*, **159**, 21; 1970) that the rate of decrease in luminosity of a remnant at any time is closely linked to the observable properties of the central neutron star, and that these parameters when measured for NP 0532 are in good agreement with the theory. The only other known supernova remnant with a radio emission which decreases measurably is Cas A, for which the model predicts an associated pulsar with period $\lesssim 0{\cdot}7$ s. Unfortunately, it seems unlikely that pulsed radiation from this source will be detected if the most widely favoured models for pulsars are at all correct, for the radiation would be highly directional and unlikely to be orientated towards the Earth.

The recent observations of NP 0532 indicating a quasi-sinusoidal variation in period suggestive of the perturbations due to a planet (D. W. Richards *et al.*, *IAU Circ. No. 2178*) and a sudden variation tentatively explained in terms of a "starquake" (D. W. Richards *et al.*, *IAU Circ. No. 2181*) can now both be explained in terms of disturbances due to a planetary companion. If a pulsar is created abruptly in a sudden explosion involving mass loss, then any associated planet initially in a circular orbit will be forced into an eccentric orbit with eccentricity $e = f\,(1 - f)$, where f is the ejected mass fraction (F. C. Michel, *Astrophys. J. Lett.*, **159**, 25; 1970). As Michel points out, an initially eccentric orbit could thus be changed to a roughly circular orbit, and although this is unlikely it seems the only way to account for the form of the sinusoidal variation in period of NP 0532, assuming that this is indeed caused by a planet.

The more likely case of a roughly circular orbit being changed to one of extreme eccentricity could well result in sudden changes in the pulsar's period as a result of close approaches to the parent body, as seen in both NP 0532 and PSR 0833. The rather short interval between the discovery of these pulsars and their observed sudden changes in period suggests that they each have a companion orbiting roughly once a year, clearly a very reasonable orbital period for planets. Such effects will be repeated regularly so there is a ready test of Michel's model, and if these variations do recur as predicted they should provide a useful tool for determining detailed structure of these pulsars.

SPECTROSCOPY

Broadening of Lines

from a Correspondent

A branch of atomic physics that is advancing rapidly in response to the demands of astronomy and plasma physics is the study of the broadening of spectral lines in gases and plasmas. This was the subject of a meeting held by the Institute of Physics and the Physical Society at Imperial College, London, on February 6, 1970.

Dr H. G. Kuhn (University of Oxford) summarized the two extreme case approximations for line broadening that dominated the theory until recently and are still very useful. These are quasi-static or statistical broadening (valid in the outer wings of a line) in which the motions of the perturbing atoms or ions are neglected, and impact broadening (applicable nearer to the line centre) in which superposition of phase shifts of the emitted radiation due to isolated encounters leads to a Lorentz profile accompanied by a small shift. Kuhn said that some lack of cohesion between theorists and experimenters, due to difficulties encountered by both, is now being overcome and understanding of interatomic forces is increasing.

Professor W. R. Hindmarsh (University of Newcastle upon Tyne) described recent studies with neutral perturbers. The classical Lindholm treatment, assuming pure van der Waals attraction, predicts incorrect line shifts, but agreement is much better when the repulsive interaction at small interatomic distances is included. Dr H. van Regemorter (Observatoire de Meudon) showed that repulsive forces probably account for the bulk of the line width as well. A further effect of complications in the interatomic potential curve is the appearance of satellites on either side of the unperturbed line, some striking examples of which were shown by Dr R. Granier (CNRS, Bellevue, Paris). When broadening is due to identical—as opposed to foreign—atoms, a stronger (dipole) interaction occurs. Dr D. N. Stacey (University of Oxford) showed that the theory of this resonance broadening effect is successful except at very low pressures where natural damping may invalidate the impact approximation. Dr C. Baron (Rank Precision Industries, Leeds) described experiments on pressure broadening of Zeeman components in a magnetic field.

The theory of broadening by charged perturbers has been greatly improved recently by the work of Baranger, Griem and others, but some problems remain.

Dr D. D. Burgess (Imperial College, London) explored a new approach based on the laws of conservation of energy and momentum which helps to define the basic assumptions and limitations of other theories. Dr Gillian Peach (University College, London) presented accurate quantum mechanical calculations of electron-scattering cross-sections required for impact broadening theory; her results agreed with semi-classical calculations, but less well with experiment.

The theory of spectral line broadening is very important in astrophysics. Dr B. E. J. Pagel (Royal Greenwich Observatory) described its application to visible effects in stellar spectra (for example, the forbidden satellite lines of helium) to the determination of stellar temperatures and gravities from hydrogen-line profiles and to abundance determinations in stellar atmospheres. The vexatious problem of solar iron abundance, in particular, was closely tied to uncertainties in collision broadening. The helium satellites also formed the subject of a laboratory investigation described by Mr C. J. Cairns (Imperial College, London) which gave results agreeing well with the theory (although theorists are not agreed about its validity). In a similar vein, Dr A. N. Prasad (University of Liverpool) showed how both temperature and density of a hot plasma could be deduced from the profile of He II λ4686.

ATOMIC BEAMS

Relativity in Action

A NEW method devised to measure the velocity of a beam of atoms exploits the relativistic relationship between an electric and a magnetic field and seems to be significantly more accurate than the usual method based on the time of flight. T. S. Stein *et al.* (*Appl. Phys. Lett.*, **16**, 1; 1970) have passed a beam of sodium atoms through a region containing electric and magnetic fields, and by comparing the magnetic effect of the electric field with a known magnetic field they have obtained an accurate measure of the mean velocity of the beam.

It is rare to find a relativistic effect that is of value at velocities as low as 10^4 cm/s, or one millionth the velocity of light. The basis of the method is to apply a constant magnetic field to separate the energy levels of the atoms, followed by a radio frequency field divided into two separate coils. Transitions of the normal Zeeman type are induced and an interference pattern appears in the counter. The magnetic effect of the constant electric field is then to shift the position of the interference pattern, and by adjusting the counter to monitor the position of largest slope on the pattern very small shifts can be detected.

Stein *et al.* point out that this method can be refined to a high degree of precision. Increased accuracy is obtained by continuously reversing the electric field, and by varying the magnetic field a plot can be obtained which gives the mean velocity of the beam as its slope. A weakness of the system, however, is that the mean velocity only is measured, whereas with time of flight techniques the whole velocity distribution is obtained.

The relativistic method was devised as a byproduct of an experiment to measure the electric dipole moment of alkali atoms, and Stein *et al.* have used it on both sodium and caesium atoms. What other particles may be tracked by this method? One suggestion is that a new attempt might be made to see if the neutron possesses a small electric dipole moment. Although no such moment would be expected, the uncertainty in nuclear conservation laws suggests that such a search may indeed be profitable.

GEOPHYSICS

Earth and Oceans

from a Correspondent

THE boom in Australian minerals provided the central theme for the international conference on geophysics of the Earth and oceans, held in Sydney from January 19 to 23, when the emphasis was on exploration. Dr F. S. Grant (Kenting Exploration), outlining the role of mineral exploration over the next ten years, stressed that, as in petroleum geophysics research in the past ten years, developments in mining geophysics would be due to refinements in data processing and techniques of interpretation, rather than any marked advances in instrumentation or field techniques.

Mr M. Talwani (Lamont–Doherty Geological Survey), who explained the principles of satellite navigation and the future world wide VLF–Omega system, presented case histories which were a favourable comment on the use of such methods in deep water geophysical surveys. The value of remote sensing techniques was brought out particularly by Mr R. Bennett (Geophoto Services), who showed how knowledge of the relative concentrations of isotopes in different formations can be used as an aid to delineating lithology by airborne gamma ray spectrometry.

Induced polarization is a very important exploration tool in Australia. Case histories reviewed by Dr S. H. Ward (University of California, Berkeley) indicated that best resolution could be obtained using the dipole–dipole array, and that the percentage frequency effect was to be preferred to the metal factor as a useful diagnostic parameter. Mr G. Omnes (Compagnie Générale de Géophysique) pointed out that induced polarization is the only satisfactory electrical conduction method to delineate massive and disseminated sulphides in areas of West Australia where there are low resistivity overburdens.

When attention turned to magnetism, Dr N. C. Steenland (GAI–GMX, Texas) showed how successful aeromagnetic surveys have been in the search for petroleum fields. He has found that an interpretation based on raw magnetic data is often more significant than results obtained from complex data analysis. Mr G. A. Young (Bureau of Mineral Resources) derived a geological map from airborne magnetic data, and on it the disposition of greenstone belts was clearly indicated, highlighting the interesting areas where the search for base metals should be concentrated.

Naturally, no geophysical conference would be complete without a discussion of continental drift. A special session on rock and palaeomagnetism was introduced by Professor S. K. Runcorn (University of Newcastle upon Tyne) with a general lecture on continental drift, mantle convection currents and tectonics. Mr G. Luck and Mr M. W. McElhinny (Australian National University) presented the first definitive palaeomagnetic results for Australia and India for the Lower Palaeozoic era. Their reconstruction of Gondwanaland was in general agreement with previous theories.

•

COMPOSITE MATERIALS

Strong Fibrous Solids

from our Materials Science Correspondent

THE growing interest and importance of strong fibrous solids were underlined by the Royal Society's discussion on this topic held in London on January 29. Mr W. Watt (Royal Aircraft Establishment, Farnborough) summarized the structural changes believed to take place in polyacrylonitrile (PAN) fibres during progressive heating in oxidizing or neutral atmospheres, with or without tensile stress, and the associated changes in properties at each stage. He emphasized that the statistical variability in tensile strength depended substantially on sample length, which indicates the importance of defects in limiting the strength. It is uncertain whether the vital defects are the inclusions, or the elongated pores recently established by diffuse X-ray scattering and electron microscopy. Watt stressed the paucity of knowledge about structural changes in PAN, for example, concerning the degree of crystallinity induced by drawing, and called for intensive investigation. The opinion was often expressed at the meeting that the technology of fibre composites has too far outstripped scientific understanding.

Dr R. J. E. Glenny (National Gas Turbine Establishment, Farnborough) recounted systematic experiments on the use of fibre composites based on refractory matrices specifically for use in gas turbine blades. On the basis of the criteria of density, strength and chemical compatibility, attention was focused on a nickel-base matrix with wires of tungsten or tungsten/rhenium alloy, Mo-RTZ alloy or alumina. Vacuum-casting of blade shapes in a cored shell-mould gave excellently uniform filament distribution and it proved possible to incorporate clear channels for coolant. Tungsten and alumina were the most promising reinforcements. Strength levels were marginally less than predicted by the rule of mixtures, and fatigue behaviour was little affected by the presence of filaments. It is too soon to be sure of the future scope of these techniques, but in view of the fact that niobium and chromium alloys are not considered to be equal to expectations filament-reinforcement seems to be the most promising way to improve on the intrinsic properties of nickel superalloys.

Dr J. Nixdorf (Battelle-Institut, Frankfurt) described the pilot-plant production of various strong metallic microwires, including wire of a marageing steel. The technique involves melting a metal rod held in a viscous glass mantle (which acts as both container and lubricant) and rapidly drawing the assembly through a die. Afterwards the glass covering is removed. The strength is attributed not only to the small diameter of a few microns but also to the rapid cooling. A particularly small statistical variability of the wire strength was claimed, and the mean strength exceeded that of boron but was less than that of E-glass. Little was said about stiffness. Various composites have been made with both metallic and polymeric matrices, and for concentrated composites a UTS better than for boron or carbon reinforcement was claimed. The production cost of microwires (on a scale resembling the present scale of production of carbon fibres) was assessed at some tens of pounds sterling per kg. Oddly enough, the audience made no reaction to these intriguing disclosures.

Describing the development of 'Hyfil' (carbon fibre-reinforced) compressor blades in Rolls-Royce jet engines, Mr J. I. Goatham (Rolls-Royce, Derby) emphasized the extensive testing procedures needed, both for development and for routine testing, including such exotic methods as holography. He also mentioned the great importance of joints and attachments, where critical losses of weight advantage can easily be sustained by faulty design. He was the only speaker to emphasize the joining problem, though the chairman, Dr A. H. Cottrell (Cabinet Office, London), expressed the view that this central problem was well on the way to being vanquished.

Dr F. J. McGarry (Massachusetts Institute of Technology) reported work on epoxy resin toughened by elastomer dispersions—following the example of rubber-reinforced polystyrene described at the Royal Society in 1963. The impressive increase in fracture toughness which was achieved seemed to depend in part on achieving an optimal degree of cross-linking of the elastomer, as well as a bimodal distribution of coarse and fine elastomer particles. McGarry postulated that coarse particles favour crazing of the matrix, which absorbs energy, while small particles promote shearing of the polymer matrix, which again absorbs energy. The ultimate aim is to use the toughened polymer as a matrix for fibre-reinforced building materials. The potential of the building industry as a consumer of composites was stressed by Dr A. J. Majumdar (Building Research Station, Watford), who showed how concrete or plaster panels could be toughened by incorporating (through a co-spraying technique) fibres of special zirconia-bearing glass which can stand up to the corrosive alkaline environment in concrete (there is no special problem with plaster). Majumdar explained that plaster panels thus reinforced will stand up to the repeated impact from primary school children.

Dr A. Kelly (National Physical Laboratory, Teddington) continued the theoretical approach by analysing the dependence of work of fracture on the length and diameter of fibres. It transpired, to the surprise of many, that from this point of view thick fibres were preferable. In brittle/ductile composites, thick fibres favour plastic deformation over large volumes of a ductile matrix, while in brittle/brittle composites, thick fibres suffer pull-out over greater lengths, with the increased associated frictional energy loss. Thus two physically quite distinct processes between them lead to a law of general validity for fibre composites.

Professor F. C. Frank (University of Bristol) assessed the ideal stiffness of a carbon chain by various means (infrared spectroscopy, calculations from the characteristics of diamond and so on) and was led to a value of 2–2·5 Mbar. The best value obtained in practice is apparently about ten times less than this, though it might have been predicted that hard-drawing, of nylon, for example, should reduce this factor of disparity. Frank went on to describe some special structures which seemed to consist of a stiff polymeric spine with built-in polymeric beads, which might open the way to a polymer–fibre-reinforced polymeric matrix. He predicted that quite soon the polymer industry, in search of higher strength, would turn to more elaborate production methods, even though this would diminish one of the chief advantages of polymers, their cheap fabrication.

Will your mind stop growing?

Will your mind stop growing when you've left university? Or will you remain flexible enough to join a company which is at the centre of change: inventing, developing and improving its products and processes all the time?

Flexibility, talent and a taste for responsibility is what ICI looks for. And if you've got it, you can go to the top with it.

Each year ICI selects about 500 graduates from British universities. That's how many are needed to maintain the present rate of diversification.

Our research, design, development and production departments demand graduates from nearly all scientific and technological disciplines: while graduates in any subject, including arts and social sciences, are considered for sales, marketing, management services, personnel, purchasing and distribution.

But that is just where it starts. Your degree subject will not limit you: peoples' talents develop in many different types of job and in many different places.

That's why, for example, you may find engineers, historians, chemists, physicists and specialists in other disciplines, all working together – building a factory in Holland, selling paint in Peterborough, giving technical advice to customers in Istanbul.

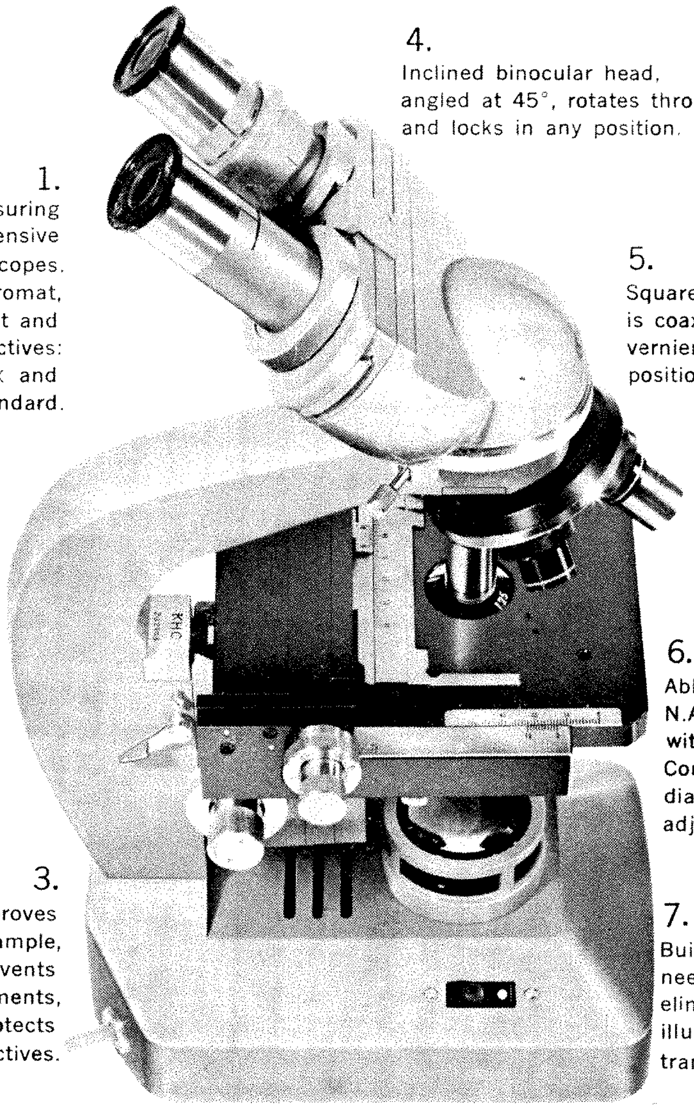
Why not talk to us?

Each spring term ICI representatives visit your university. Ask your Appointments Officer to fix up a meeting for you and learn about the opportunities which ICI can offer you.

Central Personnel Department,
Imperial Chemical Industries Ltd.,
Millbank, London, SW1.

PR500/6

Olympus KHC is top choice for latest features and low budget



4.
Inclined binocular head, angled at 45°, rotates through 360° and locks in any position.

1.
Wide field or measuring eyepieces as in expensive research microscopes. Highest quality Achromat, Plan Achromat and Fluorite objectives: Achromat 4×, 10×, 40× and 100× (oil) standard.

5.
Square mechanical stage is coaxially-controlled and vernier calibrated to give exact positioning of slides.

2.
Fine and coarse focusing knobs are coaxial and positioned low for ease of operation.

6.
Abbe type condenser, N.A: 125, is focusable with rack and pinion. Continuously variable iris diaphragm allows adjustment of illumination.

3.
Modern design improves handling ease. For example, vertical stage focusing prevents distracting head movements, and pre-set focus lock protects slides and objectives.

7.
Built-in 20-watt illuminator needs no centering, eliminates accessory illuminators and transformers.

Olympus KHC is the latest improvement for everyday laboratory work, a microscope for the lowest budget and still featuring eye-fatigue-free clearness and high precision. Its seven outstanding features have made KHC the most used and valued instrument for medical students and laboratory workers all over the world.
Total magnification: 40x~1000x

OLYMPUS TOKYO **OLYMPUS**

OLYMPUS OPTICAL CO., LTD. 7, 3-chome, Kanda-Ogawamachi, Chiyoda-ku, Tokyo, Japan
OLYMPUS OPTICAL CO., (EUROPA) GmbH. 2, Hamburg 1, Steindamm 105, Germany
OLYMPUS CORPORATION OF AMERICA 2 Nevada Drive, New Hyde Park, N.Y. 11040, U.S.A.

Problems of Expanding Populations

by
D. WOLFERS
Population Bureau,
Eland House,
London SW1

The most serious demographic problem at present is in the developing nations, where populations are growing at a rate of between twenty and forty per thousand per year. Many programmes of birth control are hampered by factors such as child mortality and cultural traditions, but this survey of the world situation shows that these problems are not insuperable if tackled realistically.

THE current human ecological crisis derives from the great technical advances by which man has placed himself in the position of a (self)-protected species for whom natural ecological regulation is suspended. Inevitably his numbers have increased to the point where all the resources of the Earth cannot supply his wants.

There have been four distinct forms of organization of human life—arboreal, pastoral, agricultural and technological—and each change from a more primitive to a more advanced organization has been accompanied by the disturbance of an established population balance, followed by a phase of rapid growth, and then the re-establishment of demographic stability. We are at present involved in the latest of these transformations and, with the limited perspective of the human life-span, have difficulty in seeing how it will end.

In each of the three past states of demographic balance, corresponding to arboreal, pastoral and agricultural society, that balance was determined by natural principles so that, growth being inhibited by the unchanging abundance of resources, death rates automatically adjusted to the prevailing birth rate. This adjustment did not mean that birth and death rates were the same every year, but there were periodic peaks of deaths, brought about by famine, epidemic or war, which irregularly restored a supportable population density (Fig. 1). The current imbalance is likely to be resolved on similar terms if direct action is not taken to avoid it. But we now have the technical and social means of restoring a balance on terms more acceptable to human aspirations than the natural balancing mechanism.

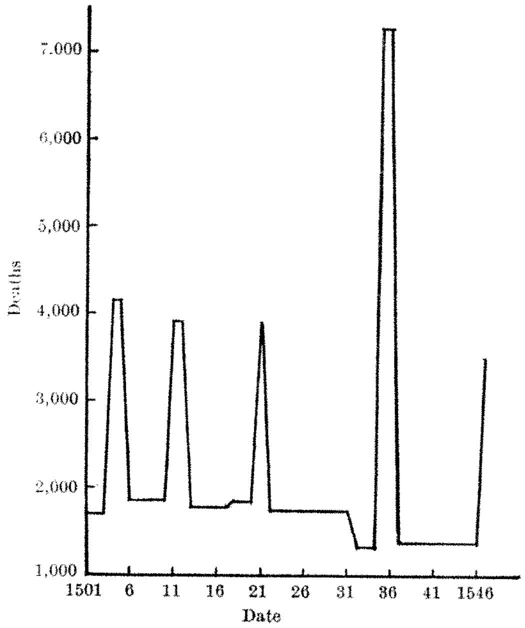


Fig. 1. Annual deaths in Augsberg from 1501 to 1547. From ref. 1.

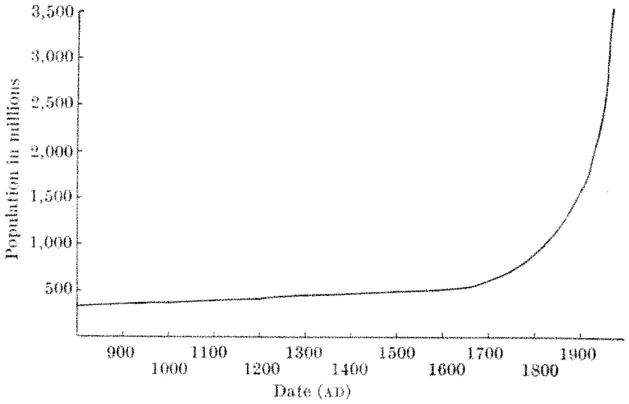


Fig. 2. The growth of the world population from AD 800 to 1969.

Population trends in the 10,000 years of the agricultural phase (Fig. 2) show that throughout this period minor improvements in the availability of resources made possible slow but distinct growth of world population at a rate somewhat less than one per thousand per year. Meanwhile, high birth and death rates (about forty per thousand) prevailed constantly, and populations were everywhere and at all times "young" (Fig. 3).

Early Disturbances of Balance

The first stirrings of disturbance of this balance are now seen to have begun in about 1650 when, in response to quite small improvements in agricultural practice, and perhaps also political organization, the rate of growth of world population more than trebled to about three per thousand per year. This change occurred simultaneously in Europe and Asia. In 1750, with the agricultural revolution in full swing and the effects of the industrial revolution beginning to be felt, it is clear that a major discontinuity had occurred in those countries that were affected; growth rates started increasing to levels like ten to fifteen per thousand per year. By now, production was leaping ahead of consumption and the determining factors of rates of

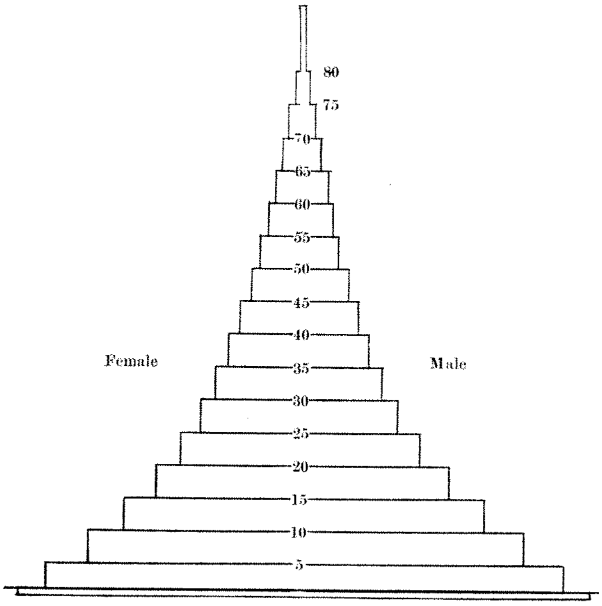


Fig. 3. The age–sex distribution of the population of Mexico in 1966. The total population was 44,145,000.

growth ceased for the time being to be economic and became biological; the link between birth and death rates was broken and they were able to act as independent variables.

The first effect of this change was the disappearance of mortality peaks. Food production and distribution had so improved that adverse seasons no longer produced famine, and nutritional standards had so changed that even though conditions of crowding and sanitation may have been worse than ever, epidemic disease was no longer able to decimate populations. Political organization had become so macroscopic that the toll of warfare became too selective to have important demographic consequences. Next came a steady decline of annual death rates in response to innovations in environmental sanitation and curative medicine and rising standards of nutrition—a process still continuing although with diminishing returns. The third effect, delayed for more than a century after the initiation of the first, was the long steady decline in birth rate, stretching in the United Kingdom over the whole period 1880–1930. No contemporary analysis of the causes of this decline exists, but it is safe to say that it reflected the adjustments made by individuals to radical changes in conditions of economic and social competition. An important secondary effect of this decline in birth rates was the transformation of the population to an older age-distribution (Fig. 4), with a consequent braking effect on the decline in death rate and a temporary alleviation of the dependency burden.

From 1930 onwards little change has been registered in the demographic indices of developed countries, growth rates fluctuating between three and ten per thousand per year, with manifest full independence of death rates from birth rates.

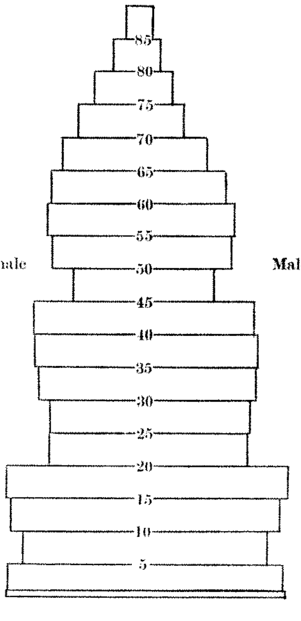


Fig. 4. The age–sex distribution of the population of France in 1965. The total population was 48,918,697.

The Developing World

Although such high growth rates cannot continue for ever, they can be sustained for some generations without bringing inevitable disaster. The current crisis arises from a recognition of the potential consequences of continuation of the quite different trends in the developing world.

By the beginning of the twentieth century, moderate growth rates of about ten per thousand prevailed in most agricultural lands. This situation reflected the pacification, aggregation and administration imposed by the colonial powers and tutelary agents. Public health advances, particularly in tropical medicine, began to reduce the force of epidemic scourges, and the concept of famine relief made some progress. It is, however, only since the Second World War that a manifestly insupportable imbalance has been recognized. Countries which in 1939 were economically and socially stagnating in subsistence agriculture emerged in 1945 with political groups determined to speed them into the maelstrom of the modern world. Not only was production set for continuous expansion and mechanization, but a new spirit of interdependence led to the limited redistribution of foodstuffs under the aegis of United Nations agencies and with the help of food surpluses in advanced countries. Although violence was involved in the achievement of political independence by many developing nations, the previous pattern of recurrent tribal raiding was prohibited by the consolidation of what were in pre-colonial days dozens or hundreds of autonomous groups into large nation states. With neither famine nor war to restrain numbers, the importance of the spectacular application of preventive medical techniques (often, as with DDT and penicillin, developed during the Second World War) was enhanced, and death rates declined throughout the world at an unprecedented rate.

Rate of Growth Today

Fig. 5 illustrates the mondiality of this trend, and its size. Growth rates in developing countries at present vary between twenty and forty per thousand per year, representing, on exponential projection, periods of doubling of between 18 and 35 years. The most generally accepted estimate of the current growth of world population is twenty-one per thousand (doubling every 33 years) with a strong accelerating trend (Fig. 2). Estimates of the probable future of development of world population

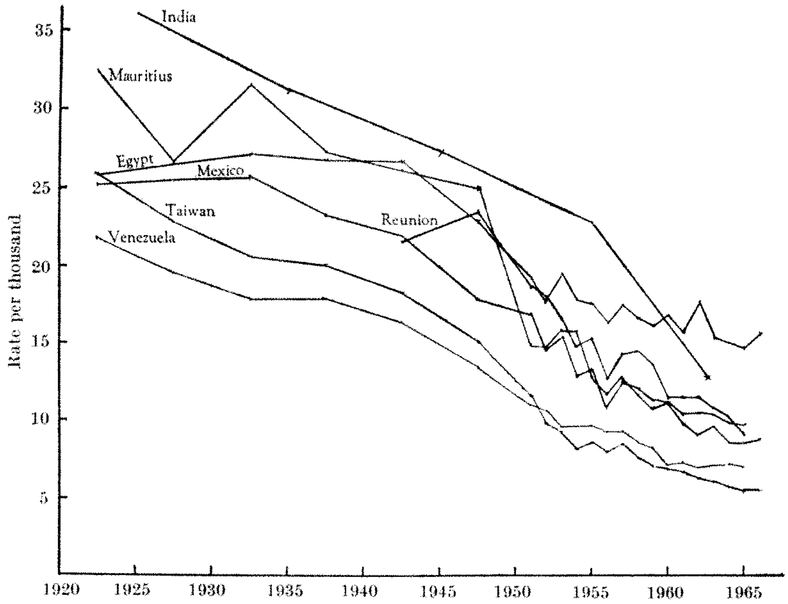


Fig. 5. Crude death rates of selected countries, 1920 to 1966. From ref. 2.

prepared by the UN Demographic Division suggest a total of 7,000 million by the year 2000 compared with the present 3,500 million.

The expansion of a species ten-fold in a mere fourteen generations is, of course, a remarkable success story—in view of the complexity of human requirements it is almost a miracle of adaptation. Furthermore, there can be no doubt that when the limits of sustenance are reached the increase will be arrested by the customary balancing mechanisms of ecological logic.

Although some people believe that the problem can therefore be safely left to solve itself, the likely resolution without deliberate action is not the introduction of low birth rates but the restoration of high death rates. The spontaneous orderly unfolding of a demographic-economic transition is most improbable in the circumstances of the developing world, for the cause and effect sequences which led to the slowing of European growth are all running in reverse. Population growth, death control and the expansion of the labour force are proceeding rapidly while those whose job it is to increase economic growth, birth control and employment opportunities strive to keep pace. The problems of capital formation are particularly acute, for not only are the demands for capital inputs to support the expanding population crippling, but the ruthless reservation of resources for capital at the expense of consumption, which darkened the lives of town dwellers in nineteenth century Europe and starved the peasants of Russia in the 1930s, is no longer morally acceptable. By increasing the denominator of every fraction, be it literacy rate, doctor-patient ratio, proportion of labour force gainfully employed, or income per head, a rapid rate of population growth leaves government and people producing frantically in order to maintain their situation. Although economies of scale may accrue as the population increases in some countries, in most the scale is already so great that no real effect of this kind can be demonstrated. While in some countries with vast hinterlands no perceptible change is taking place in the ratio of population to natural resources, in others this ratio is becoming critically high, and in yet others, principally small islands, actual space is running out. Because the flow of international migration is blocked in almost every channel and will surely remain so, these islands are experiencing the crisis in its most acute form. Because of these considerations, if no action is taken to limit population growth it is likely to continue with no substantial change in standard of living or national economy until the problem becomes too great to cope with and the system itself collapses under the administrative burden of numbers and the disappointment of perpetual deferment of hopes. If this should happen, large tracts of the Earth will return to jungle law, de-population will take place and an entirely new set of problems will arise.

This catastrophe is not an immediate prospect, nor likely to occur on a world-wide scale. Some favoured developing countries, such as Singapore, Venezuela and Israel, have rates of economic growth large enough to sustain the chain reaction of development long enough to produce the social changes which we assume to be sufficient to stimulate spontaneous fertility limitation; others are sufficiently sparsely populated in relation to their resources to be able to grow rapidly for a very long time before there is a qualitative change from rural poverty to chaotic destitution. But these countries will, if they do not act soon to limit growth rates, have missed the only foreseeable opportunity to build graciously advanced societies free of the tragic squalor which has accompanied industrialization and demographic transition in the western world.

World Situation

While each country faces its own population dilemma, the world as a whole is not exempt. The "green revolution" has given a temporary advantage in the race to keep world food supplies from becoming yet more inadequate to meet growing needs; modern public health makes it difficult to visualize uncontrollable pandemics sweeping the world; nuclear weapons appear at present to be held under firm restraint. The crucial areas of world concern are now atmospheric pollution, shortage of water and the potential exhaustion of minable minerals. In these respects we live in a fool's paradise where each has faith in the ability of the others to solve problems too difficult for himself. Aesthetically too, and in relation to the intangible "quality of life", more and more people, unable to visualize institutions which can preserve what man has held valuable in the past into a teeming future, view that future with perhaps unwarranted despair.

Action is, however, being taken. In almost all cases this takes the form of family planning programmes, fully organized by government, supported by government or permitted by government. The pattern of introduction of these programmes was, only 10 years ago, a rather unedifying spectacle in which enthusiastic voluntary agencies seemed to drag suspicious governments into a series of tentative steps. Only after feeling their way for some years did governments come to embrace programmes enthusiastically. This situation is now changing, and genuine government leadership is appearing in an encouragingly large number of countries.

Government reluctance, and in places opposition, stems from several considerations, of which the fear of provoking opposition and courting unpopularity is the least realistic and the most important. It is in dispelling the myth that most peasant populations would be outraged by exposure to birth control services and propaganda that the social survey has made its outstanding contribution to the solution of the problem. Sample surveys, referred to as KAP surveys (knowledge, attitude and practice), conducted in dozens of different settings, seem to have uncovered a very strong demand—commonly 70 to 80 per cent of female respondents—for the facilities for controlling fertility, and to have demonstrated that, almost regardless of the situation prevailing, most couples want relatively small families (three or four children). There have been some exceptions to these generalizations, particularly in tropical Africa.

Difficulty also arises in countries where political power is shared between two or more ethnic or tribal groups of approximately equal numbers. In these circumstances each group fears that their own members, by adopting family planning, will encompass their ultimate political extinction. The view is quite widely and influentially held in Africa and Latin America that "population control for the developing countries" is a manifestation of similar tribal thinking on a global scale by the developed countries, and this naturally is responsible for bitter opposition.

The unreal basis of official Roman Catholic thought on the subject hinders developments in predominantly Catholic countries, but family planning programmes with declared health and welfare objectives substituted for demographic aims are making headway.

Outcome of Programmes

It is too early to make more than the most tentative predictions of success or failure for family planning programmes, and it is likely that some will be successful and others fail. The population control objective requires that two stages be accomplished. First, the adoption of birth control by most of the population efficiently enough to ensure that births correspond closely with numbers desired, and, second, the reduction of numbers desired to the level where development success is assured.

The first is certainly an easier objective to attain than the second, but, except in a few cases, which as they appear come to be described as special, there is little encouraging to report yet. For reasons related to the austerity of Christian morality, contraceptive technology has received far too little attention from scientists in the past. The crisis has arrived when we have no satisfactory techniques of contraception to offer the clients recruited in these programmes. Methods which have proved successful for highly motivated western populations are either too difficult or unpleasant for the mildly motivated to use regularly (diaphragm, condom); have too many unwelcome side-effects (IUD, pill, injection); require an unrealistic degree of cooperation (pill) or are too ineffective (coitus interruptus, spermicidal preparations). Although many millions in the developing world are struggling to use these methods, follow-up studies show that no more than half are able to persevere with any one method for a prolonged period. The techniques of sterilization, particularly of males, are much more satisfactory, but their adoption requires a finality of decision which few can muster until their family sizes are already excessive. The uncertainty of survival in areas where rates of infant mortality are high is, of course, a further deterrent.

The demographic history of Japan in the 1950s and contemporary East Europe shows that the practice of abortion alone is capable of producing dramatic effects on the birth rate, but the applicability of this to less developed countries is dubious. Neither the level of motivation nor the availability of medical services is of the same order in most of these. Additionally, stubborn adherence to rigid interpretations of the sanctity of life, particularly in Moslem and Catholic countries, rules out this method at present.

It is not known whether the availability of more acceptable forms of contraception (and much current research is being devoted to their development) would have a favourable effect on the numbers of couples adopting contraception, but it is certain that it would enhance the effectiveness of practice of those who do.

The discrepancy between the proportions of any population studied who assert their willingness to practise contraception and those who actually do so when it is offered is very large, and offers a great challenge to the social scientist and psychologist to unravel the complexities of decision taking involved. One of the least understood factors is the part played by males in this process.

Family planning programmes with demographic objectives are recent innovations. The earliest was in 1952 (India) but remained in very low gear till 1964. The real history of such programmes dates back only to 1962. So far very few participating countries have recorded substantial gains, and there is no lack of critics who attribute these to factors other than deliberate programmes. Although no change has been recorded in the birth rates of countries such as India and Pakistan, it should be remembered that in these countries vital registration is grossly defective, that changes in the age structure of the population are producing conditions favourable to increasing birth rates and that millions of couples have attempted birth control for the first time during the past few years.

High Fertility Favoured

The complementary task of introducing the "small family norm" has, in spite of considerable effort, met even less demonstrable success. While families of three children or fewer "fit" urban, industrialized societies which put premiums on higher education and provide comprehensive welfare services, it is the large family which until recently has enjoyed advantages in the rural environment which still prevails for 80 per cent of people living in developing countries. Free labour for the farm and the security of numbers and continuity in ill health or old age were the principal economic gains, while the extended family system spread the very light burdens of child-rearing in such a way that each additional child was almost no extra burden. Because very high levels of child mortality prevailed until recently, it is not surprising that a mutually supporting structure of social, moral and religious doctrine has developed to protect and advance the ideal of high fertility. It matters little that, after a generation of rapid increase, fragmentation of farms has become more important than labour supply, and after a generation of independence the burden of education is growing to rival the benefits of mutual support; the time scale of change in social and economic institutions is now far more contracted than that of change in cultural outlook. Automatic re-acculturization cannot be relied on, and the task of changing attitudes falls to the propagandist, the educator, and perhaps soon the tax gatherer.

The "small family norm" will not, of course, make any headway where extreme levels of child mortality prevail, so that a precondition of lowering birth rates is the necessity to lower death rates. The most urgent preparatory task is to improve the coverage and standards of maternal and child health services in rural areas of the developing world; this is, of course, enormously costly. It is only, however, in the context of a secure and comprehensive health service that family planning can cease to be a foreign gimmick and become a settled part of life.

Campaign Media

Posters, films, radio talks and earnest face to face encounters multiply throughout the developing world to bring the message that two or three children are enough, but, in the hectic urgency of this campaign, time has not been found to evaluate the efficacy of the media used. The possibility of attempting to indoctrinate school children is at last being canvassed, but there is resistance because of the sexual component involved. School activity, to be effective, must take place in primary schools, because too few children attend higher grades for a campaign to have worthwhile results. Numerous suggestions for giving financial incentives to parents to have small families or imposing penalties on those who have large ones have been made. Almost without exception* these prove relatively easy to implement in the highly organized money economy of an industrial state, which does not need them, but quite impossible in areas of subsistence agriculture where taxpayers form a small élite of the population. Meanwhile, the world is not ready to surrender another of its primitive freedoms by accepting the introduction of licences for childbirth.

If no way has been found to manipulate family size norms, the same may be said for the determination of optimum levels to aim for. Although in a technologically stagnant world, with abundant space and goods, a case might be made for aiming at a zero rate of growth, this magic figure has no applicability to any actual situation in the world today.

Although many programmes express their objectives in terms of the attainment of specific rates of growth by a certain date, few if any of these objectives have a more rational basis than the hope that the objective can be attained. So many ecological studies have been made too late that there is clearly a great urgency for the revival of studies of optimal population growth so that, when conditions are achieved which permit the induction of massive changes in fertility, a rational policy will be possible.

The situation at present is undeniably confused, with hope and despair about evenly balanced. This is inevitable in the first decade of serious attempts to deal with a problem of this size. Clear outlines of the tasks to be performed are, however, beginning to emerge. Of these, surely the most difficult and most important is to reduce child mortality as far as possible and provide a comprehensive network of health services throughout the developing world. This is the carrier wave for broadcasting family planning. A process of re-acculturization must be accomplished, and this implies indoctrination of populations at impressionable ages—in early school years, and as a corollary it implies bringing the whole child population to school. To these extents, medical and educational, population control depends on progress in development. There are, however, activities which may bring results in relatively unpromising situations. It is agreed that too frequent child-bearing is physically harmful to women, and this may form the basis for the adoption of a more authoritarian attitude to the prescription of contraception by members of the medical profession and their agents. Were the provision of contraceptive techniques as routine a part of the after-care of childbirth as iron for the treatment of anaemia, or insulin for diabetes, great results might be achieved even in areas now hopelessly unprepared for radical social change.

Impact of Abortion

The liberalization of abortion laws will likewise have an impact, although necessarily one which is dependent on the availability of competent services to perform operations. Meanwhile the search for reliable and safe medical abortifacients must be pursued, as must the search for better contraceptives. For all its moral dubiety, abortion enjoys the enormous psychological advantages of the nine late over the timely one stitch.

It is not easy to assess the ultimate impact of the torrent of "educational" material now inundating adult populations in the developing world. There are good reasons to believe that this insistent reiteration may, after a relatively long time, quite suddenly bring about a change in popular attitudes. Familiarity and legitimation are powerful agents for the dissolution of resistance. Patience is therefore important.

If we look more than a generation or two ahead, we are forced to come to terms with the ecological basis of the problem. At present the human race is appalled by the recognition of its dramatic rise to maturity with the accompanying achievement of the potential for self-destruction, for irretrievable pollution of the environment, and for exhaustion of key materials as well as for overcrowding itself. An age of centralized planning is on us as part of the unavoidable logic of development and progress. That planning already involves production and agriculture, education and welfare, research and development, distribution and transportation, the siting and distribution of population and countless other aspects of our lives. It is inevitable that it will soon come to embrace the numbers and then the quality of population, for all else will come to depend on these.

---

* In the highly organized economy of Communist China a system of economic sanctions seems to have been successfully imposed.

[1] Sussmilch, J. P., *Die Göttliche Ordnung in den Veränderungen des Menschlichen Geschlechts* (Berlin, 1765).

[2] *UN Demographic Year Book*, 348 (1967).

RNA Polymerase σ-Factor and the Selection of Initiation Site

by
M. SUGIURA
T. OKAMOTO
M. TAKANAMI
Institute for Chemical Research,
Kyoto University, Kyoto

RNA polymerase lacking σ-factor can transcribe the replicative form of phage fd DNA quite well, but loses the ability to select the appropriate initiation sites on the template, and both strands are transcribed. When σ-factor is added, the specificity and asymmetry of transcription are restored. The σ-factor restricts initiation to specific sites on the DNA template.

BURGESS *et al.*[1] separated *Escherichia coli* RNA polymerase into a core enzyme and a σ-factor by chromatography on phosphocellulose. The factor appears to be identical with the "γ-protein" described by Krakow *et al.*[2]. RNA synthesis on certain templates such as the DNA of bacteriophage T4 was greatly stimulated by addition of σ-factor to the core enzyme. This discovery led to a number of studies suggesting that the σ-factor acts at the level of initiation of RNA synthesis[3–7]. Burgess *et al.*[1] have shown, however, that the core enzyme alone can transcribe calf thymus DNA quite well. This is also the case with the circular double stranded replicative form DNA (RF-I DNA) of phage fd, which was the subject of this study. The species of RNA transcribed on RF-I DNA *in vitro* by the undissociated enzyme have been well characterized[8,9]. Accordingly, we analysed the starting nucleotide sequences as well as the size of the RNA synthesized in the presence and absence of σ-factor. Like the complete enzyme, the core enzyme could initiate RNA synthesis with purine ribonucleoside triphosphates using RF-I DNA as a template. The core enzyme, however, transcribed both strands of the template, and the reaction products were very heterogeneous, in both their starting sequences and their size. If σ-factor was added back, the limitations on transcription were restored and only the minus strand served as a template.

RNA polymerase was prepared from *E. coli* A19 and purified by sucrose density gradient centrifugation[8]. The enzyme was then fractionated into the core enzyme and σ-factor by phosphocellulose column chromatography, according to the method of Burgess *et al.*[1]. RF-I DNA was prepared from *E. coli* K38 cells infected with phage fd as described previously[8]. T4 DNA was prepared from purified phage T4 by the method of Richardson *et al.*[10]. The methods for analysing the starting nucleotide sequences and for measuring the size distribution by band centrifugation have been described[8,9].

Kinetics of RNA Synthesis

The addition of the σ-factor to core enzyme greatly stimulated the RNA-synthesizing activity with T4 DNA and *E. coli* DNA as template (Table 1). By contrast, the core enzyme alone was able to transcribe RF-I DNA with about half the efficiency of the complete enzyme. The difference in template response became more striking at low substrate concentration. A double reciprocal plot of RNA synthesis as a function of the substrate concentration (Fig. 1) was nonlinear, as also noted by Anthony *et al.*[11], who suggested that the concentration of substrates affects both the initiation and polymerization steps. When the concentration of one nucleoside triphosphate was varied and the other three substrates kept at a high concentration (0·4 mM), the double reciprocal plot became nearly linear. The plots for ATP and GTP are shown in Fig. 2. With the complete enzyme, the apparent K_{m} values were low and almost identical for T4 and RF-I DNA templates. By contrast, high apparent K_{m} values were found with the core enzyme, and the values with T4 DNA were conspicuously higher than those with RF-I DNA. The difference in the stimulatory effects of σ-factor on transcription of T4 and RF-I DNA may be a reflexion of these K_{m} values. A detailed study of the kinetics and their implication will be published elsewhere. Vogt[7] suggested that breaks in the DNA template stimulate transcription by the core enzyme. This is not the case with RF-I DNA, for RF-I DNA remained unchanged during transcription[9].

Table 1. STIMULATING ACTIVITY OF σ-FACTOR ON VARIOUS DNA TEMPLATES

DNA	AMP incorporated (nmoles)	
	Core	Core + σ-factor
T4	0·51	5·8
E. coli	0·27	2·8
RF-I DNA of fd	2·7	4·6

The assay mixture (0·5 ml.) contained 40 mM Tris-HCl (*p*H 7·9), 8 mM $MgCl_2$, 40 mM KCl, 0·4 mM each of CTP, UTP, GTP and ^{3}H-ATP (generally labelled, 1,000 c.p.m./nmole), 5 μg DNA and 5 μg core enzyme. About 3 μg of σ-factor was added. Following incubation for 10 min at 37° C, the reaction was terminated by adding 10 per cent trichloroacetic acid–2 mM pyrophosphate, and the ^{3}H-AMP incorporated was assayed by a 'Millipore' filter method.

The 5′-Termini of Products synthesized with and without σ-Factor

Incorporation of the four γ-^{32}P-labelled nucleoside triphosphates into the 5′-termini of RNA was studied. RNA chains formed by the core enzyme were initiated

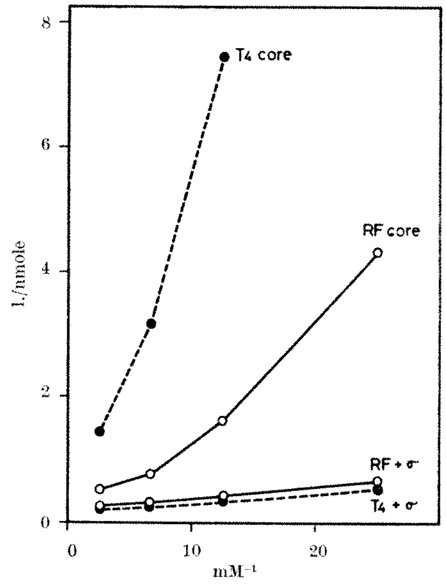


Fig. 1. The effect of varying the concentration of all four nucleoside triphosphates on the rate of RNA synthesis. The assay mixture (0·5 ml.) contained 40 mM Tris-HCl (*p*H 7·9), 8 mM $MgCl_2$, 40 mM KCl, 10 μg DNA, 5 μg core enzyme and increasing concentrations of GTP, CTP, UTP and ^{3}H-ATP from 0·04 mM to 0·4 mM. About 3 μg of σ-factor was added. Following incubation for 10 min at 37° C, the reaction was terminated and the ^{3}H-AMP incorporated was assayed as in the legend to Table 1.

predominantly with purine nucleoside triphosphates (Table 2). The ratio of the terminal ATP to GTP incorporated was similar to that for synthesis by the complete enzyme.

Table 2. INCORPORATION OF NUCLEOSIDE γ-^{32}P-LABELLED TRIPHOSPHATES INTO RNA

Enzyme	Nucleoside γ-^{32}P-triphosphates incorporated (pmoles) CTP	GTP	UTP	ATP	^{3}H-AMP incorporated (nmoles)
Core	0·9	7·2	0·7	4·3	2·4
Core + σ-factor	1·5	14·0	1·3	9·0	5·6

The assay mixture (0·5 ml.) contained 40 mM Tris-HCl (*p*H 7·9), 8 mM $MgCl_2$, 40 mM KCl, 0·2 mM each of nucleoside triphosphates, including a γ-^{32}P-labelled nucleoside triphosphate (about 10^6 c.p.m./nmole) and ^{3}H-ATP, 10 μg RF-I DNA and 5 μg core enzyme. About 3 μg of σ-factor was added. Following incubation for 10 min at 37° C, the mixtures were passed through 'Sephadex *G*-100' columns (1 cm × 20 cm) to remove radioactive substrates. The RNA fractions were pooled, precipitated with cold 10 per cent trichloroacetic acid, and collected on 'Millipore' filters, and the radioactivity was assayed.

We have shown previously[8] that three different species of RNA starting with the sequences pppApUpGp - - - , pppGpUpAp - - - and pppGpUpUp - - - , are synthesized under the direction of RF-I DNA. Accordingly, RNA labelled with either γ-^{32}P-ATP or γ-^{32}P-GTP was prepared in the presence and absence of σ-factor. Following hydrolysis with pancreatic ribonuclease, the resulting radioactive fragments were resolved chromatographically, as described previously[8]. The results are shown in Table 3. With core enzyme, less than 45 per cent of the ^{32}P applied was recovered in the expected areas (pppApPyp and pppGpPyp), and the greatest amount was found in other chromatographic regions. On adding σ-factor the ^{32}P recovered in the expected pppApPyp and pppGpPyp regions was markedly increased and accounted for more than 70 per cent of the total termini. When RNA formed by the core enzyme (labelled with γ-^{32}P-ATP) was hydrolysed with T1 ribonuclease, only 15 per cent of the radioactivity was recovered in the pppApPypGp region. These results indicate that, although the core enzyme could initiate RNA synthesis with purine nucleotides, the chief products differed in their subsequent nucleotide sequences from those formed by the complete enzyme.

Characterization of RNA Products

Hybridization tests were performed according to the procedure previously described[8]. The RNA synthesized by the core enzyme–σ-factor complex could hybridize only with denatured RF DNA, as observed earlier[8]. By contrast, RNA synthesized by isolated core enzyme hybridized with both the single stranded DNA from mature phage and with denatured RF DNA (Table 4). The amount of RNA which hybridized with the denatured RF DNA was about twice that which hybridized with the single stranded DNA (compared on a molar basis). This indicates that, whereas the complete enzyme selectively transcribes only the minus strand of the template, the core enzyme lacks this specificity and transcribes both strands of the template.

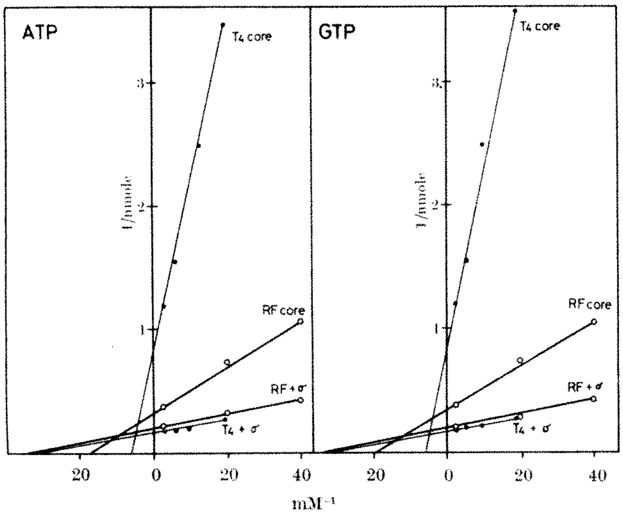


Fig. 2. The effect of varying a single nucleoside triphosphate concentration on the rate of RNA synthesis. The reaction volume, contents and incubation were as described in the legend to Fig. 1, except that one nucleotide, ^{3}H-ATP or ^{3}H-GTP, was varied and the concentrations of the other three nucleotides were fixed at 0·4 mM.

The RNA species starting with pppA - - - has a size of about 26S, and represents nearly one turn of the template[9]. Accordingly, RNA containing γ-^{32}P-ATP was synthesized with and without σ-factor and analysed by band centrifugation. An excess template condition (see ref. 9) was used for the RNA synthesis, because the core enzyme can transcribe both strands of the RF-I DNA template and thus move in either direction on the template. The molar ratio of enzyme to template in the reaction mixtures was estimated to be roughly 1 : 1, assuming that the molecular weights of the core enzyme and RF-I DNA are 4×10^5 and 3×10^6 daltons, respectively.

The sedimentation profiles of the synthesized products are shown in Fig. 3. The undissociated enzyme gave rise to a major peak at about 26S, with a shoulder toward the lighter region (Fig. 3*A*). The shoulder was assumed to correspond to the nascent RNA chains, because the 26S peak became much sharper and the shoulder disappeared when the termini of RNA chains were pulse-labelled as described previously[9]. By sharp contrast with this pattern, the RNA formed by the core enzyme was found distributed throughout the gradient (Fig. 3*B*). Although synthesis of RNA with the core enzyme was performed for an additional 20 min, no increase in ^{32}P at the 26S region was observed. On adding σ-factor to the core

Table 3. RECOVERY OF ^{32}P IN THE FRAGMENTS PRODUCED BY DIGESTION OF RNA LABELLED WITH γ-^{32}P-ATP AND γ-^{32}P-GTP WITH PANCREATIC RIBONUCLEASE

Fragments	(γ-^{32}P-ATP)-RNA Core	Core + σ-factor	Fragments	(γ-^{32}P-GTP)-RNA Core	Core + σ-factor
pppApPyp	44·3	79·1	pppGpPyp	40·3	73·1
pppApPupPyp	13·6	11·6	pppGpPupPyp	13·5	6·8
pppAp(Pup)$_2$Pyp	8·2	4·2	pppGp(Pup)$_2$Pyp	8·4	8·0
pppAp(Pup)$_3$Pyp	12·4	1·6	pppGp(Pup)$_3$Pyp	6·8	4·2
≥pppAp(Pup)$_4$Pyp	21·5	3·5	≥pppGp(Pup)$_4$Pyp	30·9	7·9

RNA labelled with γ-^{32}P-ATP and γ-^{32}P-GTP was synthesized by RNA polymerase with and without σ-factor, as in the legend to Table 2. The products were extracted by treatment with 80 per cent phenol and then passed through 'Sephadex *G*-100' columns (1 cm × 20 cm) to remove radioactive substrates. The labelled RNA preparations were hydrolysed with pancreatic ribonuclease, and the resulting radioactive fragments were separated by 'DEAE-Sephadex' column chromatography according to the procedure previously described[8]. Values are expressed in per cent.

Table 4. HYBRIDIZATION TESTS WITH RNA SYNTHESIZED BY RNA POLYMERASE WITH AND WITHOUT σ-FACTOR

DNA	C.p.m. hybridized* Core	Core + σ-factor
Denatured calf thymus DNA	32	10
Denatured RF DNA	572	623
Single stranded DNA from mature phage	256	25

* Average of two experiments.

For the preparation of ^{3}H-RNA, the reaction mixture (1 ml.) contained 40 mM Tris-HCl (*p*H 7·9), 8 mM $MgCl_2$, 40 mM KCl, 0·4 mM each of CTP, UTP, GTP and ^{3}H-ATP (5,000 c.p.m./nmole), 10 μg RF-I DNA and 5 μg core enzyme. About 3 μg of σ-factor was added. Following incubation for 10 min at 37° C, 10 μg of pancreatic deoxyribonuclease was added, and the incubation was continued for an additional 10 min. The mixture was chilled, treated twice with 80 per cent phenol, and then passed through a 'Sephadex *G*-100' column (1 cm × 20 cm) equilibrated with 0·15 M NaCl 20 mM Tris-HCl (*p*H 7·9). The RNA fraction eluted was collected. For the hybridization tests, the mixtures contained, in a final volume of 1 ml., 0·5 M NaCl, 20 mM Tris-HCl (*p*H 7·9), about 2,000 c.p.m. ^{3}H-RNA, and either 10 μg single stranded DNA from mature phage fd or 20 μg denatured RF DNA (sonicated, heated at 100° C for 10 min and quickly cooled). As control, 20 μg of denatured calf thymus DNA was added in place of phage DNA. The mixtures were incubated for 30 min at 67° C and cooled to room temperature. Pancreatic ribonuclease (10 μg) was added. After 20 min at room temperature, the mixtures were diluted with 0·5 M NaCl, 20 mM Tris-HCl (*p*H 7·9), and passed through 'Millipore' filters (type *HA* 0·45 μm). The filters were washed twice with 5 ml. each of the same buffer, and bound radioactivity was determined.

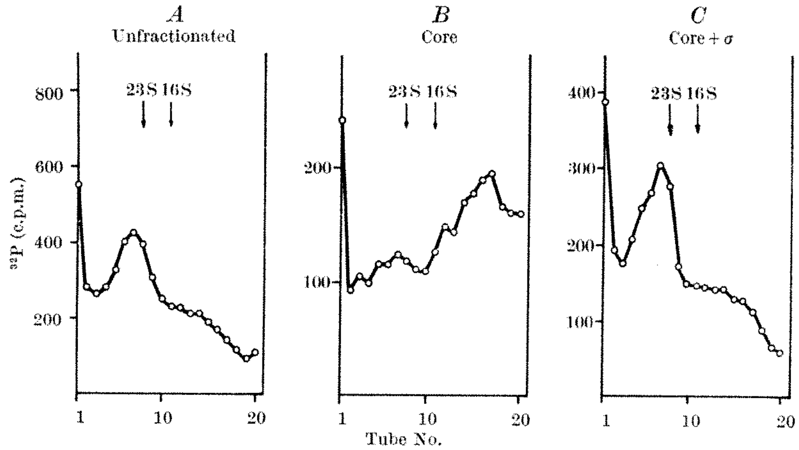


Fig. 3. Sedimentation analysis of RNA labelled with γ-^{32}P-ATP synthesized by RNA polymerase with and without σ-factor. The reaction mixture (1 ml.) contained 40 mM Tris-HCl (*p*H 7·9), 8 mM $MgCl_2$, 40 mM KCl, 0·2 mM each of CTP, GTP, UTP and γ-^{32}P-ATP (10^6 c.p.m./nmole), 20 μg RF-I DNA and (*A*) 10 μg undissociated enzyme, (*B*) 3 μg core enzyme, and (*C*) 3 μg core enzyme + 2 μg σ-factor. After incubation for 10 min at 37° C, the reaction was terminated by chilling. The reaction mixtures were treated with 80 per cent phenol, and then passed through 'Sephadex *G*-100' columns (1 cm × 20 cm). To the RNA fraction eluted, *E. coli* ribosomal RNA was added as marker. The mixtures were placed on sucrose density gradients (5 to 20 per cent in 0·15 M NaCl–20 mM Tris-HCl, *p*H 7·9) formed in Spinco *SW*25 tubes. After centrifugation for 16 h at 25,000 r.p.m. and 5° C, drops were collected from the tube bottom. The radioactivity and absorbance at 260 nm were determined. The arrows in figures indicate the positions of *E. coli* ribosomal RNA.

enzyme, the major peak was again found at the 26S region (Fig. 3*C*). The core enzyme therefore seems to initiate RNA synthesis at random along the template.

Role of σ-Factor in Transcription on RF-I DNA

The primary reactions involved in the initiation of RNA synthesis are the association of RNA polymerase to the promotor sites of the template, and the binding of the initiating ATP or GTP to the DNA–enzyme complex. Like the complete enzyme, the core enzyme alone initiated RNA synthesis with purine nucleoside triphosphates. The RNA synthesized in the absence of σ-factor was, however, very heterogeneous both in size and in the character of the initiating nucleotide sequences. It was also shown that the core enzyme transcribed both strands of the template. Addition of σ-factor to the core enzyme restored the specificity of the enzyme so that it transcribed the minus strand of the template at limited regions.

These observations would imply that the core enzyme has an intrinsic ability to initiate RNA synthesis with purine nucleoside triphosphates by associating with the template. The function of the σ-factor seems to be the prevention of random initiation by the core enzyme and the selection of promotor sites at which synthesis of "sense" RNA is initiated. A similar conclusion has been advanced by Bautz *et al.*[6], who demonstrated that the σ-factor selectively stimulated the transcription of a group of T4 genes which are expressed *in vivo* immediately after infection.

Questions remain concerning the configuration of DNA forming the promotor sites and how these sites are recognized preferentially by RNA polymerase containing σ-factor. It should be mentioned that the σ-factor loses its specificity after storage, though still retaining its ability to stimulate incorporation significantly.

We thank Dr J. S. Krakow, of the University of California, for his interest and for reading the manuscript.

Received December 18, 1969.

[1] Burgess, R. R., Travers, A. A., Dunn, J. J., and Bautz, E. K. F., *Nature*, **221**, 43 (1969).

[2] Krakow, J. S., Daley, K., and Karstadt, M., *Proc. US Nat. Acad. Sci.*, **62**, 432 (1969).

[3] Travers, A. A., and Burgess, R. R., *Nature*, **222**, 537 (1969).

[4] Mauro, E., Snyder, L., Parino, P., Lamberti, A., Coppo, A., and Tocchini-Valentini, G. P., *Nature*, **222**, 533 (1969).

[5] Summers, W. C., and Siegel, R. B., *Nature*, **223**, 1111 (1969).

[6] Bautz, E. K. F., Bautz, F. A., and Dunn, J. J., *Nature*, **223**, 1022 (1969).

[7] Vogt, V., *Nature*, **223**, 854 (1969).

[8] Sugiura, M., Okamoto, T., and Takanami, M., *J. Mol. Biol.*, **43**, 299 (1969).

[9] Okamoto, T., Sugiura, M., and Takanami, M., *J. Mol. Biol.*, **45**, 101 (1969).

[10] Richardson, C. C., Inman, R. B., and Kornberg, A., *J. Mol. Biol.*, **9**, 46 (1964).

[11] Anthony, D. D., Wu, C. W., and Goldthwait, D. A., *Biochemistry*, **8**, 246 (1969).

Inactivation of Prostaglandins by the Lungs

by
PRISCILLA J. PIPER
J. R. VANE
J. H. WYLLIE

Department of Pharmacology,
Institute of Basic Medical Sciences,
Royal College of Surgeons of England,
Lincoln's Inn Fields, London WC2;
and Department of Surgery,
University College Hospital Medical School,
London WC1

Prostaglandins E_1, E_2, $F_{2\alpha}$ and A_2 are enzymically inactivated to different extents during passage through the pulmonary circulation. The enzyme responsible is probably 15-hydroxyprostaglandin dehydrogenase.

Ferreira and J. R. V.[1] discovered that the activity of prostaglandins E_1 and E_2, although stable in blood, almost completely disappeared in a single transit through the pulmonary circulation of dogs, cats and rabbits. This work has now been confirmed and extended by several workers; prostaglandins E_1, E_2 and $F_{2\alpha}$ are almost completely inactivated by the pulmonary circulation of cat, dog, rat and guinea-pig[2-5]. In contrast, the vasodilator activity of prostaglandins A_1 and A_2 survives passage through the pulmonary circulation of the cat and dog[3,4].

The mechanism by which the lungs inactivate prostaglandins has not been defined; this might be by uptake into cells in the lungs, uptake into blood cells or chylomicrons as these pass through the capillaries, binding to plasma proteins, or metabolism. Samuelsson *et al.*[6-9] demonstrated the presence of 15-hydroxyprostaglandin dehydrogenase in lung homogenates and we have now found that the prostaglandins are enzymatically inactivated as they pass through the pulmonary circulation. We have also compared the rates of metabolism for prostaglandins E_1, E_2, $F_{2\alpha}$ and A_2.

Two Types of Experiments

Guinea-pigs (0·5–1·2 kg) of either sex were killed and their heart and lungs were excised. A polyethylene cannula was inserted through the right ventricle into the pulmonary artery and the lungs were perfused with Krebs solution supplied at 10 or 20 ml./min by a roller pump. The Krebs solution was previously gassed with 5 per cent carbon dioxide in oxygen and was warmed to 37° C by a water jacket, except in three experiments in which the temperature was varied. The lungs were initially inflated with air through a cannula in the trachea; this helped to flush out all the visible blood.

The first of the two types of experiments were single pass experiments. In these a known concentration of a prostaglandin was added to the Krebs solution entering the lungs and the prostaglandin emerging in the venous effluent was estimated by bioassay. In some experiments the bioassay was made continuously by superfusing the effluent from the lungs over various tissues[10,11]—a rat stomach strip[12], a rat colon[13] and a chick rectum[14]. In other experiments the venous effluent from the lungs was sampled serially and each sample was then assayed for prostaglandin content on a rat stomach strip suspended in a 5 ml. organ bath, or on a series of organs superfused with Krebs solution. The second type of experiment involved recycling 50 or 100 ml. of Krebs solution (gassed with 5 per cent carbon dioxide in oxygen) from a small reservoir through the lungs for up to 30 min. Inactivation of prostaglandins added to the reservoir was estimated by assay of 1 ml. samples removed from the reservoir at various times.

Samples of perfusate were also collected for examination by thin-layer chromatography on silica gel *G* with the A II or A III solvent systems of Samuelsson *et al.*[6,15].

In twenty-five pairs of guinea-pig lungs prostaglandins were infused into the pulmonary artery and the effluent superfused over assay tissues. The disappearance of prostaglandins E_2, $F_{2\alpha}$ and A_2 in the pulmonary circulation was estimated by comparing the responses of the assay organs to infusions given into the pulmonary circulation or directly to the assay organs (Table 1). With prostaglandin E_2 (0·05–0·5 μg/ml.) a mean of 92 per cent disappeared in one transit through the pulmonary circulation (Fig. 1) and the disappearance was not related to the dose (fourteen experiments). With prostaglandin $F_{2\alpha}$ (0·1–1 μg/ml., six experiments), 90 per cent disappeared in the pulmonary circulation, and with prostaglandin A_2, 64 per cent (0·1–0·2 μg/ml., five experiments).

Recovery of Prostaglandins from Guinea-pig Lungs

To find out whether the prostaglandins which disappeared in the lungs were stored in some way, four pairs of perfused lungs were homogenized in ethanol and extracted. After thin-layer chromatography using the A II solvent system, material was recovered from consecutive regions of the thin-layer plates and resuspended in Krebs solution. Prostaglandins were then estimated on a rat stomach strip and a rat colon. Three of the pairs of perfused lungs had caused the disappearance of about 50 μg E_2, 50 μg $F_{2\alpha}$ and 50 μg E_2 + 50 μg $F_{2\alpha}$ respectively. The quantities of prostaglandin $F_{2\alpha}$ recovered were 200, 210, 100 ng (25, 25 and 13 ng/g wet weight), and of prostaglandin E_2, 125, 50, 160 ng (10, 6 and 20 ng/g wet weight).

The fourth pair of lungs was perfused with Krebs solution without addition of prostaglandins. The left and right lungs were processed separately and prostaglandin E_2 (equivalent to 1 μg/g) was added to one extract at the initial homogenization. Forty-two per cent of this added prostaglandin was recovered in the final assay. The other lung, to which no prostaglandin had been added, yielded prostaglandin E_2 at a concentration of 10 ng/g. These results showed that the prostaglandins which disappeared in the lungs were not taken up and stored.

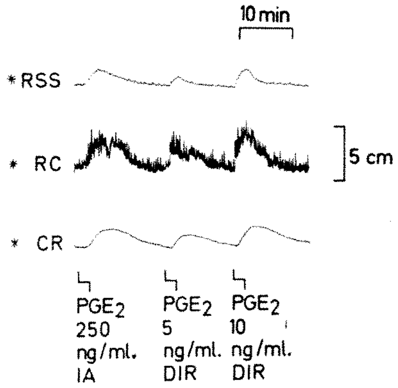


Fig. 1. Contractions of a rat stomach strip (RSS), rat colon (RC) and chick rectum (CR) superfused with Krebs solution after it had passed through lungs from an unsensitized guinea-pig. The contractions of the assay organs during infusion of prostaglandin E_2 (PGE_2) into the pulmonary artery (IA) were bracketed by contractions caused by infusions of PGE_2 5 and 10 ng/ml. given directly to the assay tissues (DIR). This shows that 96 per cent of PGE_2 was removed in the pulmonary circulation. Time scale 10 min; vertical scale 5 cm.

Evidence that the Lungs metabolize Prostaglandins

Guinea-pig lungs were perfused with Krebs solution from a reservoir and the venous effluent was returned to the reservoir. When prostaglandins were added to this recycling system, large quantities disappeared in a short time. This is shown in Fig. 2, which is a tracing of contractions of a rat stomach strip superfused with Krebs solution. The strip was sensitive to 10 or 20 ng of prostaglandin E_2 (end panels), but Krebs solution alone from the perfused lungs was inactive in a dose of 0·5 ml. Prostaglandin E_2 (1 μg/ml.) was then added to the circuit. Samples of 0·05 ml. of the recycled Krebs solution now showed activity. This activity declined to a low level within 15 min after which the residual activity (tested in 0·5 ml. samples) no longer declined. That this residual activity was due to a metabolite of prostaglandin E_2 was shown by recycling 50 μg of prostaglandin E_2 through perfused lungs for 30 min. Prostaglandins were then extracted from the Krebs solution and were submitted to thin-layer chromatography on the A III system. Prostaglandins recovered from the thin-layer plate by elution into methanol were resuspended in Krebs solution after evaporation of the methanol *in vacuo*. Bioassay then showed two peaks

Table 1. DISAPPEARANCE OF PROSTAGLANDINS IN GUINEA-PIG ISOLATED PERFUSED LUNGS

Prostaglandin	Concentration (ng/ml.)	%	Mean disappearance (% ± *S.E.*)
E_2	50	99	
		93	
	100	98	
		93	92 ± 2·7
		93	
		95	
		98	
	200	97	
		96	
	500	98	
		96	
		88	
		84	
		60	
$F_{2\alpha}$	100	80	
		85	90 ± 3·3
	200	98	
		98	
		83	
	1,000	95	
A_2	100	75	
		60	64 ± 3·1
	200	62	
		57	
		65	

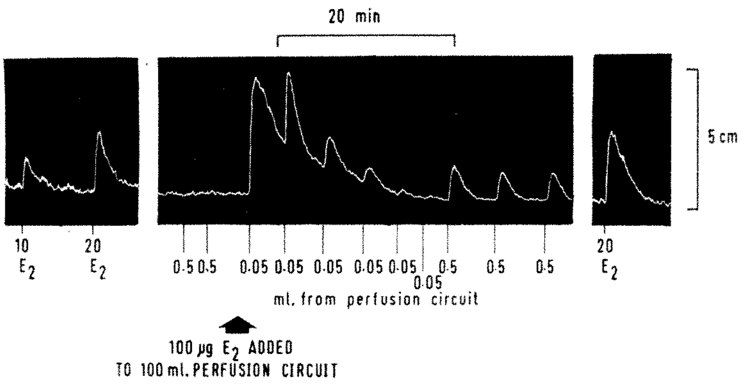


Fig. 2. Contractions of a rat stomach strip (RSS) superfused with Krebs solution. The first and third panels show contractions of the RSS induced by injection of 10 ng and 20 ng of prostaglandin E_2 (E_2) into the Krebs solution. 100 ml. Krebs solution was recycled through guinea-pig isolated lungs. Samples of Krebs solution were removed from this system and assayed on the RSS. The second panel shows the contractions of RSS induced by 0·05 or 0·5 ml. samples from the recycling system. At arrow 100 µg E_2 was added to the recycling Krebs solution. The contractions of RSS show disappearance of E_2 until a small contraction only was produced by samples of 0·5 ml. Horizontal scale 20 min; vertical scale 5 cm.

of activity (Fig. 3); one (R_F 0·18) represented unchanged prostaglandin E_2 in about 1/2,000 of the original amount. The other (R_F 0·50) represented the metabolite and possessed about 3 per cent of the activity of the original prostaglandin E_2 on the rat stomach strip.

Table 2

Experiment No.	R_F of parent prostaglandins					R_F of metabolites of prostaglandins			
	$F_{2\alpha}$	E_2	E_1	A_2	A_1	$F_{2\alpha}$	E_2	E_1	A_2
1 (single pass)	0·054	0·14	0·21		0·54	0·17	0·38		(0·75)
						(double spot)			
2 (single pass)		0·18					0·43		
3 (recycled)							0·40		(0·76)
							0·41		(0·78)
				0·54					0·77
4 (recycled)	0·046				0·56				
		0·13		(0·51)			0·43		(0·79)
		0·14		(0·50)			0·41		(0·79)
				0·48					0·75
5 (recycled)		0·22		(0·54)			0·48		(0·81)
				0·55					0·81
6 (recycled; expt. of Fig. 3)		0·18					0·50		
7 (standards only)		0·14		(0·48)					
		0·055	0·22						
				0·49					

Parentheses indicate weak spots.

The metabolism of prostaglandins in perfused lungs from the guinea-pig was temperature dependent, as shown in Fig. 4. A rat stomach strip and a rat colon, superfused with Krebs solution at 37° C, were calibrated by injections of prostaglandin E_2 (10–100 ng). A pair of guinea-pig lungs were also perfused with Krebs solution at 20 ml./min from a reservoir of 100 ml.; the venous effluent was recycled and the whole system was kept at 6° C. The contractions of the assay tissues, induced by 0·5 or 0·05 ml. of Krebs solution assayed immediately after withdrawal from the lung circuit, indicate the time course of decay of prostaglandin E_2 added to the circuit. The activity remained constant while the lungs were kept at 6° C, but when the circuit was quickly warmed to 37° C the prostaglandin activity disappeared in about 10 min. A second experiment at 6° C gave a similar result and in a third at 14° C the metabolism of prostaglandin E_2 was greatly slowed.

Chromatography of Metabolites

Prostaglandins (200 or 500 ng/ml.) were either passed once through the isolated lungs (two experiments) or recycled for 20 min (initial concentration of 1 µg/ml., three experiments). The Krebs solution in the venous effluent or in the reservoir was then extracted into small volumes of methanol and submitted to thin-layer chromatography on silica gel *G* with the A III solvent. After developing the chromatograms for a distance of 13 cm, the plates were dried in air and sprayed with phosphomolybdic acid (10 per cent in ethanol) and heated in a hot air oven at 120° C for 20 min. Prostaglandins appeared as bluish-grey spots and were detected in all five experiments, provided that at least 10 µg prostaglandin or its metabolite had been applied to the plate. As well as staining added prostaglandins, phosphomolybdic acid stained a considerable quantity of material from recycled Krebs solution when no prostaglandin had been added. The bulk of this unidentified material ran near the solvent front. It was present in extracts of untreated Krebs solution, so it did not originate in the lungs. Added prostaglandins, however, were detectable against this background (Table 2). Prostaglandin E_2 showed as a dense compact spot with an R_F of 0·16 (range 0·13–0·22). Prostaglandin A_2 was similarly obvious, with an R_F of 0·51 (range 0·48–05·5). Prostaglandin E_2 also gave a weak spot at R_F 0·55. This was seen when prostaglandin E_2 was chromatographed by itself (experiment 7, Table 2) as well as in other experiments in which it was extracted from Krebs solution and chromatographed (experiments 1 and 4 of Table 2). This suggests that the prostaglandin E_2 sample was contaminated with prostaglandin A_2.

Chromatography of the extracts after the prostaglandins had passed through the pulmonary circulation gave a different picture. The spots due to prostaglandin E_2 vanished and were replaced by two new spots ($R_F = 0·43$ and 0·78). The slower moving of these two metabolites of prostaglandin E_2 was not prostaglandin A_2, for it had a lower mobility in experiments 4 and 5 of Table 2. Besides, prostaglandin A_2 also disappeared in the lungs and was replaced by a metabolite which ran nearly to the solvent front (R_F 0·78). We were thus unable to decide whether the faster moving metabolite was derived from prostaglandin E_2 itself or from traces of prostaglandin A_2. As with prostaglandins A_2 and E_2, prostaglandin $F_{2\alpha}$ was

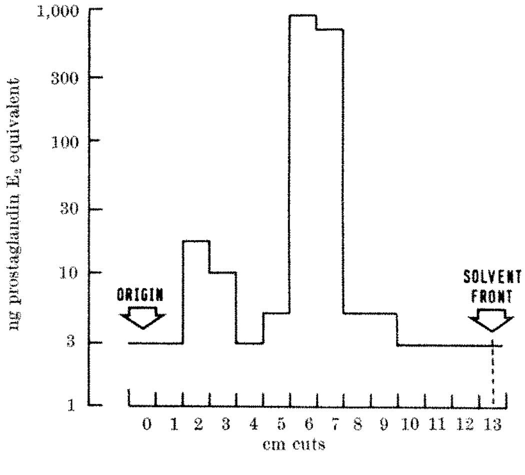


Fig. 3. Chromatography of prostaglandins. Prostaglandins were extracted from Krebs solution which had originally contained 50 µg E_2 in the recycling system and submitted to thin-layer chromatography in the A III solvent system. Centimetre cuts were made in the silica gel from the origin to the solvent front (horizontal scale). Prostaglandins were recovered by elution with methanol and estimated by bioassay in terms of prostaglandin E_2 equivalent (vertical scale). The first peak (R_F 0·18) corresponds to prostaglandin E_2 and the second (R_F 0·50) to its metabolite.

transformed into a less polar metabolite (R_F 0·17). We have not identified any of these metabolites except to show that neither prostaglandin E_2 nor A_2 is metabolized to prostaglandin A_1, A_2, E_2 or $F_{2\alpha}$.

Samuelsson *et al.*[6,16,17] have elucidated the structures of metabolites of E-type prostaglandins which were formed by enzyme systems found in the high speed supernatant of homogenates of guinea-pig lung. The two metabolites of prostaglandin E_2 had R_F values in the A III system of 0·32 (metabolite I) and 0·65 (metabolite II) while that of prostaglandin E_2 was 0·14. Thus although we obtained a similar R_F for prostaglandin E_2 there is a discrepancy between these values and those for the metabolites we obtained (R_F 0·43 and 0·78). This may be because of differences in chromatographic conditions, or may indicate that metabolites produced by intact lungs are different from those produced by homogenized tissue.

Rapidity of Metabolism

The rapidity of metabolism and completeness of inactivation of prostaglandins by the lungs are of importance. If, in a single transit, the prostaglandins are almost completely inactivated at all concentrations which might be encountered in life, it is unlikely that they have a function as circulating hormones. It has long been known that extracts of renal medulla contain vasodepressor substances identified as prostaglandin E_1 (refs. 18 and 19), as prostaglandin E_2 (ref. 20), as prostaglandin A_2 (ref. 21), and as a mixture of prostaglandins E_2, $F_{2\alpha}$ and A_2 (ref. 22) with some evidence that the A_2 was formed from E_2 during extraction procedure. In the cat and dog, the vasodilator activity of prostaglandin A_1 and A_2 passes the lungs without being decreased[3,4]. We have now compared the rate of inactivation of prostaglandin A_2 with that for prostaglandin E_1, E_2 and $F_{2\alpha}$ in perfused lungs from the guinea-pig. This was done in two ways. In the first, known concentrations of prostaglandins were infused into guinea-pig lungs and the activity leaving the lungs was estimated by the contractions of superfused tissues. The results of these experiments are shown in Table 1; inactivation of prostaglandin A_2 is clearly less than that of prostaglandin E_2. This difference could be due to a substantial fraction of prostaglandin A_2 evading metabolism, or it could be due to its complete transformation to metabolites which were still active biologically. The second type of experiment was designed to investigate the extent to which each of these mechanisms contributed to the results.

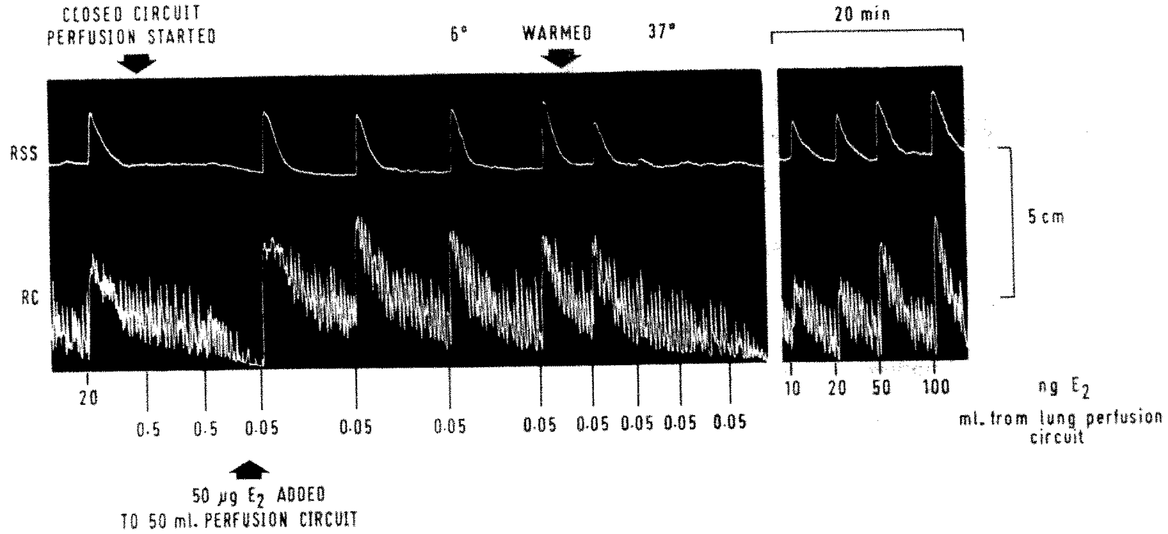


Fig. 4. Contractions of a rat stomach strip (RSS) and a rat colon (RC) superfused with Krebs solution. 50 ml. of Krebs solution was recycled through a pair of guinea-pig isolated lungs. Samples of 0·5 or 0·05 ml. of this solution were withdrawn and immediately assayed for prostaglandin content on RSS and RC. The recycling system was initially kept at 6° C and 50 µg E_2 added to the Krebs. The recycling Krebs was later warmed to 37° C. The prostaglandin content of the recycling Krebs solution changed little at 6° C but declined rapidly when the system was warmed to 37° C. Horizontal scale 20 min; vertical scale 5 cm.

In recycling experiments a known quantity of prostaglandin was added to the system and samples were removed at intervals thereafter. These were subsequently assayed against standard prostaglandins on a rat stomach strip in a 5 ml. bath. This system should produce an exponential decay of prostaglandin activity if the lungs metabolize a constant proportion of prostaglandin passing through them for widely different concentrations. We have not found clear evidence that the lungs depart from this behaviour, even with starting concentrations as high as 2·5 µg/ml. This implies that the enzyme systems are not saturated by these high concentrations. Fig. 5 shows the result of such an experiment (Table 2, experiment 3) in which prostaglandin A_2 (1·0 µg/ml.) was added to the 50 ml. circuit and observations were made for 15 min. After lung perfusion for 10 min with fresh Krebs solution, a second recycling experiment was performed using prostaglandin E_2 (1·0 µg/ml.). Fig. 5 shows plots of log N/N_0 against time for these two experiments (N = prostaglandin activity at time t; N_0 = prostaglandin activity corresponding to 1·0 µg/ml.). Regression lines were drawn by the method of least squares. The slope of each line, multiplied by 2·3, gives k in the expression $N = N_0e^{-kt}$. The value for k depends on the percentage of prostaglandin inactivated in a single passage through the lungs. In this experiment $k = 0·51$ corresponds to 100 per cent inactivation of prostaglandin in a single passage through the lungs, and $k = 0·088$ corresponds to 23 per cent inactivation.

This method also allowed measurement of residual activity after recycling the Krebs solution for 30 min. Whereas prostaglandin E_2 activity declined to 0·7–3·2 per cent of the original level, that of prostaglandin A_2 ceased to decline when it reached about 25 per cent of the original activity (Table 3). Making allowance for this residual activity which we assume represents the bioactivity of the metabolite(s), it is possible to calculate the percentage metabolism of A_2 in a single transit of the lungs (Table 3). It appears that prostaglandin A_2 is metabolized at a slower rate than E_1, E_2 or $F_{2\alpha}$.

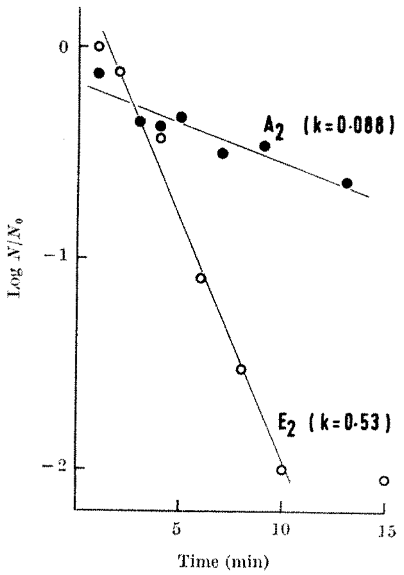


Fig. 5. Inactivation of prostaglandins A_2 (A_2) and E_2 (E_2) by guinea-pig lungs (from experiment 3, Table 3). Prostaglandin A_2 (1·0 μg/ml.) was added to a 50 ml. recycling system and the change in concentration during repeated passage through the pulmonary circulation estimated by assay on a rat stomach strip. After perfusing the lungs with fresh Krebs solution for 10 min, the experiment was repeated using prostaglandin E_2 (1·0 μg/ml.). N = Prostaglandin activity at time t. N_0 = Prostaglandin activity corresponding to 1·0 μg/ml. The slope of each line, multiplied by 2·3, gives k in the expression $N = N_0e^{-kt}$. The value for k depends on the percentage of prostaglandin inactivated in a single passage through the lungs. In this experiment $k = 0·51$ corresponds to 100 per cent inactivation and $k = 0·088$ corresponds to 23 per cent inactivation. Vertical axis log N/N_0 for A_2 and E_2; horizontal axis time in min.

Applying a similar correction to the data in Table 1, it is found that a mean percentage inactivation of 64 per cent for prostaglandin A_2 implies 84 per cent metabolism in a single transit of the lungs. This is still clearly less than that of prostaglandin E_2 ($P < 0·01$). Thus all the prostaglandins we studied underwent extensive metabolism in a single passage through the lungs. The apparently smaller effect on prostaglandin A_2 is due partly to the activity of the metabolite on the rat stomach strip, and partly to less avid metabolism of this prostaglandin by the lungs.

Table 3

Exp.	Prostaglandin (initial concentration, μg/ml.)	Volume (ml.)	Pump rate (ml./min)	k (min^{-1})	Calculated percentage inactivation in one pass	Observed final activity (at 30 min) (percentage of original)	Calculated percentage metabolized in one pass
1	E_2 (1·0)	100	20	0·23	102	1·0	103
	$F_{2}\alpha$ (2·0)	100	20	0·073	35	—	—
2	E_2 (1·0)	50	20	0·43	88	0·7	89
	E_1 (1·0)	50	20	0·46	92	1·8	94
	$F_{2}\alpha$ (1·0)	50	20	0·32	68	1·0	69
	E_2 (1·0)	50	20	0·36	76	0·9	77
3	A_2 (1·0)	50	20	0·088	23	23	30
	E_2 (1·0)	50	20	0·53	107	0·9	108
4	A_2 (2·5)	50	20	0·105	25	24	33
5	A_2 (2·5)	50	20	—	—	27	—

We conclude that the disappearance of the activity of prostaglandins in the pulmonary circulation is due to their metabolism to less polar derivatives. The products of the metabolism of prostaglandin E_1 and E_2 in cat, dog, rabbit and guinea-pig have insignificant activity on the rat stomach strip[1,5], on renal blood flow in the dog[3] or on blood pressure in cat or dog[4]. Prostaglandin $F_{2\alpha}$ is also metabolized, perhaps at a slightly slower rate. Its products in the dog are inactive on renal blood flow[3], and in the perfused guinea-pig lungs the products are inactive on the rat stomach strip, rat colon and chick rectum.

With prostaglandins A_1 and A_2, the situation is different. In the cat and dog, there is little or no change in vasodilator activity of prostaglandin A_1 and A_2 on passage through the lung[3,4]. Our results in the guinea-pig perfused lungs show 23–75 per cent inactivation of prostaglandin A_2 in one passage, as measured by activity on the rat stomach strip. Some of the residual activity was due to the metabolite(s); some was due to unchanged A_2. This suggests either that the guinea-pig isolated lungs behave differently from the lungs of the other two species, or that there is similar metabolism in cat and dog, but that the products have as potent a vasodilator activity as does prostaglandin A_2.

We did not identify the products of the metabolism of the prostaglandins, but the simplest explanation of our results is that the enzyme isolated from swine and guinea-pig lung, 15-hydroxyprostaglandin dehydrogenase[6–9], is responsible. The fact that this enzyme metabolizes A_1 and A_2 at a slower rate than E_1 and E_2 (refs. 6 and 7) fits with our results. The lack of correspondence between the R_F values of the metabolic products might have been due to different chromatographic conditions.

Our results support the previous conclusion[23] that the E-type prostaglandins are unlikely to be circulating hormones, but leave the possibility that A_1 and A_2 might be.

It is interesting to speculate on the possible role of the pulmonary circulation in control of blood pressure. In the case of the renin–angiotensin system the kidney secretes renin and angiotensin I is generated in the plasma; but it is the converting enzyme in the lungs[24–26] which brings about the release of the potent vasoconstrictor angiotensin II. If the kidneys are also able to secrete prostaglandin A_2 and if the lungs possess a mechanism to regulate the degree of metabolism of this substance, a comparable situation would exist for presenting a potent vasodilator substance to the arterial circulation.

We thank Miss Leslie Gruby and Mr M. A. Palmer for technical assistance and the MRC and Wellcome Trust for grants. We also thank Dr J. E. Pike of the Upjohn Company, Kalamazoo, for a gift of prostaglandins.

Received December 17, 1969.

[1] Ferreira, S. H., and Vane, J. R., *Nature*, **216**, 868 (1967).
[2] Biron, P., *Clin. Res.*, **16**, 511 (1968).
[3] McGiff, J. C., Terragno, N. A., Strand, J. C., Lee, J. B., Lonigro, A. J., and Ng, K. K. F., *Nature*, **223**, 742 (1969).
[4] Horton, E. W., and Jones, R. L., *Brit. J. Pharmacol.*, **37**, 705 (1969).
[5] Piper, P. J., and Vane, J. R., in *Prostaglandins, Peptides and Amines* (edit. by Mantegazza, P., and Horton, E. W.), 15 (Academic Press, London, 1969).
[6] Anggård, E., Gréen, K., and Samuelsson, B., *J. Biol. Chem.*, **240**, 1932 (1965).
[7] Anggård, E., Gréen, K., and Samuelsson, B., *J. Biol. Chem.*, **240**, 3518 (1965).
[8] Anggård, E., Gréen, K., and Samuelsson, B., *Arkiv Kemi*, **25**, 293 (1966).
[9] Anggård, E., Gréen, K., and Samuelsson, B., in *Prostaglandins*, Proc. Second Nobel Symp., Stockholm, June 1966, 97 (Interscience, New York).
[10] Gilmore, N., Vane, J. R., and Wyllie, J. H., *Nature*, **218**, 1135 (1968).
[11] Piper, P. J., and Vane, J. R., *Nature*, **223**, 29 (1969).
[12] Vane, J. R., *Brit. J. Pharmacol.*, **12**, 344 (1957).
[13] Regoli, D., and Vane, J. R., *Brit. J. Pharmacol.*, **23**, 35 (1964).
[14] Mann, M., and West, G. B., *Brit. J. Pharmacol.*, **5**, 173 (1950).
[15] Gréen, K., and Samuelsson, B., *J. Lipid Res.*, **5**, 117 (1964).
[16] Anggård, E., and Samuelsson, B., *J. Biol. Chem.*, **239**, 4097 (1964).
[17] Anggård, E., and Samuelsson, B., *Biochemistry*, **4**, 1864 (1965).
[18] Lee, J. B., Covino, B. G., Takman, B. H., and Smith, E. R., *Circulation Res.*, **17**, 57 (1965).
[19] Strong, C. G., Boucher, R., Nowaczyuski, W., and Genest, J., *Mayo Clinic Proc.*, **41**, 433 (1966).
[20] Daniels, E. G., Hinman, J. W., Leach, B. E., and Muirhead, E. E., *Nature*, **215**, 1298 (1967).
[21] Lee, J. B., Gougoutas, J. Z., Takman, B. H., Daniels, E. G., Grostic, M. F., Pike, J. E., Hinman, J. W., and Muirhead, E. E., *J. Clin. Invest.*, **45**, 1036 (1966).
[22] Lee, J. B., Crowshaw, K., Takman, B. H., and Attrep, K. A., *Biochem. J.*, **105**, 1251 (1967).
[23] Vane, J. R., *Brit. J. Pharmacol.*, **35**, 209 (1969).
[21] Ng, K. K. F., and Vane, J. R., *Nature*, **216**, 762 (1967).
[25] Ng, K. K. F., and Vane, J. R., *Nature*, **218**, 144 (1968).
[26] Bakhle, Y. S., Reynard, A. M., and Vane, J. R., *Nature*, **222**, 956 (1969).

Novel Correlations between Global Features of the Earth's Gravitational and Magnetic Fields

by

R. HIDE

Meteorological Office,
Bracknell, Berkshire

S. R. C. MALIN

Institute of Geological Sciences,
Herstmonceux Castle, Sussex

There is a significant correlation between the Earth's gravitational field and the non-dipole part of the geomagnetic field, provided the latter is displaced in longitude.

It is more than forty years since seismological studies first revealed the existence of a liquid core within the Earth at depths greater than 2,900 km ($=0{\cdot}455\ R$ where $R=6{,}371{\cdot}2$ km is the Earth's mean radius), but little is so far known of the interface between the core and the overlying solid mantle[1], where there is a more or less abrupt change of density from 5·6 g cm^{-3} to 9·6 g cm^{-3}.

Undulations of the interface (bumps) on a planetary scale a few kilometres in vertical amplitude (and therefore well below the present limit of resolution by seismological methods) would, according to a recent suggestion[2,3], interact so strongly with fluid motions in the core as to produce significant distortions in the geomagnetic field **B** as measured at and near the Earth's surface (and to account for the horizontal stresses at the core–mantle interface that are implied by the most pronounced changes observed in the length of the day[4]). Such bumps would also distort the gravitational field **g**, contributing significantly to the low-order harmonics (of degree n less than 5 or 6) of **g** at the surface of the Earth[5].

Qualitatively, distortions in **B** could be displaced relative to the bumps through distances at least comparable with the horizontal scale of the bumps—thousands of kilometres. This suggests that the variation of the degree of correlation of **g** and **B** with the horizontal displacement of one field relative to the other is worth studying[2]. Attention is confined to displacements in longitude alone, a procedure which avoids the complications of transforming spherical harmonic series from one polar axis to another, but a physically reasonable one because core motions are probably mainly zonal[2]; this restriction should be relaxed in future work.

Spherical Harmonics and Truncation

The gravitational field of the Earth at a point with spherical polar coordinates (r, φ, λ) can be expressed in terms of spherical harmonics by introducing a potential U such that $\mathbf{g}=-\nabla U$, where[1,6,7]

$$U = \frac{MG}{R}\left\{\frac{R}{r} + \sum_{n=1}^{\infty}\sum_{m=0}^{n} S(A_n, A_n^m, B_n^m)\right\} + U_e \qquad (1)$$

Here r denotes distance from the Earth's centre of mass, φ co-latitude, λ east longitude, G is the universal gravitational constant, M is the total mass of material contained within a sphere of radius r centred on the origin of coordinates, U_e is the potential due to sources lying outside that sphere and $S(A_n, A_n^m, B_n^m)$ is a typical term in the spherical harmonic expansion of U (see appendix A for exact definition), where m denotes the order of the harmonic and n the degree, m and n being positive integers. A_n, A_n^m and B_n^m are coefficients appearing in S, which is also a function of $(R/r)^{n+1}$, $\cos m\lambda$, $\sin m\lambda$ and $\cos\varphi$. (The coefficients in the spherical harmonic expansion of the topography of the core–mantle interface[5] are, apart from an unimportant constant, the same as those in the expansion for $U-U_e$ evaluated at $r=0{\cdot}545\ R$.)

The main geomagnetic field in regions where local electric currents are negligible can be similarly expressed. Thus $\mathbf{B}=-\nabla V$ where[8,9]

$$V = \frac{L}{R^2}\sum_{n=1}^{\infty}\sum_{m=0}^{n} S(a_n, a_n^m, b_n^m) + V_e \qquad (2)$$

a_1L (see appendix A) being the resolute parallel to the Earth's rotation axis of the moment of the effective magnetic dipole component of sources within a sphere of radius r centred on the origin of coordinates and V_e is the potential due to magnetic sources lying outside the sphere. As magnetic materials in the Earth's mantle and crust[9,10], sources outside the Earth and very slight leakage of electric currents out of the liquid core into the solid mantle make an insignificant contribution to global-scale features of the main geomagnetic field, we can suppose that sources of V are largely confined to the core. Consequently equation (2) with $V_e=0$ should constitute a satisfactory representation of V throughout the mantle, including the extreme levels $r=0{\cdot}545\ R$ and $r=R$.

The main geomagnetic field **B**, unlike **g**, undergoes measurable fluctuations on time-scales of a few years and upwards[8,9]. The potential of the so-called "geomagnetic secular variation" may be expressed in terms of an expansion similar to equation (2) by changing V to $\dot{V}$ and (a_n, a_n^m, b_n^m) to $(\dot{a}_n, \dot{a}_n^m, \dot{b}_n^m)$, where the dot denotes differentiation with respect to time. A significant part of the geomagnetic secular variation can be represented in terms of a uniform westward drift[11-13] of the main geomagnetic field at $u_0=0{\cdot}0035$ rad yr^{-1} ($=0{\cdot}20$ degrees of longitude yr^{-1}). We may remove this part of the secular variation, thus leaving a (hypothetical) non-drifting part with coefficients (α_n^m, β_n^m), where

$$\alpha_n^m = \dot{a}_n^m - u_0 m b_n^m,\ \beta_n^m = \dot{b}_n^m + u_0 m a_n^m \qquad (3)$$

Global-scale features of U and V are described by the foregoing series truncated at some low value n^* of n. We take $n^*=4$ in the first instance, for reasons given in the previous section, before examining systematically the sensitivity to the value chosen for n^* of the principal results obtained.

Because the coordinate system for gravitational measurements has its origin at the Earth's centre of mass and the Earth rotates about its axis of maximum moment of inertia, the terms A_1, A_1^1, B_1^1, A_2^1 and B_2^1 are all zero[6]. Thus, we are left with A_2^2, B_2^2, A_3^1, B_3^1, A_3^2, B_3^2, A_3^3, B_3^3, A_4^1, B_4^1, A_4^2, B_4^2, A_4^3, B_4^3, A_4^4 and B_4^4. Corresponding to this set of sixteen (equals $n^*(n^*+1)-4$) coefficients required to specify the tesseral part of the spherical harmonic series for U truncated at $n=n^*=4$, we have sets for V and $\dot{V}$, respectively the potentials of the geomagnetic field and the geomagnetic secular variations, and for the non-drifting part of the secular variation (see equation (3)).

Correlation Coefficients

One method of investigating correlations between U and V is to evaluate grid-point values (at the level

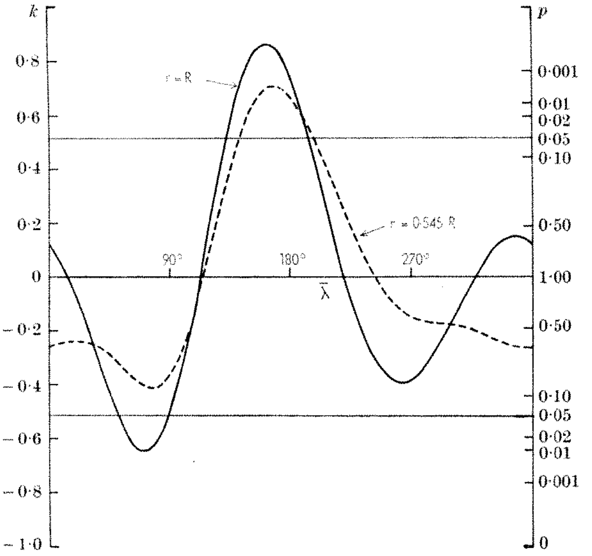


Fig. 1. The dependence of the degree of correlation k between the large-scale features of the non-dipole part of the Earth's magnetic field, **B**, for epoch 1965 and gravitational field, **g**, as described by spherical harmonic coefficients of degree not greater than 4, on the eastward displacement in longitude of **B** relative to **g** through an angle $\bar{\lambda}$. A value of unity for k would correspond to perfect correlation. The solid curve corresponds to the fields at the Earth's surface and the broken curves to hypothetical fields obtained by extrapolating the surface spherical harmonics down to the level $r = 0{\cdot}545R$. On the Student t test (see significance levels p given at the right-hand side of the diagram), the odds that the maximum value of k, found at $\bar{\lambda} = 160°$, represents a true physical connexion between **B** and **g** exceed 1,000 : 1. When deficiencies in the Student t test are taken into account (see text) the odds are lower, 100 : 1, but still well within the generally accepted limit of significance (20 : 1).

required, surface or core–mantle boundary) and calculate correlation coefficients from these (indeed, contingency tests along these lines were carried out as a preliminary to the present work). But because we are representing U and V by spherical harmonic series we can estimate correlation coefficients k at $r = R$ directly in terms of the coefficients (a_n^m, b_n^m) and (A_n^m, B_n^m), by means of equation (B1) (see appendix B). That equation applies when $\bar{\lambda}$, the eastward displacement of V relative to U, is zero; when $\bar{\lambda} \neq 0$ the coefficients a_n^m and b_n^m should be changed as follows:

$$(a_n^m, b_n^m) \longrightarrow ([a_n^m \cos m\bar{\lambda} - b_n^m \sin m\bar{\lambda}], \quad [b_n^m \cos m\,\bar{\lambda} + a_n^m \sin m\bar{\lambda}]) \qquad (4)$$

To find k at other levels $r \neq R$ where no sources of tesseral harmonics are present, (a_n^m, b_n^m) and (A_n^m, B_n^m) should be multiplied by $(R/r)^{n+1}$.

Values of k have been calculated for $\bar{\lambda} = 0°$ to $360°$ at 2° intervals and for two values of r/R, namely 1·000 and 0·545 (see Fig. 1), using coefficients given by various workers (see Table 1); the principal results are summarized in Table 2.

At epoch 1965 the maximum degree of correlation, coefficient $\hat{k}$, of the gravitational field with the displaced magnetic field occurs when $\bar{\lambda}$ has the value $\hat{\lambda} = 160°$ (see the section on drift of the geomagnetic field). $\hat{k}$ and $\hat{\lambda}$ are apparently insensitive to the particular field models chosen (the variations between models being slightly greater for U than for V). Thus we need only discuss one pair of models and "CA" for U and "LME" for V (see Table 1) are chosen. The corresponding values of $\hat{k}$ and $\hat{\lambda}$ at $r = R$ are 0·8381 and 160° respectively (see Fig. 1). That this value of $\hat{k}$ represents a statistically significant correlation will be demonstrated in the next section. Tables 1 and 2 also show that $\hat{k}$ is a good deal less for the secular variation than for the geomagnetic field itself. In all cases (except the non-drifting geomagnetic secular variation) $\hat{k}$ is larger at $r = R$ than at $r = 0{\cdot}545\ R$.

As an additional exercise the correlation between $|U|$ and $|V|$ was investigated. In this case it was not possible to work with spherical harmonic coefficients directly, so 10° × 10° grid point values were synthesized at $r = R$ and $r = 0{\cdot}545\ R$ and k investigated as a function $\bar{\lambda}$, as before. The results are shown in Table 3.

For the case of the magnetic field, taking the modulus makes little difference to $\hat{k}$ and $\hat{\lambda}$; inevitably $\hat{k}$ must be larger when moduli are considered, but it is not much larger. The greatest effect of taking moduli occurs for the non-drifting secular variation, but, even so, $\hat{k}$ does not in this case attain a statistically interesting value.

Significance Tests

The next step is to compare the values of $\hat{k}$ found in the analysis (see Fig. 1 and Table 2) with those expected by pure chance and thus evaluate the extent to which the results must be regarded as evidence for a true physical association between the magnetic and gravitational fields. It is instructive to apply first the Student t test before considering complications due to factors that might invalidate the test.

Because we are considering 16 $(= n^*(n^* + 1) - 4)$ independent pairs of quantities we have $N = 16$ degrees of freedom when $\bar{\lambda}$ is fixed, but we lose one of these, so that $N = 15$, when we allow $\bar{\lambda}$ to vary. The t test is applied by calculating the quantity

$$t = k/[(1 - k^2)/(N - 2)]^{\frac{1}{2}} \qquad (5)$$

Table 1. POTENTIAL MODELS USED IN THE CORRELATION INVESTIGATION

Gravity	
GN	Guier and Newton[14]. Gravity potential deduced from doppler tracking of 5 satellites. Coefficients up to $m = n = 8$. These coefficients must be divided by $(4n + 2)^{1/2}$ to correct them to the fully normalized form.
G8	Gaposhkin[15]. Gravity potential from Baker–Nunn observations of 13 satellites. 98 coefficients; complete up to $m = n = 8$. Fully normalized. Kaula[16] considers this to be the best single solution.
K8	Kaula[16]. Gravity potential. 72 coefficients; complete up to $m = 5$, $n = 7$. Fully normalized.
C	Kaula[16]. Gravity potential. Arithmetic mean of Anderle[17], GN, K8 and Gaposhkin G6 (ref. 15) (all based on satellite data). 42 coefficients; complete up to $n = 7$, $m = 2$. Fully normalized. Better than any individual model[16].
CA	Kaula[16]. Gravity potential from C (above) and gravimetry. 42 coefficients; complete up to $n = 7$, $m = 2$. Fully normalized. Should be the best overall representation.
Magnetic Main Field	
LME	Leaton, Malin and Evans[18]. Magnetic potential deduced from surface and airborne observations. 80 coefficients; up to $m = n = 8$. Earth assumed to be spherical. Coefficients in Schmidt quasi-normalized form, must be divided by $(2n + 1)^{1/2}$ to correct them to the fully normalized form.
M IGRF	Malin[19]. Magnetic potential deduced from surface, airborne and satellite observations. 80 coefficients up to $m = n = 8$. Earth assumed to be an oblate spheroid. Coefficients in Schmidt quasi-normalized form.
C IGRF	Cain[20]. Magnetic potential deduced from surface, airborne and satellite observations. 120 coefficients, in Schmidt quasi-normalized form, up to $m = n = 10$. Earth assumed to be an oblate spheroid.
F IGRF	Fougère[19]. Magnetic potential, data unspecified. 120 coefficients, in Schmidt quasi-normalized form, up to $m = n = 10$. Earth assumed to be an oblate spheroid.
O IGRF	Orlov[21]. Magnetic potential deduced from Cosmos 49 total field observations. 48 coefficients, in Schmidt quasi-normalized form, up to $m = n = 6$. Earth assumed to be an oblate spheroid.
Magnetic Secular Variation	
M SV	Malin[22]. Magnetic secular variation potential deduced from observatory data, 1940–1965. 48 coefficients, in Schmidt quasi-normalized form, up to $m = n = 6$. Earth assumed to be spherical.
Magnetic Non-drifting Secular Variation	
M NDSV	Malin (unpublished). Magnetic secular variation after removal of the part which would arise from a westward drift of the main field of 0°·2 yr^{-1}. Deduced from M SV and main field coefficients deduced from the same set of observatory data[21].
N NDSV	Nagata[13]. Similar to M NDSV. The secular variation model was that of Nagata and Syono[23]; the main field model was that of Nagata and Oguti[24].

The four magnetic main field models denoted IGRF are those from which the International Geomagnetic Reference Field[25] was derived.

Table 2. CORRELATION BETWEEN GRAVITATIONAL POTENTIAL AND RECENT MAGNETIC POTENTIAL EVALUATED AT $r = 0·545\ R$ AND $r = R$

					Gravity						
Magnetic	GN		G8		K8		C		CA		r/R
LME	0·7081	143 168 197	0·6516	145 166 191	0·6408	145 166 191	0·6620	143 166 191	0·6427	145 166 189	0·545
	0·8640	133 162 195	0·8366	133 160 191	0·8412	131 160 191	0·8516	133 160 191	0·8381	133 160 189	1·000
M IGRF	0·7094	143 168 197	0·6528	143 166 191	0·6414	145 166 199	0·6631	145 166 191	0·6438	143 166 191	0·545
	0·8666	133 162 195	0·8390	133 160 191	0·8435	131 160 191	0·8541	133 160 191	0·8405	133 160 189	1·000
C IGRF	0·7072	143 168 197	0·6510	143 166 191	0·6393	145 166 199	0·6610	145 166 191	0·6423	143 165 191	0·545
	0·8669	133 162 193	0·8408	133 160 189	0·8451	131 158 189	0·8556	131 160 191	0·8425	131 160 189	1·000
F IGRF	0·7085	143 168 197	0·6516	143 166 191	0·6416	145 166 199	0·6621	145 166 191	0·6423	145 166 189	0·515
	0·8671	133 162 193	0·8392	133 160 191	0·8440	131 160 189	0·8543	133 160 191	0·8405	133 160 189	1·333
O IGRF	0·7124	143 170 199	0·6545	145 168 193	0·6365	147 166 193	0·6611	147 168 193	0·6455	145 168 191	0·545
	0·8706	133 162 193	0·8387	133 160 191	0·8429	131 160 191	0·8541	133 160 191	0·8411	133 160 189	1·000
M SV	0·4187	— 206 —	0·4992	— 198 —	0·4203	— 194 —	0·4417	— 200 —	0·4777	— 202 —	0·545
	0·7150	179 204 229	0·7607	177 200 227	0·7399	177 202 229	0·7414	177 202 229	0·7496	177 202 227	1·000
M NDSV	0·5693	121 136 151	0·5019	— 144 —	0·5041	— 142 —	0·5177	135 140 143	0·4763	— 138 —	0·545
	−0·3299	— 318 —	0·3972	— 192 —	−0·4188	— 312 —	−0·3773	— 314 —	−0·3960	— 312 —	1·000
N NDSV	−0·4737	— 38 —	−0·3804	— 46 —	−0·3250	— 38 —	−0·3941	— 44 —	−0·3698	— 42 —	0·545
	0·3071	— 224 —	0·4079	— 224 —	0·4173	— 224 —	0·3733	— 224 —	0·3884	— 226 —	1·000

The numbers are arranged as follows: A $\begin{matrix} \text{C} \\ \text{B} \\ \text{D} \end{matrix}$

where A is the maximum correlation coefficient, $\hat{k}$,

B is the eastward displacement in longitude at which $\hat{k}$ occurs ($\hat{\lambda}$), to the nearest even degree,

C is the smallest value of $\bar{\lambda}$ for which the correlation coefficient exceeds 0·5139 (corresponding to the 5 per cent significance level, judged by the Student t test), to the nearest odd degree,

and D is the largest value of $\bar{\lambda}$ for which the correlation coefficient exceeds 0·5139, to the nearest odd degree.

and finding the significance level for t and N from standard tables[26,27]. Alternatively, we may reverse the procedure and find what value of k is likely to arise from a random set of data for a chosen significance level when $N = 15$.

Significance level	0·50	0·10	0·05	0·02	0·01	0·001
k	0·189	0·4409	0·5139	0·5923	0·6411	0·761

These significance levels are indicated in Fig. 1 and the values of $\bar{\lambda}$ at which k enters and leaves the 5 per cent significance level are given in Table 2.

The maximum correlation coefficient for the case $r = R$ illustrated in Fig. 1 is apparently significant at the 0·1 per cent level; $\hat{k}$ at $r = 0·545\ R$ is apparently significant at the 1·0 per cent level. These levels fall within the 5 per cent limit, so that if the t test is applicable then the corresponding values of $\hat{k}$ must be considered physically significant. The test may not be applicable here, however, and therefore the results of its application represent no more than a *prima facie* case for carrying out more stringent tests.

Suppose we are correlating two sets of data where the spherical harmonic coefficients are all zero except for a single pair of values of (m, n). By a suitable choice of $\bar{\lambda}$ we could then produce a value 1·0 for $\hat{k}$ (see, for instance, Table 4), apparently indicating perfect correlation. In the more realistic case when one value of

$$[(a_n^m)^2 + (b_n^m)^2]^{1/2}\ [(A_n^m)^2 + (B_n^m)^2]^{1/2}$$

Table 3. CORRELATION COEFFICIENT FOR RECENT EPOCH DEDUCED FROM SYNTHETIC GRID POINT VALUES

	Gravity model CA				
Magnetic model	Signed grid-point values		Modulus of grid-point values		r/R
	$\hat{k}$	$\hat{\lambda}$	$\hat{k}$	$\hat{\lambda}$	
M IGRF	0·8405	160°	0·8642	160°	1·000
	0·6383	170	0·7561	180	0·545
M SV	0·7483	200	0·7864	200	1·000
	0·4766	200	0·6999	30	0·545
M NDSV	−0·3952	310	0·7003	200	1·000
	0·4762	140	0·7402	130	0·545

is much larger than the other non-zero values we would still expect a very large, but probably spurious, value of $\hat{k}$.

A test in which this difficulty is recognized, which not only provides a more robust test than the t test but also permits an evaluation of the dependence of the degree of correlation on the largest terms in the original sets of coefficients, has been proposed by Brier (private communication, see appendix C). For the correlation between the gravitational and magnetic fields at $r=R$ using the "CA" model for U and the "LME" model for V (see Table 1), for which $\hat{k}=0\cdot8381$ and $\hat{\lambda}=160°$ (and the t test indicates a highly significant correlation, $p<0\cdot001$ where p is the probability that the data are uncorrelated), Brier's test gives $y=-2\cdot58$ and $p=0\cdot01$, where y is the weighted standard deviate (see equation (C6)), which is still well within the 5 per cent limit. The correlation is significant at the 5 per cent level when $\bar{\lambda}$ lies between 150° and 170°, but not otherwise. If we remove the pair of harmonics that make the largest contribution to the correlation coefficient, for which $(m, n)=(1,3)$, and repeat Brier's test using the same value of $\bar{\lambda}$, the correlation is still significant at the 3·4 per cent level (that is, $p=0\cdot034$, $y=-2\cdot12$). Only when the two largest pairs of harmonics, for which $(m, n)=(1,3)$ and $(1,4)$, are removed does p rise to a level bordering on insignificance (namely, 0·083, corresponding to $y=-1\cdot74$).

A high degree of correlation is thus established between those parts of the Earth's gravitational and non-dipole magnetic fields for epoch 1965 described by tesseral harmonics of degree $n\leq 4$ when the magnetic pattern is displaced 160° to the east but not otherwise. This correlation apparently stands up well to severe significance tests and therefore requires an explanation in geophysical terms. The harmonics used are all well determined, as shown by the consistency between models, and our results are unlikely to be affected substantially by new data or methods of analysis. For reasons that are not very clear (having regard for the arguments that prompted the investigation) the correlation is not quite so high at the core–mantle boundary.

Though less notable, a correlation is also established between gravity and the geomagnetic secular variation. This result is undoubtedly associated with the remarkably good correlation between the geomagnetic field and the geomagnetic secular variation, which was discovered incidentally during this investigation but will be discussed elsewhere.

Table 4 summarizes the results of an investigation of how the truncation procedure affects $\hat{k}$ and $\hat{\lambda}$ at $r/R=1$ and $r/R=0\cdot545$ for epoch 1965, carried out after the foregoing results for $n^*=4$ had been obtained. For this purpose it was convenient to use model G8 for the gravitational field, considered by Kaula[16] to be the best single solution, and model IGRF[25] for the magnetic field, both of which are complete up to $n=8$. The number of coefficients needed to represent each field, $n^*(n^*+1)-4$, increases rapidly with n^*, but because the coefficients fall off in amplitude with increasing n^*, it is not surprising to find at $r=R$ that $\hat{k}$ does not differ significantly from 0·8, $\hat{\lambda}$ does not differ materially from 160° at this epoch.

It is perhaps worth pointing out that if the correlation is caused by processes at the core–mantle interface, then there is no physical reason for expecting terms beyond $n=5$ or 6 to contribute to the correlation coefficient[5]; the high-degree terms in the gravitational field almost certainly originate in the upper part of the mantle.

Table 4. VARIATION OF $\hat{k}$ AND $\hat{\lambda}$ WITH n^* FOR EPOCH 1965

n^*	$\hat{k}(r/R=1)$	$\hat{\lambda}(r/R=1)$	$\hat{k}(r/R=0\cdot545)$	$\hat{\lambda}(r/R=0\cdot545)$
2	1·0000	162·8°	1·0000	162·8°
3	0·9117	158·9°	0·8919	156·7°
4	0·8375	160·9°	0·6525	167·0°
5	0·8146	161·3°	0·5568	170·0°
6	0·7944	161·2°	0·3290	172·5°
7	0·7868	161·2°	0·2612	174·0°
8	0·7820	161·2°	0·2351	169·9°

Table 5. VARIATION OF $\hat{k}$ AND $\hat{\lambda}$ WITH TIME FOR $n^*=4$

Model No.	Epoch	$\hat{k}$	$\hat{\lambda}°$	$\Delta\hat{\lambda}°$ (see note)
1[29]	1835	0·6425	125·5	−0·7
2[29]	1835	0·6024	127·4	1·2
3[29]	1845	0·7018	128·8	−0·1
4[29]	1880	0·7204	138·4	−0·1
5[29]	1885	0·7097	138·7	−1·1
6[29]	1885	0·7358	138·0	−1·8
7[29]	1905	0·7974	146·0	0·7
8[29]	1915	0·8262	149·2	1·2
9[29]	1922	0·8383	151·6	1·6
10[29]	1925	0·8409	151·8	1·0
11[29]	1935	0·8494	153·7	0·2
12[29]	1942·5	0·8614	155·4	−0·1
13[23]	1942·5	0·8409	156·2	0·7
14[29]	1945	0·8475	155·6	−0·6
15[22]	1947·5	0·8385	157·2	0·3
16[22]	1952·5	0·8345	158·4	0·1
17[29]	1955	0·8354	158·8	−0·2
18[22]	1957·5	0·8344	159·7	0·1
19[20]	1960	0·8409	159·7	−0·6
20[22]	1962·5	0·8347	160·7	−0·3
21[25]	1965	0·8376	160·9	−0·8

Note: $\Delta\hat{\lambda}°$ is the difference between $\hat{\lambda}$ and the best straight line relationship between $\hat{\lambda}$ and T (see equation (6)).

Drift of the Geomagnetic Field

The first spherical harmonic analysis of the geomagnetic field was done by Gauss for epoch 1835 (see Table 5). This and subsequent analyses revealed details of slow secular changes in **B**, including the westward drift of the field pattern first noted by Halley in the seventeenth century.

Comparable gravitational data are not available, but it is reasonable to suppose that **g** remains fixed on this time scale. Distortions in **g** at the Earth's surface must be due to density variations in the solid part of the Earth, concomitant stresses being roughly 10^8 N m^{-2}. Stresses of this magnitude could be supported[5] elastically or by viscous forces (associated with very slow motions, on geological time scales) in the mantle and crust, but they are too large, by several orders of magnitude, to be associated with processes in the core, where the geomagnetic field originates.

Values of $\hat{k}$ and $\hat{\lambda}$ when $r=R$ and $n^*=4$ evaluated for different epochs going back to 1835 are given in Table 5, and the corresponding variations of $\hat{k}$ and $\hat{\lambda}$ with time T are illustrated in Figs. 2 and 3. The tendency for $\hat{k}$ to increase with T prior to 1920 before flattening out at a value somewhat greater than 0·8 is presumably due largely to the general improvement in the accuracy and coverage of the basic magnetic data. The tendency for $\hat{\lambda}$ to increase with time T is a manifestation of the westward drift. The data of Table 5 and Fig. 3 fit the straight line

$$\hat{\lambda}=(126\cdot2\pm0\cdot2)°+(0\cdot273\pm0\cdot005)[T\pm10-1835]° \quad \text{(standard errors)} \quad (6)$$

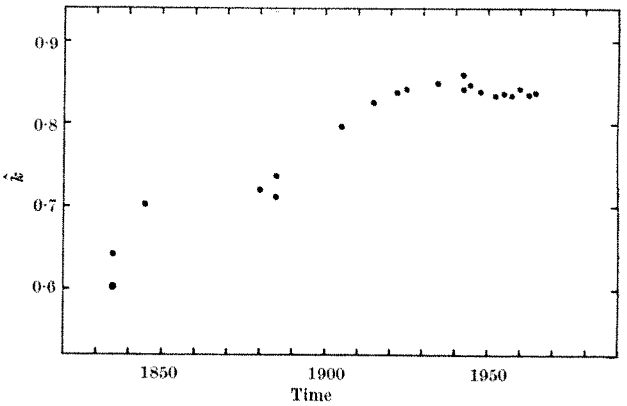


Fig. 2. The variation of $\hat{k}$ with time when $n^*=4$ (see Table 5).

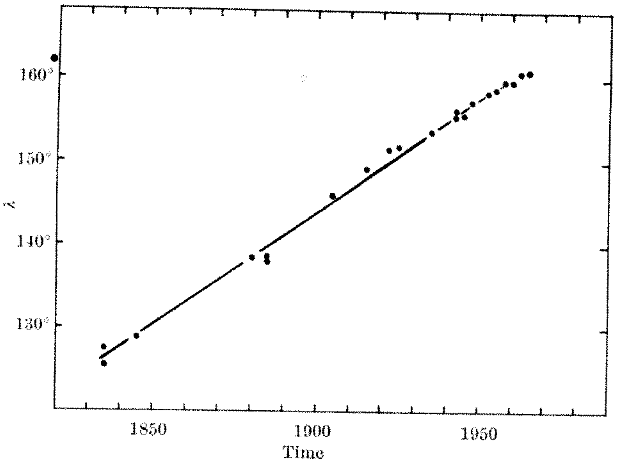


Fig. 3. The variation of $\hat{\lambda}$ with time when $n^* = 4$ (see Table 5 and equation (6)).

very closely indeed, where T is the epoch in year AD.

If the correlation between **B** and **ĝ** is a manifestation of strong magnetohydrodynamic interactions between core motions and bumps on the core–mantle interface[2,3], then the main features of the drifting part of the non-dipole component of the present geomagnetic field might have been produced by such an interaction about 500 years ago, when (if we assume that equation (6) was at least roughly applicable at and since that time) $\hat{\lambda}$ was still positive but very small. This tentative conclusion is consistent both with theoretical ideas concerning the rates of propagation and dispersion of free magnetohydrodynamic oscillations of the core[2] and with archaeomagnetic data[28], which indicate that 0·23 ± 0·06 degrees of longitude per year has been the westward drift rate since about 1400 but that the drift was much slower and possibly even eastward between 1100 and 1400.

Received November 24, 1969; revised January 22, 1970.

Appendices

(A) The infinite series introduced in equation (1) is defined as follows

$$\sum_{n=1}^{\infty}\sum_{m=0}^{n} S(A_n, A_n^m, B_n^m) \equiv \sum_{n=1}^{\infty} A_n\left(\frac{R}{r}\right)^{n+1} P_n(\cos\varphi) + \sum_{n=1}^{\infty}\sum_{m=1}^{n}\left(\frac{R}{r}\right)^{n+1} P_n^m(\cos\varphi)[A_n^m \cos m\lambda + B_n^m \sin m\lambda] \qquad \text{(A1)}$$

where

$$P_n(x) \equiv \frac{1}{2^n n!}\,\frac{\mathrm{d}^n}{\mathrm{d}x^n}\,(x^2-1)^n \qquad \text{(A2)}$$

and

$$P_n^m(x) \equiv \left[\frac{2(2n+1)(n-m)!}{(n+m)!}\right]^{1/2}(1-x^2)^{m/2}\,\frac{\mathrm{d}^m}{\mathrm{d}x^m}\,P_n(x) \qquad \text{(A3)}$$

The terms involving the dimensionless coefficients A_n are the zonal harmonics, which are independent of λ, and those involving the pairs of coefficients (A_n^m, B_n^m) are the tesseral harmonics[6,7].

The dimensionless coefficients (a_n^m, b_n^m) appearing in equation (2) are related as follows to the Schmidt quasi-normal coefficients[8,9] generally used in geomagnetic work

$$(a_n^m, b_n^m) = [R^3/a_1 L(2n+1)^{1/2}](g_n^m, h_n^m) \qquad \text{(A4)}$$

(B) The coefficient of correlation between U and V at $r = R$ is given by the equation

$$k = \frac{\sum_{m,n}(a_n^m A_n^m + b_n^m B_n^m)}{[\sum_{m,n}[(a_n^m)^2+(b_n^m)^2]]^{1/2}[\sum_{m,n}[(A_n^m)^2+(B_n^m)^2]]^{1/2}} \qquad \text{(B1)}$$

with corresponding expressions involving $(\dot{a}_n^m, \dot{b}_n^m)$ and (α_n^m, β_n^m) for the drifting and non-drifting secular variation fields (see equation (3) and compare equation (6)).

(C) Introduce the following quantities:

$$c_n^m \equiv [(a_n^m)^2+(b_n^m)^2]^{1/2},\quad \theta_n^m \equiv \tan^{-1}(b_n^m/a_n^m) \qquad \text{(C1)}$$

$$C_n^m \equiv [(A_n^m)^2+(B_n^m)^2]^{1/2},\quad \Theta_n^m \equiv \tan^{-1}(B_n^m/A_n^m) \qquad \text{(C2)}$$

$$\hat{\theta}_n^m \equiv \theta_n^m - m\hat{\lambda} \qquad \text{(C3)}$$

$$\delta_n^m \equiv \hat{\theta}_n^m - \Theta_n^m \qquad \text{(C4)}$$

and

$$Q_{m,n} \equiv |\delta_n^m|/\pi \qquad \text{(C5)}$$

Brier's test is based on the fact that if the angles δ_n^m are distributed uniformly and independently of each other over the interval $-\pi$ to π, then the coefficients $Q_{m,n}$ should average 0·5.

The weighted standard deviate y is given by[27]

$$y = \frac{\sum_{m,n}(c_n^m C_n^m Q_{m,n}) - \frac{1}{2}\sum_{m,n}(c_n^m C_n^m)}{[\sum_{m,n}(c_n^m C_n^m)^2/12]^{1/2}} \qquad \text{(C6)}$$

On the null hypothesis that (a_n^m, b_n^m) and (A_n^m, B_n^m) are not correlated, the most probable value of y from a large sample would be zero. The probability p that the data are uncorrelated may be related to y via q, the proportion of the total area under the normal error curve lying to the left of $|y|$, which can be found from tables[26].

$$q = 1 - \tfrac{1}{2}p \qquad \text{(C7)}$$

Brier's test is much more stringent than the Student t test and seems to take into account all the factors in the particular problem in hand that renders the t test inadequate.

[1] Jeffreys, H., *The Earth*, fourth ed. (Cambridge Univ. Press, London, 1959). Bullard, E. C., in *The Earth as a Planet* (edit. by Kuiper, G. P.) (Univ. Chicago Press, 1954).

[2] Hide, R., *Phil. Trans. Roy. Soc.*, A, **259**, 615 (1966); *J. Fluid Mech.*, **39**, 283 (1969).

[3] Hide, R., *Science*, **157**, 55 (1967).

[4] Hide, R., *Nature*, **222**, 1055 (1969).

[5] Hide, R., and Horai, K. I., *Phys. Earth Planet. Interiors*, **1**, 305 (1968).

[6] Kaula, W. M., *An Introduction to Planetary Physics* (John Wiley and Sons, London, 1968).

[7] Cook, A. H., *Phil. Trans. Roy. Soc.*, A, **262**, 119 (1967).

[8] Chapman, S., and Bartels, J., *Geomagnetism* (Univ. Press, Oxford, 1940).

[9] Mauersberger, P., *Geophysikalisches Institut Potsdam, Abhandlungen Nr. 5*, 1 (1952).

[10] Malin, S. R. C., and Pocock, S. B., *Pure Appl. Geophys.*, **75**, 117 (1969).

[11] Bullard, E. C., Freedman, G., Gellman, H., and Nixon, J., *Phil. Trans. Roy. Soc.*, A, **243**, 67 (1950).

[12] Yukutake, T., *Bull. Earth. Res. Inst.*, **40**, 1 (1962).

[13] Nagata, T., *Benedum Earth Magnetism Symposium* (Pittsburgh, 1962).

[14] Guier, W. H., and Newton, R. R., *J. Geophys. Res.*, **70**, 4613 (1965).

[15] Gaposhkin, E. M., *Space Res.*, **7**, 685 (North Holland Pub. Co., Amsterdam, 1967).

[16] Kaula, W. M., *J. Geophys. Res.*, **71**, 5303 (1966).

[17] Anderle, R. J., *US Naval Weapons Lab. Rep. No. 1978* (1966).

[18] Leaton, B. R., Malin, S. R. C., and Evans, M. J., *J. Geomag. Geoelect. (Kyoto)*, **17**, 187 (1965).

[19] Intern. Assoc. Geomag. Aeron., *World Magnetic Survey 1957–69* (in the press).

[20] Cain, J. C., *J. Geomag. Geoelect. (Kyoto)*, **19**, 335 (1967).

[21] Orlov, V. P., *Catalogue of Total Field Variations from Cosmos-49*, **1** (Akad. Nauk., Moscow).

[22] Malin, S. R. C., *Geophys. J. Roy. Astron. Soc.*, **17**, 415 (1969).

[23] Nagata, T., and Syono, Y., *J. Geomag. Geoelect. (Kyoto)*, **12**, 84 (1961).

[24] Nagata, T., and Oguti, T., *J. Geomag. Geoelect. (Kyoto)*, **14**, 125 (1962).

[25] Intern. Assoc. Geomag. Aeron., *J. Geophys. Res.*, **74**, 4407 (1969).

[26] Fisher, R. A., *Statistical Methods for Research Workers* (Oliver and Boyd, London, 1938).

[27] Brooks, C. E. P., and Carruthers, N., *Handbook of Statistical Methods in Meteorology* (Meteorological Office Publication 538, 1953).

[28] Burlatskaya, S. P., Nechayeva, T. B., and Petrova, G. N., *Izv. Earth Physics, No. 12*, **62** (1968). Kawai, N., Hirooka, K., and Tokieda, K., *Earth Planet. Sci. Lett.*, **3**, 48 (1967). Aitken, M. H., and Weaver, G. M., *J. Geomag. Geoelect.*, **17**, 391 (1965).

[29] McDonald, K. L., and Gunst, R. H., *ESSA Tech. Rep.* IER46–IES-1 (1967).

Abundance of Iron in the Solar Photosphere

by
JOHN E. ROSS
Department of Astronomy,
University of California,
Los Angeles

Recent measurements of iron *f*-values indicate that the iron abundance derived from neutral iron lines formed in the solar photosphere based on older *f*-values should be increased by a factor of ~ 5. This increase is partially offset if the damping constant is taken into account.

THE solar iron abundance has been intensively investigated because photospheric determinations seem to give a substantially smaller iron/silicon ratio than do the coronal. The ratio found by Pottasch[1] agrees with an average ratio for the chondritic meteorites, and some writers argue that the photospheric result is spurious.

Recent measurements of iron *f*-values (unpublished work of S. J. Wolnik *et al.* and ref. 2) indicate systematic errors in *f*-values of transitions arising from high upper levels ($\chi_u > 3$ eV) in earlier *f*-value compilations[3,4]. A straightforward application of these new *f*-values by Garz *et al.*[5] in a curve of growth analysis yields a solar iron abundance $\log [N(\mathrm{Fe})/N(\mathrm{H})] + 12 = \{\mathrm{Fe}\} = 7{\cdot}5$, a value in agreement with the coronal and meteoric determinations.

These authors have used the total intensities (equivalent widths, W_λ) of the lines, ignoring the information contained in the shape or profile of the line. It is the purpose of this article to emphasize the importance of the line profile in abundance analysis.

The Emergent Line Profile

From the most elementary point of view, the emergent (observable) profile of a spectrum line is governed intrinsically by the mean profile of the line absorption coefficient, and extrinsically by large-scale photospheric motions.

The photospheric absorption coefficient of a neutral iron line is broadened simultaneously by collisions with neutral hydrogen atoms (the width at half-intensity characterized by Γ_v), by thermal doppler effects, and by small-scale photospheric motions commonly called microturbulence. By contrast, large-scale (that is, no relative motion within the region of line formation) photospheric motions involved in extrinsic broadening are called "macroturbulence". The most probable microturbulent and macroturbulent velocities are denoted by ξ and Ξ respectively.

If the physical state of the solar atmosphere in the region of line formation is known, the uncertain quantities governing the theoretical line profiles are the kinematical parameters ξ and Ξ, the atomic parameters, Γ_v, and the product of the abundance $N(\mathrm{Fe})$ and the Ladenburg f, $N(\mathrm{Fe})f$. Inadequacy in theory and observations prevents the extraction of these parameters from a single line; instead, one must obtain ξ and Ξ from judiciously chosen lines and then deduce Nf and Γ_v for each line, subject to uncertainties in ξ and Ξ. We now consider each of these effects in turn.

Macroturbulence in the solar photosphere has little effect on the equivalent width of the line. Significantly, the effect on the line profile is to mimic both ξ and Γ_v, thus rendering any empirical evaluation of the latter parameters by profile analysis extremely difficult. To some extent, a determination of Ξ seems straightforward. If we take $\xi = 2$ km s^{-1} and increase Γ_v to ten times its theoretical value, $\Xi \sim 1{\cdot}8$ km s^{-1} is required to bring theoretically computed, crowded spectral regions into accord with observations. If we take $\xi = 0$ and $\Gamma_v = 0$ we obtain an upper limit $\Xi \sim 2{\cdot}5$ km s^{-1}. Preliminary studies indicate $\xi^2 + \Xi^2 \sim 5$ km s^{-1} with the most probable values being $\Xi = 2{\cdot}2$ km s^{-1} and $\xi = 1$ km s^{-1}, which are adopted for this analysis. The instrumental profile inserts a smearing effect similar to macroturbulence. The effective smearing corresponds to a doppler velocity < 1 km s^{-1} which would make the true macroturbulent velocity ~ 2 km s^{-1}.

Microturbulence in the photosphere gives rise to a doppler effect analogous to the thermal doppler broadening of the line absorption coefficient. The appropriate value of ξ as a function of optical depth in the photosphere has not been satisfactorily determined, and the depth independent value $\xi = 1$ km s^{-1} has been arbitrarily adopted. The equivalent widths of medium-strength lines are strongly dependent on ξ; in any instance where ξ affects the results of the analysis, the quantity $\mathrm{d}[\log (N)]/\mathrm{d}\xi$ is given, enabling the results to be adjusted within the range $0{\cdot}5 < \xi < 1{\cdot}5$ km s^{-1}.

The damping constant affects the equivalent widths of medium-strength to strong (saturated) lines. In the limiting case of strong lines, the line strength and profile are determined by the product $Nf\Gamma_v$, and separation of Nf and Γ_v becomes impossible. In the limiting case of weak lines, Nf determines the line strength, and ξ, Ξ and Γ_v determine the line profile. The value of Γ_v in the case of weak lines is determinable only if the damping half-width is comparable with the combined ξ, Ξ half-width. In the case of medium-strength lines, Γ_v is determinable by profile analysis within limits imposed by observations and uncertainties in Ξ; the larger the damping constant, the more precise the determination.

The chief contribution to the damping constant of a neutral iron line in the solar photosphere arises from collisions with neutral hydrogen atoms. The impact approximation as proposed by Unsold[6] or used by Aller[7], for example (utilizing Coulomb wave functions, or scaled Thomas–Fermi (STF) wave functions[8]), is invariably used to evaluate Γ_v. In the case of neutral iron the approximation is questionable and there is evidence which indicates that the theory predicts damping constants that are too low by factors ranging from two to ten for $\chi_u < 6$ eV.

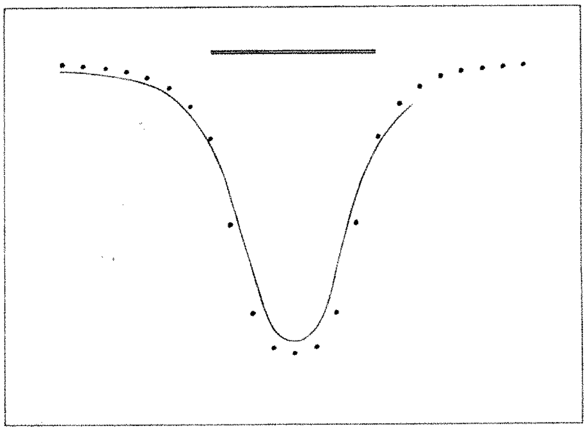


Fig. 1. Fe I $\lambda 5{,}434{\cdot}5$ a^5F–$z^5D°$ transition. The solid line represents both the observed profile and a synthetic profile for $\{\mathrm{Fe}\} = 6{\cdot}7$, $\Gamma_v = 12\Gamma_v^{\mathrm{stf}}$. The dotted line is a synthetic profile with $\{\mathrm{Fe}\} = 7{\cdot}7$ and $\Gamma_v = \Gamma_v^{\mathrm{stf}}$. The residual intensity in the observed line core is 0·15 that of the continuum (double horizontal bar).

Kusch[9] has determined Γ_v experimentally for a number of neutral iron lines. If the ratio $\Gamma^0_v = \Gamma_v$ (Kusch)/$\Gamma_v{}^{\mathrm{stf}}$ versus χ_u is plotted, a fit to the lower envelope of the points gives

$$\Gamma_v \sim 30{\cdot}7 - 11{\cdot}5\ \chi_u + 1{\cdot}524\ \chi_u{}^2 - 0{\cdot}07111\ \chi_u{}^3\ (3 < \chi_u < 5{\cdot}5\ \mathrm{eV})$$

for s–p transitions (that is, transitions arising from even-parity lower levels). The damping constants for p–s transitions with $\chi_u \sim 5{\cdot}5$ eV scatter around $2 < \Gamma^0_v < 8$.

Wolnik *et al.*[10] present an empirical curve of growth obtained from shock-tube measurements of neutral chromium. The damping to doppler ratio indicated by the curve of growth suggests damping in excess of that predicted by theory. Because Cr and Fe are similar in atomic structure, one would expect that a similar excess occurs in iron.

Abundance Determination

The photospheric abundance determination has been attacked from several points of view by a combination of the direct–profile–integration curve of growth method[11], and by spectrum line synthesis[12]. Equivalent widths were taken from the second revision of Rowland's table[13]. Line profiles were obtained at the McMath–Hulbert Observatory. Corliss and Tech[4] *f*-values are denoted by "CT", *f*-values from unpublished work of Wolnik *et al.* by "W" and Garz and Kock[2] *f*-values by "GK". Unless otherwise specified, two values of the damping constant were used: theoretical[8] ($\Gamma_v{}^{\mathrm{stf}}$), and half the value given by the fit to Kusch's[9] lower envelope fit ($\Gamma_v{}^{\mathrm{K}}$).

The photospheric model is due to Elste[14], but the results are insensitive to the model employed.

(i) A curve of growth analysis of fifty weak ($W_\lambda < 0{\cdot}03$ Å) high excitation lines in the wavelength region $5000 < \lambda < 6000$ Å gives $\{\mathrm{Fe}\} = \log\ [N(\mathrm{Fe})/N(\mathrm{H})] + 12 = 6{\cdot}4 \pm 0{\cdot}2$ based on CT *f*-values. Adopting the correction indicated by Wolnik *et al.*, $\Delta gf = \log\ [gf(\mathrm{CT})/gf(\mathrm{W})] \lesssim 0{\cdot}7$, these lines give $\{\mathrm{Fe}\} \lesssim 7{\cdot}1$. Dependence on Γ_v and ξ is negligible for these lines.

(ii) A curve of growth analysis of thirty-five low excitation lines belonging to multiplets 1, 12, 13, 15 and 16 gives $\{\mathrm{Fe}\} \sim 6{\cdot}8$ for the weaker lines and $\{\mathrm{Fe}\} \sim 7{\cdot}5$ for the stronger lines using CT *f*-values and Γ^{stf}. If $\Gamma_v{}^{\mathrm{K}}$ is employed, $\{\mathrm{Fe}\} \sim 6{\cdot}9 \pm 0{\cdot}2$, with a marked reduction in the scatter of abundances about the mean value. $\Delta gf \sim 0{\cdot}2$ for these multiplets (with the exception of multiplet 15) (ref. 15) resulting in $\{\mathrm{Fe}\} \sim 7{\cdot}1$ with $\mathrm{d}\{\mathrm{Fe}\}/\mathrm{d}\xi = 0{\cdot}3$.

(iii) A curve of growth analysis of twenty-six s–p and forty-four p–s lines, for which W *f*-values are available, gives $\{\mathrm{Fe}\} \sim 7{\cdot}4 \pm 0{\cdot}25$, $\mathrm{d}\{\mathrm{Fe}\}/\mathrm{d}\xi = 0{\cdot}3$, a value in accord with the analysis of Garz *et al.*[5] whose results were reproduced as a procedural check. If $\Gamma_v{}^{\mathrm{K}}$ is adopted, the s–p transitions give $\{\mathrm{Fe}\} = 7{\cdot}15 \pm 0{\cdot}2$, $\mathrm{d}\{\mathrm{Fe}\}/\mathrm{d}\xi\ 0{\cdot}2$. If $\Gamma^0_v = 4$, as Kusch indicates for p–s transitions, the p–s lines give $\{\mathrm{Fe}\} = 7{\cdot}15 \pm 0{\cdot}2$, $\mathrm{d}\{\mathrm{Fe}\}/\mathrm{d}\xi = 0{\cdot}25$.

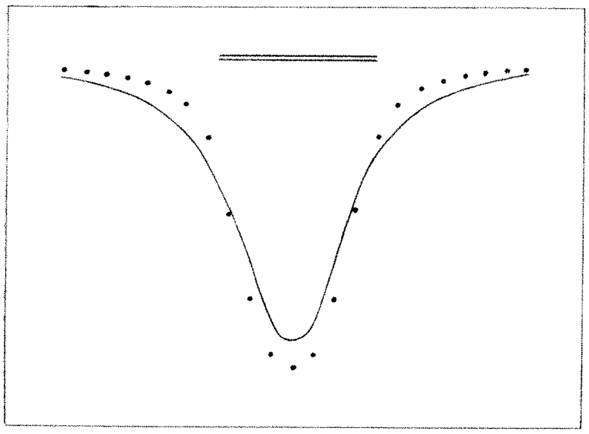


Fig. 2. Fe I λ5,568·6 z^5F°–e^5D transition. The solid line represents both the observed profile and a synthetic profile for $\{\mathrm{Fe}\} = 6{\cdot}85$, $\Gamma_v = 12L_v{}^{\mathrm{stf}}$. The dotted line is a synthetic profile for $\{\mathrm{Fe}\} = 7{\cdot}7$ and $\Gamma_v = \Gamma_v{}^{\mathrm{stf}}$. The intensity in the observed line core is 0·18 that of the continuum (double horizontal bar).

(iv) The synthesis method was applied to five of the least blended s–p lines having W and GK *f*-values. The damping constant is intrinsically small[8] for these lines and separation of $N(\mathrm{Fe})$ from Γ is difficult, but the use of $\Gamma_v{}^{\mathrm{K}}$ gives systematically better profile fits (see Fig. 1).

(v) The synthesis method was applied to ten of the least blended p–s lines having W and GK *f*-values. These lines naturally have theoretical damping constants which are an order of magnitude greater than the s–p lines studied[8]. The damping constants for medium-strength p–s lines are easily separable from $N(\mathrm{Fe})$ and there is little doubt that these transitions have damping constants several times that predicted by theory (see Fig. 2).

(vi) The appropriate damping constants for multiplets 15, 16, 553 and 1,146 were derived by a (non-linear) least-squares analysis. Assuming the damping constant to be the same for all members of a given multiplet, the minimum abundance scatter occurs with $\Gamma_v > \Gamma_v{}^{\mathrm{stf}}$ for multiplets 15, 553 and 1,146 (W *f*-values) and $\Gamma_v = 6\Gamma_v{}^{\mathrm{stf}}$ for multiplet 16 (CT *f*-values).

Discussion

A straightforward curve-of-growth analysis using γ-values from Wolnick *et al.* or from Garz and Kock[2] and theoretical (STF) damping constants yield

$$\{\mathrm{Fe}\} = 7{\cdot}4 \pm 0{\cdot}3,\quad \mathrm{d}\{\mathrm{Fe}\}/\mathrm{d}\xi = -0{\cdot}3$$

A profile analysis indicates Γ_v should be increased over the theoretical value for s–p transitions, and shows conclusively that Γ_v must be increased for p–s transitions. If Γ_v is increased four times for p–s transitions, and half the damping given by the fit to Kusch's[9] data is employed for s–p transitions, then $\{\mathrm{Fe}\} = 7{\cdot}15 \pm 0{\cdot}2$, $\mathrm{d}\{\mathrm{Fe}\}/\mathrm{d}\xi = -0{\cdot}25$, and the computed profiles are in better agreement with observations. If the damping is indeed as large as that indicated by Kusch, a value which would not conflict with profile analysis, $\{\mathrm{Fe}\} \lesssim 6{\cdot}9$ is indicated.

The photospheric iron abundance must be considered uncertain until reliable damping constants are obtained for lines having reliable *f*-values, or until reliable *f*-values for weak lines are found. This analysis indicates, however, that $\{\mathrm{Fe}\} = 7{\cdot}2$ should be considered an upper bound based on the newer *f*-values (unpublished work of Wolnick *et al.* and ref. 2).

I thank Professor L. H. Aller for guidance in the abundance problem, Dr Gordon Wares for *f*-values in advance of publication, and Dr Orren Mohler and Dr Walter S. Mitchell for observational data in advance of publication. Part of the computing costs and use of the computing facilities were provided by Campus Computing Network, University of California, Los Angeles. The programme is supported by a NASA grant to the University of California, Los Angeles.

Received January 8, 1970.

[1] Pottasch, S. R., *Astrophys. J.*, **137**, 945 (1963).

[2] Garz, T., and Kock, M., *Astron. Astrophys.*, **2**, 274 (1969).

[3] Corliss, C. H., and Bozman, W. R., *NBS Monograph No. 53* (US Dept of Commerce, Washington DC, 1962).

[4] Corliss, C. H., and Tech, J. L., *NBS Monograph No. 108* (US Dept of Commerce, Washington, DC 1968).

[5] Garz, T., Holweger, H., Kock, M., and Rochter, J., *Astron. Astrophys.*, **2**, 446 (1969).

[6] Unsold, A., *Physik der Sternatmosphären* (Springer-Verlag, Berlin, 1955).

[7] Aller, L. H., *Astrophysics, the Atmospheres of the Sun and Stars* (second ed.) (Ronald Press Co., New York, 1963).

[8] Warner, B., *Mon. Not. Roy. Astron. Soc.*, **136**, 381 (1967).

[9] Kusch, H. J., *Z. Astrophys.*, **45**, 1 (1958).

[10] Wolnik, S. J., Berthel, R. O., Carnevale, E. H., and Wares, G. W., *Astrophys. J.*, **157**, 983 (1969).

[11] Auer, L. H., Mihlas, D., Aller, L. H., and Ross, J. R., *Astrophys. J.*, **145**, 153 (1966).

[12] Ross, J. E., and Aller, L. H., *Astrophys. J.*, **153**, 235 (1968).

[13] Moore, C. E., Minnaert, M. G. J., and Houtgast, J., *NBS Monograph No. 61*, US Dept of Commerce, Washington, DC, 1966).

[14] Elste, G., *Solar Phys.*, **3**, 106 (1968).

[15] Corliss, C. H., and Warner, B., *Astrophys. J. Suppl.*, **83**, 395 (1964).

Possible Mechanism for the Pulsar Radio Emission

by
M. M. KOMESAROFF
Division of Radiophysics,
CSIRO, Sydney,
Australia

An attempt to account quantitatively for the pulse width, polarization and spectrum of the pulses.

ANY explanation of the pulsar radio emission must take account of the fact that although a pulsar may emit highly variable individual pulses, the average properties of a large number observed at a fixed wavelength are characteristic of that particular pulsar and show the following features (some of which come from the unpublished work of myself and my colleagues). (*a*) The pulse duration is a small fraction of the interval between pulses. (*b*) The pulse energy is extremely intense and usually varies approximately at the wavelength (λ) for values of λ down to about 0·2 m. For shorter wavelengths the spectral decrease may be much steeper[1]. (*c*) The average pulse is usually single or double, although higher order multiplicities are sometimes observed. (*d*) The pulse shape is a slowly varying function of wavelength. (*e*) For pulsars which emit pulses with double structure (such as NP 0527, CP 1133 and PSR 2045–16), the spacing between components varies[2,3] approximately as $\lambda^{1/4}$. Drake has indicated that at very long wavelengths the intrinsic pulse shapes of several pulsars become double (Sixteenth General Assembly of URSI, Ottawa, 1969). (*f*) In several cases the pulse is almost completely linearly polarized. Examples are PSR 0833–45 and PSR 1929+10 (ref. 4). (*g*) For PSR 0833–45, the plane of polarization rotates through a large angle during the pulse, and the rate of rotation is almost independent of wavelength[5,6]. (*h*) In the case of the double pulses emitted by MP 0736 and PSR 2045–16 a very rapid change in the direction of polarization is observed near the time of bifurcation of the pulse.

In explanation of the PSR 0833–45 results (*f*) and (*g*), Radhakrishnan and Cooke[5] have suggested that the radiation is sharply beamed from the vicinity of a magnetic pole, and that the position angle reflects the instantaneous direction of the magnetic field lines in the emitting region. More recently Radhakrishnan[7] has proposed that the radiation is produced by relativistic particles moving almost directly along the magnetic field lines and radiating by virtue of the acceleration associated with the curvature of these lines, in contrast with the synchrotron radiation produced by particles gyrating around the field lines.

The present argument carries Radhakrishnan's suggestions a step further and attempts to account quantitatively for the pulse width, polarization and spectrum of the pulses.

Assumptions for the Pulsar Model

Almost any attempt to explain the intensity of the pulsar radio emission must have recourse to the concept of coherence, that is, of large numbers of particles emitting "in phase". In view of the spectrum this implies correlated fluctuations of particle density extending over only a few centimetres along the line of sight. To account for the intensity of the radiation without invoking prohibitively large densities of particles, we must assume that in the direction perpendicular to the line of sight the correlated fluctuations extend over some hundreds of metres. I therefore suggest the following model of a pulsar:

(i) Pulsars are rotating neutron stars (radius $\approx 10^4$ m) with surface magnetic fields as large as 10^{12} G. The magnetic field near the stellar surface is assumed to be approximately dipolar.

(ii) The magnetic axes make large angles (≈ 1 rad) with the rotational axes[8].

(iii) The radiation is produced by short-duration fluctuations of charged particle density extending over only a fraction of a metre in the direction of the field lines, and extending over a few hundred metres in the direction perpendicular to the field lines.

(iv) The particles are accelerated to relativistic velocities within a very short distance of the stellar surface, and those responsible for the radio emission move almost directly along the magnetic field lines with negligible velocity of gyration.

(v) The relevant particles are predominantly those moving along particular groups of field lines having radii of curvature of the same order as those lines which would be tangential to the velocity-of-light cylinder if the dipole configuration were maintained out to that distance.

(vi) The fluctuating component of particle density, N, falls off less rapidly than does the magnetic field. I assume that N is approximately proportional to $B^{1/2}$, which implies that the fluctuations are enhanced as they move outward.

Assumptions (v) and (vi) will be discussed in more detail later. They imply that most of the radio emission originates within a few radii of the stellar surface and well within the velocity-of-light cylinder.

Radiation from the Model Pulsar

A charged particle moving along a curved field line emits a "spike" of energy toward a distant observer from that point at which the tangent to the field line is most nearly parallel to the observer's line of sight. The radiation is beamed into a small angle along the tangent, mainly at frequencies below a "critical frequency" f_c, given by[9]

$$f_c = \frac{3\gamma^3 c}{\pi \rho} \tag{1}$$

where ρ is the radius of curvature of the line and γ is the ratio of the total energy to the rest energy of the particle. Within a few degrees of a magnetic pole

$$\rho = \frac{4r}{3\theta} \tag{2}$$

where r and θ are polar coordinates measured with respect to the magnetic axis. Combining equations (1) and (2),

$$f_c = \frac{9\gamma^3 c\theta}{16\pi r}$$

For $\gamma \gtrsim 10^3$, $\theta \gtrsim 1/30$ rad, and $r \lesssim 3 \times 10^4$ m, $f_c \gtrsim 10^{11}$ Hz, and so most of the emission would be in the radio domain.

The E-vector of the radiation received at any instant will be approximately in the plane of the field line pointing toward the observer. Because this plane necessarily contains the magnetic axis, the position angle (P.A.) of the E-vector will be nearly the same as the position angle of the magnetic axis. Measured with respect to the rotational axis

$$\tan(\text{P.A.}) = \frac{\sin\chi \sin l}{\sin\varphi \cos\chi - \cos\varphi \sin\chi \cos l} \tag{3}$$

$$\left. \mathrm{d}(\text{P.A.})/\mathrm{d}l \right|_{l=0} = \frac{\sin\chi}{\sin(\varphi - \chi)}$$

where χ and φ are the angles made by the magnetic axis and the line of sight respectively with the rotational axis; l is the longitude of the magnetic axis with respect to the plane containing the rotational axis and the observer.

From equation (3) it follows that if the line of sight lies exactly on the cone swept out by the magnetic axis ($\chi = \varphi$), the position angle changes discontinuously from $-\pi/2$ to $+\pi/2$ as l passes through zero. In this situation a double pulse will be observed. If the line of sight only grazes the beam of emission, a single pulse will be observed. As shown below, the total emission beam has a narrow angular radius, and because according to assumption (ii), $\sin\chi \approx 1$, $\mathrm{d(P.A.)}/\mathrm{d}l \gg 1$.

For frequencies well below the critical frequency, in the plane "tangential" to the field line (see Fig. 1), the emission from a single particle is confined chiefly to an angle of about $2\xi_c$, where

$$\xi_c = \left(\frac{3\lambda}{2\pi\rho}\right)^{1/3} \tag{4}$$

Taking into account equation (2) and the equation of a field line, which is

$$r = r_e \sin^2\theta = r_e\theta^2, \text{ for small } \theta \tag{5}$$

(see Fig. 2), then

$$\xi_c = \left(\frac{9\lambda}{8\pi r_e\theta}\right)^{1/3} \tag{6}$$

The energy density emitted by the particle[9] is approximately

$$\frac{\mathrm{d}I(f)}{\mathrm{d}\Omega} = K\xi_c^{-2} \text{ J sr}^{-1} \text{ Hz}^{-1}$$

where $K = 1{\cdot}2 \times 10^{-36}$ if the particle carries one electronic charge, and Ω represents solid angle.

The radius of curvature of the surface perpendicular to the field lines in the polar region is

$$\rho_V = (2{\cdot}3)r = (2/3)r_e\theta^2$$

and the angular radius of the first Fresnel zone associated with this surface can be seen from Fig. 1 to be

$$\xi_F = \left(\frac{\lambda}{\rho_V}\right)^{1/2} = \left(\frac{3}{2}\frac{\lambda}{r_e}\right)^{1/2}\theta^{-1} \tag{7}$$

In general, for small values of θ, $\xi_c \ll \xi_F$ at the stellar surface, but as θ increases ξ_F decreases more rapidly than ξ_c. For $\xi_c < \xi_F$, the volume containing all those particles which emit in-phase is

$$V = \pi\rho_V^2\,\xi_c\,\xi_F\,d$$

d being the "thickness" of the bunch. By virtue of the in-phase emission the total energy density is proportional to the square of the number of emitting particles and is given by

$$J = N^2(\theta)\,V^2\frac{\mathrm{d}I(f)}{\mathrm{d}\Omega} = \pi^2 K N^2(\theta)\,d^2 \cdot \rho_V^3 \cdot \lambda$$

According to assumption (vi), the particle density along a particular field line may be written $N(\theta) = N_0\theta^{-3}$, where N_0 is the particle density at the stellar surface. Thus substituting for ρ_V

$$J = \pi^2 K N_0^2 (2/3\,r_e)^3\,\lambda d^2 \tag{8}$$

This will hold provided $\lambda > d$, and hence yields the approximate spectral law mentioned in (*b*).

Equation (8) only applies if $\xi_c < \xi_F$. For larger values of θ, $\xi_c > \xi_F$, and in-phase radiation is emitted only from the volume $\pi\rho_V^2\,\xi_F^2$, because contributions from outside the first Fresnel zone will be in antiphase with the main emission (Fig. 1). For this region the value of J given by equation (8) must be multiplied by ξ_F^2/ξ_c^2, which varies as $\theta^{-4/3}$, and therefore $\xi_c = \xi_F$ defines the region of strong emission. We define θ_m to be that angle for which $\xi_c = \xi_F$. From equations (6) and (7)

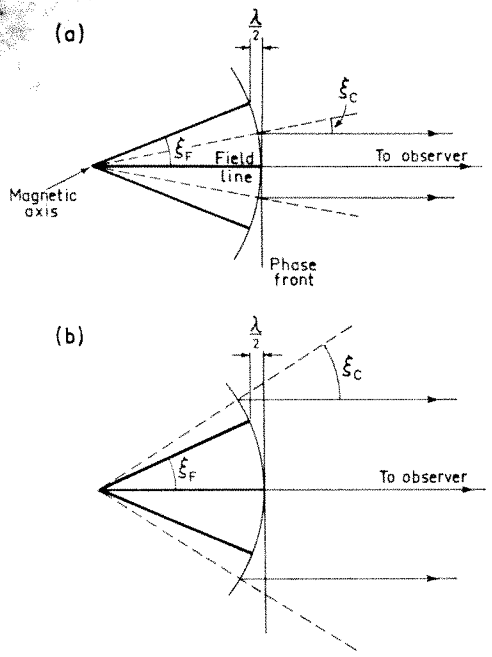


Fig. 1. The plane of this figure (the "tangent plane") contains the tangent to a field line and is perpendicular to the plane of the line. The field lines as projected on this plane are shown as radii of the circular arc, which is a section of the surface normal to the lines. Two cases are shown: (*a*) $\xi_c < \xi_F$. All the radiation reaching the observer is emitted within the first Fresnel zone and is therefore in-phase. (*b*) $\xi_c > \xi_F$. Some of the radiation is emitted from beyond the first Fresnel zone and is therefore in antiphase with the main emission. (Sizes of angles have been exaggerated for the sake of clarity.)

$$\theta_m = 2{\cdot}26\left(\frac{\lambda}{r_e}\right)^{1/4} \text{ rad}$$

The tangent to the field line at the point (r, θ_m) makes an angle $\psi = 3/3\,\theta_m$ with the magnetic axis (see Fig. 2). Thus the spacing between emission maxima is

$$2\psi = 3\theta_m = 6{\cdot}8\left(\frac{\lambda}{r_e}\right)^{1/4} \text{ rad} \tag{9}$$

The form of this wavelength dependence is that required by (*e*) above.

Assuming that the field lines involved are those approximately tangential to the velocity-of-light cylinder, then

$$r_e = \frac{cP}{2\pi}$$

(Fig. 2), where P is the rotation period in seconds and

$$2\psi = 8 \times 10^{-2}\left(\frac{\lambda}{P}\right)^{1/4} \text{ rad} \tag{10}$$

Because the dependence on P is so slow, it could be masked by variations of geometry from pulsar to pulsar.

Finally, it should be noted that for typical values of P and λ most of the radiation is emitted within a few stellar radii of the neutron star, well within the velocity-of-light cylinder.

Discussion

According to the model just outlined, pulsar radiation is emitted in a hollow beam coaxial with the magnetic axis, and the pulses are produced by the motion of this beam past the line of sight as a result of rotation. The pulses are single or double depending on whether the beam merely grazes the line of sight or intersects it twice. The model provides an explanation for the observed variation of polarization position angle within the pulse, and also

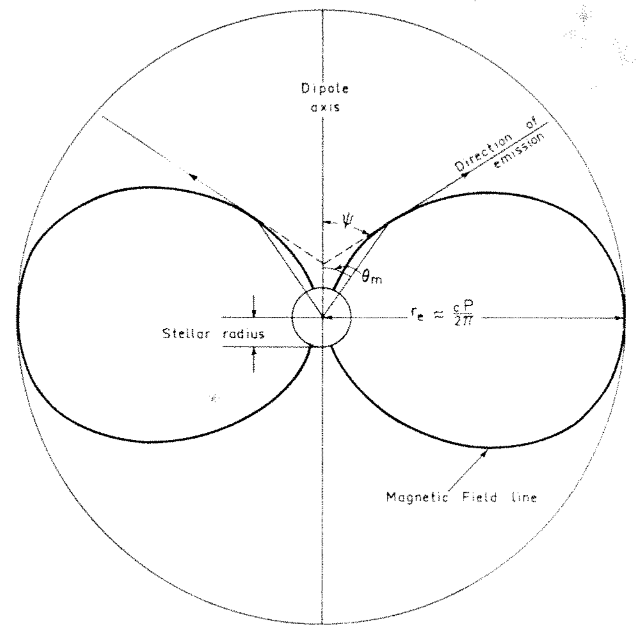


Fig. 2. The plane containing the magnetic axis, assumed to be at a large angle to the rotational axis. The larger circle represents a section of the velocity-of-light cylinder. The diagram is not drawn to scale (the radius of the light cylinder is actually hundreds or thousands of times greater than that of the star, and the angles θ_m and ψ are shown very much enlarged).

accounts for the wavelength dependence of the component spacing in double pulses. If we let $\lambda = 1$ m and $P = 1$ s, we find from equation (10) that $2\psi = 4°{\cdot}6$, which is of the right order but smaller by a factor of about 1·5 than the value actually observed for CP 1133 ($P = 1{\cdot}18$ s). The discrepancy is in the same sense but greater in the case of NP 0527, MP 0736 and PSR 2045–16. This could indicate that we were mistaken in assuming that the angle between the magnetic and rotational axes is necessarily "large" (that is, ≈ 1 rad); alternatively the explanation may be in terms of departures from a dipole configuration.

The form of the spectrum given by equation (8) is in rough agreement with observation down to $\lambda \approx 0{\cdot}2$ m. If we assume that d is of this order, the break in the spectrum of many pulsars can be explained.

Equation (8) permits an estimate of the required value of N_0, the fluctuating component of particle density at the stellar surface. Large *et al.*[10,11] find that for many pulsars the energy density per pulse at $\lambda = 0{\cdot}75$ m is about 10^{-27} J m^{-2} Hz^{-1}. Assuming that each pulse represents the radiation from a hundred or more fluctuations and that the average distance of a pulsar from the Earth is about 0·5 kpc ($1{\cdot}5 \times 10^{19}$ m), we find that $N_0 \leq 10^{10}$ cm^{-3}. Goldreich and Julian[12], considering the case of an axisymmetric neutron star, find that the space charge density is $7 \times 10^{-2}\ B_z/P$, where B_z is the component of magnetic field parallel to the rotation axis. For $P = 1$ s and $B_z \approx 10^{12}$ G, the space charge density in the co-rotating domain is 10^{11} electronic charges per cm^3.

The assumption of correlated density fluctuations has been introduced without physical justification. Some such assumption seems almost essential on any theory, in order to account for the enormously intense radio emission, without invoking prohibitively large numbers of radiating particles. The fact that, according to the present model, the fluctuations move along those field lines approximately tangential to the light cylinder suggests that they may be established as a result of disturbances propagated inward along these lines due to the kind of effect discussed by Gold[13].

Because the polarized optical emission observed in the case of NP 0532 (refs. 14–16) is presumably synchrotron radiation due to particle gyration around the field lines, it would have its E-vector approximately perpendicular to that of the radiation produced by the mechanism described earlier. Observations of the radio polarization from this pulsar could provide a critical test of the present model.

I thank several of the staff of the Division of Radiophysics for helpful criticism.

Received November 3, 1969.

[1] Moffet, A. T., and Ekers, R. D., *Nature*, **220**, 756 (1968).
[2] Craft, W. D., and Comella, J. M., *Nature*, **220**, 676 (1968).
[3] Zeissig, G. A., and Richards, D. W., *Nature*, **222**, 151 (1969).
[4] Radhakrishnan, V., Cooke, D. J., Komesaroff, M. M., and Morris, D., *Nature*, **221**, 443 (1969).
[5] Radhakrishnan, V., and Cooke, D. J., *Astrophys. Lett.*, **3**, 225 (1969).
[6] Gardner, F. F., and Whiteoak, J. B., *Nature*, **224**, 891 (1969).
[7] Radhakrishnan, V., *Proc. Astron. Soc. Austral.*, **1**, 254 (1969).
[8] Woltjer, L., *Astrophys. J. Lett.*, **152**, L179 (1968).
[9] Jackson, J. D., *Classical Electrodynamics* (Wiley and Sons, 1963).
[10] Large, M. I., Vaughan, A. E., and Wielebinski, R., *Nature*, **220**, 753 (1968).
[11] Large, M. I., Vaughan, A. E., and Wielebinski, R., *Astrophys. Lett.*, **3**, 123 (1969).
[12] Goldreich, P., and Julian, W. H., *Astrophys. J.*, **157**, 869 (1969).
[13] Gold, T., *Nature*, **221**, 25 (1969).
[14] Cooke, W. J., Disney, M. J., and Taylor, D. J., *Nature*, **221**, 525 (1969).
[15] Warner, B., Nather, R. E., and MacFarlane, M., *Nature*, **222**, 233 (1969).
[16] Wampler, E. J., Scargle, J. D., and Miller, J. S., *Astrophys. J. Lett.*, **157**, L1 (1969).

Observations of Atmospheric Turbulence with a Radio Telescope at 5 GHz

by
R. A. HINDER
Mullard Radio Astronomy Observatory,
Cavendish Laboratory,
University of Cambridge

Variations of the radio refractive index of the atmosphere are important to radio astronomers and in satellite communications. This article describes the situation at 5 GHz, based on work with the one-mile telescope at Cambridge.

CHANGES of pressure, temperature and humidity cause variations of the radio refractive index of the troposphere. Such variations are naturally of interest to meteorologists, but have recently become of practical importance in setting a limit to the resolving power of radio telescopes. They may also affect the performance of satellite communications systems. The next generation of radio telescopes using the technique of aperture synthesis may well achieve the maximum resolution which is attainable in the presence of the variations. In this context it is important to know how the electrical path length from an extraterrestrial radio source to the ground is influenced by the refractive index variations occurring along lines of sight through the atmosphere, and in particular how the resulting changes in path length are correlated on adjacent lines of sight.

Some investigators are studying refractive index variations with airborne refractometers[1], but their results apply only to local changes. An alternative technique, using a radio interferometer, measures the effect on path length directly. In this method (Fig. 1), waves from a radio source pass through the atmosphere to two aerials A_1 and A_2 spaced laterally on the ground. A line P_1P_2 above the atmosphere is drawn parallel with the line joining the two aerials so that P_1A_1 and P_2A_2 are the ends of ray paths from the radio source to the telescope. Knowing the position of the source, the relative phase of the signals at P_1 and P_2 can be continuously computed so that departures of the measured phase differences between signals at A_1 and A_2 from the computed values allow variations in the integrated phase path through the atmosphere to be found.

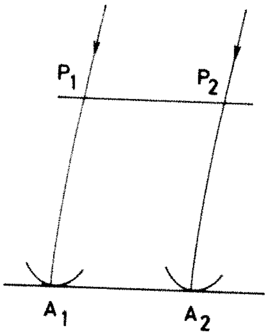


Fig. 1. Radio paths through the atmosphere from a point source to an interferometer.

For very small separations the ray paths are nearly coincident and the phase departures are common to both so that the observed phase differences show little fluctuation due to the atmosphere. As the aerials are separated the correlation between the phase fluctuations along the two paths becomes smaller, and the observed phase differences increase. The variance of the phase difference for a given separation therefore depends on the autocovariance of fluctuations in the refractive index. Because the refractive index of air is non-dispersive at radio wavelengths, the phase differences are proportional to the observing frequency.

This method has been used by Baars[2] and Basart *et al.* (unpublished) at the US National Radio Astronomy Observatory using a frequency of 2·7 GHz and baselines up to 35 km, and more recently by Gent *et al.*[3] with the RRE interferometer, also at 2·7 GHz. Their observations have demonstrated the existence of the phase variations, and suggest that they may be associated with meteorological phenomena. But no detailed evidence on the scale of the variations and how they are related to the time of day or season has yet been put forward.

This article describes the results of a more extensive series of observations carried out since 1967 at Cambridge with the one-mile radio telescope operating at 5 GHz, in order to investigate the incidence and nature of refractive index fluctuations over the UK. For these observations the three aerials comprising the telescope provide three overlapping baselines having different separations.

It has been found that the phase variations originate in a pattern of refractive index variations which moves across the telescope at wind speeds and does not as a rule evolve and decay in the time taken to move across the telescope. The observed time variations can therefore be related to spatial variations of the refractive index of the air. In this way observations made at many different aerial separations from 0·05 to 1·6 km have allowed a direct measurement of structure in this range of scale sizes; furthermore, as the pattern is effectively frozen, useful information on larger scale sizes up to about 10 km has been obtained by looking at time variations which last longer than the time taken for a feature to move across the telescope. The observations have also shown that both the scale size and the magnitude of the irregularities vary from day to day and from season to season.

Observations

A detailed description of the one-mile telescope has already been published[4]. The phases of signals received at each of three aerials are compared every 20 s and the differences between these values are related in a computer to the values expected in the absence of atmospheric refraction. The phase departures are automatically presented on a curve plotter attached to the computer, and results for typical runs are shown in Fig. 2. Three such curves are derived simultaneously, one for each pair of aerials in the telescope. Most of the observations have been made using the sources 3C 84 and 3C 454·3 at hour angles such that the path length of the radio waves through the atmosphere does not exceed the path length at the zenith by more than a factor of three.

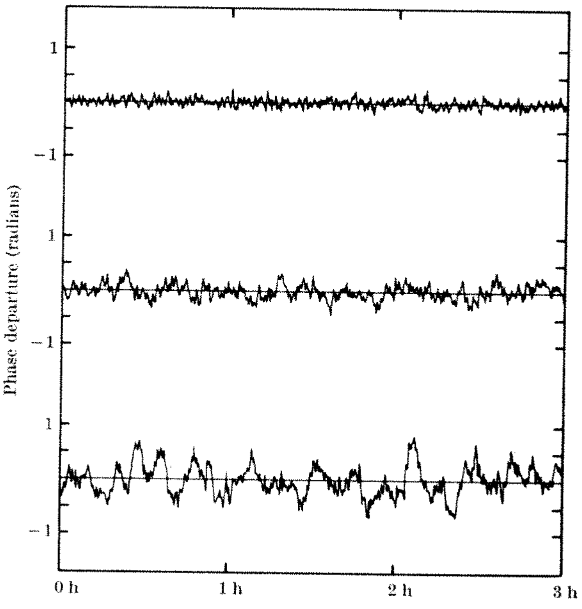


Fig. 2. Typical observations of phase fluctuations from the radio source 3C 84 at 5 GHz during 1969. Top: September 15–16, 2324–0224 UT, separation 47 m, $\sigma(\theta) = 0{\cdot}04$ rad. Centre: June 14, 1145–1445 UT, separation 469 m, $\sigma(\theta) = 0{\cdot}17$ rad. Bottom: June 6, 1012–1312 UT, separation 1,126 m, $\sigma(\theta) = 0{\cdot}38$ rad.

The phase departures observed with the three baselines are generally correlated, and a detailed analysis shows that the departures are caused by a pattern of turbulence which moves across the telescope, affecting each radio path in turn depending on the direction of the wind. The time scale of a phase fluctuation therefore depends on the spatial scale of the refractive index pattern and on the speed with which this is moving relative to the axis of the interferometer. It is typically about 5–10 min when the aerials are separated by a kilometre, as is shown in the records in Fig. 2.

The probability distribution of the phase departures recorded during an observation shows that it is Gaussian; the results have therefore been analysed in terms of the r.m.s. of the recorded phase departure. Although this quantity is determined principally by the variations of refractive index in the troposphere, there are two further contributions for which corrections can be made. The first is a component due to the receiver noise, and the second comes from the phase-sampling procedure. The r.m.s. phase departure resulting from these two effects is typically 0·11 rad, but this value may be found accurately, and hence the r.m.s. phase departure, $\sigma(\theta)$, due to the troposphere alone may be calculated. A further analysis includes the calculation of autocorrelograms for each run, cross-correlograms for pairs of separations which have been observed simultaneously, and frequency spectra. The results of these more detailed analyses will be discussed in a subsequent paper together with the results of some experiments, carried out at larger zenith angles using the source 3C 279, to find the vertical extent of the irregularities.

The observations show a wide range of $\sigma(\theta)$, depending strongly on the time of day and the season. During the summer day-time, large variations of $\sigma(\theta)$ occur from day to day, and these are found to be associated with the weather—the largest values of $\sigma(\theta)$ tending to occur during hot, clear weather conditions. The dependence of $\sigma(\theta)$ on meteorological parameters is complicated, however, and is the subject of a separate paper.

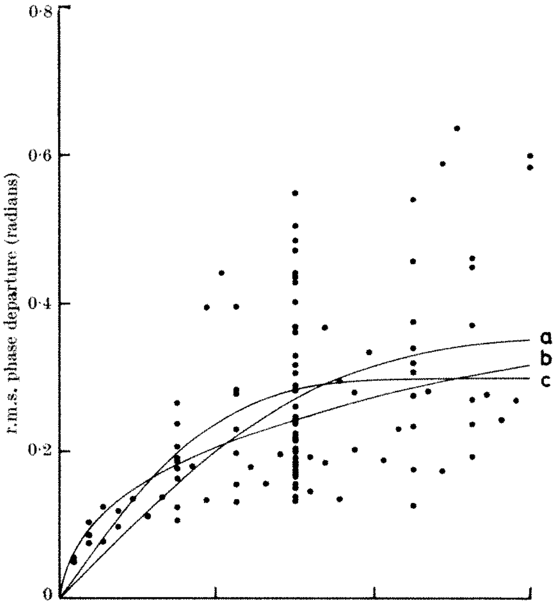


Fig. 3. The effect of aerial separation on the observed r.m.s. phase departures $\sigma(\theta)$ for the summer day-time. The curve *a* represents a Gaussian model having $a=0·36$ rad and a scale size of 800 m, curve *b* has $a=0·44$ rad and a scale size of 2,000 m for an exponential model, and curve *c* represents a Gaussian model having $a=0·30$ rad and a scale size of 500 m.

The results of some 1,473 h of observation during the period January–September 1969 are presented in Figs. 3 and 4. Observations of less than 2 h duration have been rejected from the analysis in order not to restrict the investigation to fluctuations of short period and hence small scale sizes. Values of $\sigma(\theta)$ for each run are plotted as a function of aerial separation in each figure. Observations recorded during the summer day-time, between 0600 UT and 1800 UT from the beginning of April until the end of August 1969, are shown in Fig. 3. Results for (i) summer night-time, (ii) September night-time, and (iii) winter day-time are shown separately in Fig. 4. For comparison some theoretical curves are shown with the summer day-time points and the September night-time points. They have been calculated on the basis of an atmospheric turbulence model assuming either a Gaussian or an exponential autocovariance function of refractive index variations, both of which are often considered in theoretical analyses[5]. The Gaussian autocovariance function for a scale size L gives an r.m.s. phase departure which varies as

$$\sigma(\theta)=\alpha(1-\exp(-X^2/L^2))^{1/2}$$

where α is $\sqrt{2}$ times the r.m.s. departure for a single path through the atmosphere, and X is the distance between the aerials. For an exponential autocovariance function the form of $\sigma(\theta)$ is the same except that the argument of the exponential is $(-X/L)$. These two forms can be distinguished by noting that the rate of change of σ with X as X tends to zero is much larger for the exponential model than it is for the Gaussian model.

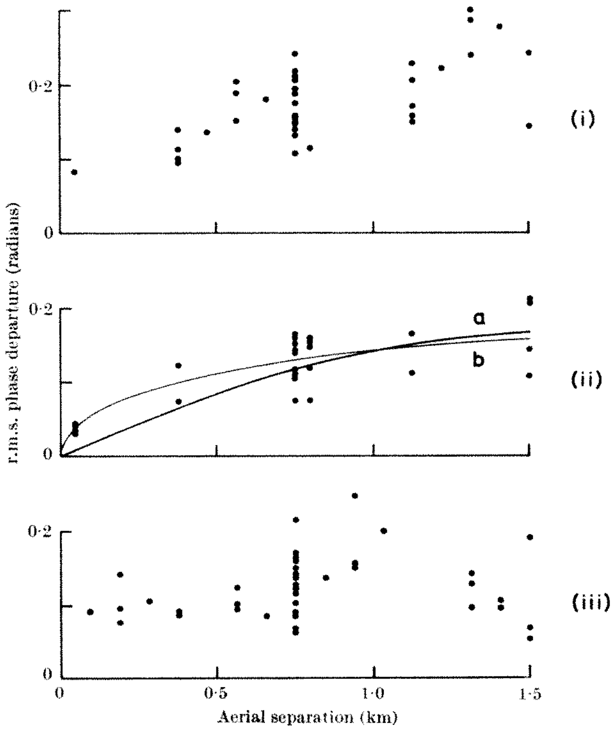


Fig. 4. The r.m.s. phase departure $\sigma(\theta)$ versus aerial separation for (i) summer night-time, (ii) September night-time and (iii) winter day-time. The curve *a* represents a Gaussian model and *b* represents an exponential model. Both curves have $a=0·18$ rad and a scale size of 1,000 m.

During the summer day-time (Fig. 3), $\sigma(\theta)$ varies markedly from day to day and this result is attributed to the wide range of weather conditions which occurs at this time of year. There is a strong dependence of $\sigma(\theta)$ on separation and observations recorded simultaneously at three separations show that the scale size on different days varies from a few hundred metres to several kilometres. Three curves which are compatible with the mean $\sigma(\theta)$ observed at different separations are given to illustrate the wide range of scale sizes and magnitudes of the irregularities which can exist during the summer day-time.

During the summer night-time (Fig. 4 (i)) and throughout the winter, the values of $\sigma(\theta)$ are smaller than those which occur in the summer day-time, and have a much smaller dispersion. During the winter day-time (Fig. 4 (iii)) there is little variation of $\sigma(\theta)$ with aerial separation, indicating that the irregularities have a very small scale size, less than a hundred metres. Observations during the winter night-time indicate that the phase departures are smaller than those occurring in the winter day-time.

A series of observations recorded at night-time during September 1969 is shown in Fig. 4 (ii). For this period the variation of $\sigma(\theta)$ shows less scatter. Two theoretical curves, (*a*) representing the Gaussian model and (*b*) the exponential model, are given for comparison, both having a scale size of 1 km and $\alpha = 0{\cdot}18$ rad. It seems that the exponential model provides the better fit for this set of observations.

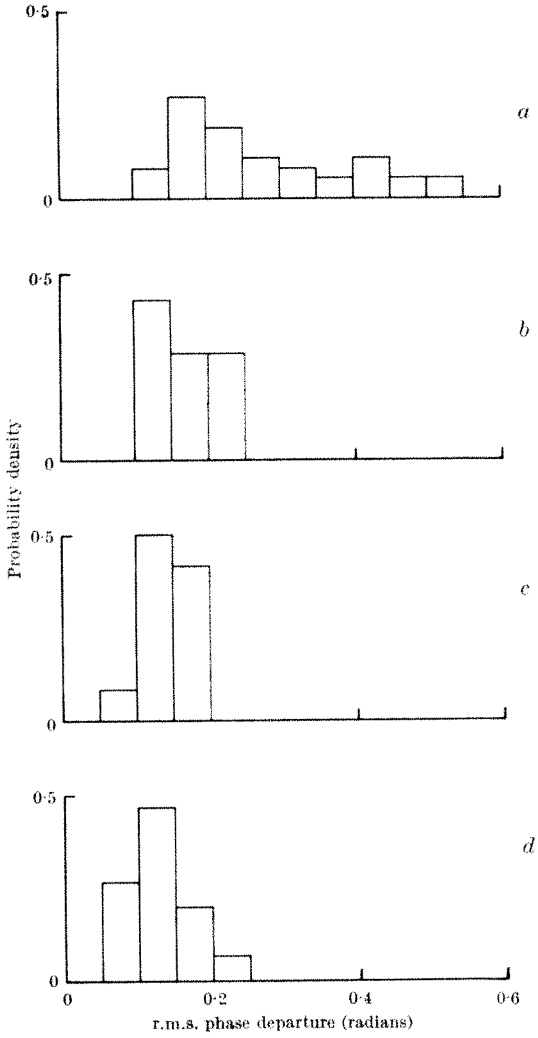


Fig. 5. The observed probability density of r.m.s. phase departure $\sigma(\theta)$ for an aerial separation of 751 m in (*a*) summer day-time, (*b*) summer night-time, (*c*) September night-time, and (*d*) winter day-time.

It is interesting to note that the values of $\sigma(\theta)$ at the smaller separations vary little with the time of day or season, whereas the values at larger separations vary quite radically. An examination of the frequency with which different values of $\sigma(\theta)$ occur is given in Fig. 5, where the probability density of r.m.s. phase departure at a particular separation of 751 m is given for each of the times and seasons considered here. The analysis is again restricted to observations of greater than 2 h duration, of which there are thirty-seven in the summer day-time (Fig. 5*a*); fourteen in the summer night-time (Fig. 5*b*); twelve in the September night-time (Fig. 5*c*); and fifteen in the winter day-time (Fig. 5*d*). The distributions emphasize the outstanding values of $\sigma(\theta)$ which occur at the larger separations during the summer day-time, and show that the probability density is largest near the minimum value of r.m.s. phase departure observed in each case.

Taking the present observations *in toto*, the mean r.m.s. phase departure observed at Cambridge is between 0·1 and 0·2 rad at 5 GHz. For comparison the observations made by Baars and by Basart *et al.* at the National Radio Astronomy Observatory were typically 0·1 rad at 2·7 GHz, which would be about 0·2 rad at 5 GHz. There is no further information for a more detailed comparison.

Discussion

The results, particularly over the period January–September 1969, have given detailed data on the irregularities of the wavefront of 5 GHz radiation passing through the atmosphere. The r.m.s. phase departure is highly variable, but usually increases with baseline, and has a marked dependence on the time of day at the larger separations, particularly during the summer. This result is to be used in a separate paper which discusses the ultimate limitations set both by the atmosphere and by the ionosphere on radio observations of celestial objects from the ground. It turns out that observations in the UK at 5 GHz with radio telescopes having apertures of several kilometres will not be seriously affected for much of the time. Observations at higher frequencies will be possible during the night and particularly during the winter months.

It is possible to use the results of this investigation to predict how the observed refractive index irregularities will influence the behaviour of millimetre-wave satellite communications systems which are proposed for the near future. The principal effect on dish aerials will be a frequency independent variation of the angle of arrival of radio waves rather than a degradation of the beam due to phase irregularities across the aperture. A direct observation of this phenomenon was made by Unger[6] using the Early Bird satellite at 4 GHz, and the 20 m wide horn-reflector aerial at the American Telephone and Telegraph Station near Andover, Maine. About 20 min of data were analysed and the r.m.s. deviation of the angle of arrival was found to be about 2×10^{-5} rad. The present results, taken over a much longer period of time, predict that the variation observed by aerials having apertures of less than a hundred metres in the UK will not change much with season or time of day and will be of the order of 10^{-5} rad r.m.s. Because the phase departures are observed to be normally distributed, the angle of arrival is expected to be within 3×10^{-5} rad of its mean value for 99 per cent of the time. The angle variations will increase rapidly as the elevation angle decreases, however, because of the longer path through the atmosphere, and will be some ten times the zenith value for an elevation angle of 5°. The variations have a time-scale of several seconds and servo-controlled pointing is easily capable of following such deviations. It is concluded therefore that variations in the refractive index of the troposphere will not form a serious hazard to reliable Earth to satellite communications systems as long as the satellite is more than a few degrees above the horizon. The communications systems will, however, still be limited by more serious tropospheric effects such as the absorption and scattering of radio waves by clouds and precipitation.

I thank many colleagues for help and the Science Research Council for a maintenance award.

Received December 2, 1969.

[1] Lane, J. A., *Proc. IEE*, **115**, 1227 (1968).
[2] Baars, J. W. M., *IEEE Trans. AP-15*, 582 (1967).
[3] Gent, H., Adgie, R. L., and Crowther, J. H., *RRE Res. Rev.*, **8** (1969).
[4] Elsmore, B., Kenderdine, S., and Ryle, M., *Mon. Not. Roy. Astron. Soc.*, **134**, 87 (1966).
[5] Chernov, L. A., *Wave Propagation in a Random Medium* (trans. by Silverman, R. A.) (McGraw-Hill, New York, 1960).
[6] Unger, J. H. W., *Bell Syst. Tech. J.*, **45**, 1439 (1966).

LETTERS TO THE EDITOR

PHYSICAL SCIENCES

Level Populations of Excited States of Hydrogen in Gaseous Nebulae

THE radiation from hydrogen atoms which is observed at radio and millimetre wavelengths due to atomic transitions between levels of high quantum number n cannot be accounted for without accurate calculations of the population densities of all the levels[1,2]. In order to interpret the observed intensities[3], and to deduce physical parameters such as temperatures and abundances, accurate calculations of level populations are needed. In gaseous nebulae the physical processes which may populate and depopulate a quantum level are radiative capture and cascade, collisional ionization and three-body recombination, collisional transitions ($n \rightarrow n'$) induced by electrons and protons, and radiative transitions induced by the free-free continuum within the nebula. Calculations have been made over a large range of temperatures and densities and for all n. The results should be much more accurate than any available previously.

Dyson[4] has shown that, for any reasonable emission measure, induced radiative transitions have a negligible effect on the level populations. The cross-sections for n to $n \pm 1$ transitions due to proton collisions have been computed. Their effect is negligible, if $T_e < 10^5$ K, in the region in which populations are not completely determined by collisional processes.

Allowing for the remaining processes, the equation of statistical equilibrium is[5]

$$N_e N_+ \{\alpha_n + C_{i,n}\} + \sum_{n'=n+1}^{\infty} N_{n'} A_{n',n} + \sum_{n'=n_0}^{\infty} N_{n'} C_{n',n}$$
$$= N_n \sum_{n'=n_0}^{n-1} A_{n,n'} + N_n C_{n,i} + N_n \sum_{n'=n_0}^{\infty} C_{n,n'} \quad (1)$$

The symbols are defined by Seaton[5]. Equation (1) may be expressed in terms of b_n factors

$$\sum_{n'=n+1}^{\infty} (A_{n',n} \frac{n'^2}{n^2} e^{x_{n'} - x_n} + C_{n,n'}) b_{n'}$$
$$+ (-\sum_{n'=n_0}^{n-1} A_{n,n'} - \sum_{n'=n_0}^{\infty} C_{n,n'} - C_{n,i}) b_n + \sum_{n'=n_0}^{n-1} C_{n,n'} b_{n'}$$
$$= -\frac{\alpha_n}{n^2} \left(\frac{h^2}{2\pi m k T_e}\right)^{-3/2} e^{-x_n} - C_{n,i} \quad (2)$$

or, more conveniently,

$$\sum_{n'=n_0}^{\infty} K_{n,n'} b_{n'} = L_n \quad (3)$$

The procedure adopted is to solve the set of simultaneous equations of the form

$$\sum_{n'=n_0}^{N} K_{n,n'} b_{n'} = L_n \quad (N > 300) \quad (4)$$

using the matrix condensation technique first suggested by Burgess and Summers[6]. For $n > N$ the solutions are fitted to analytical expressions, subject to the boundary condition that $b_n \rightarrow 1$ as $n \rightarrow \infty$. Allowance is made for transitions from levels above N, by corrections to the condensed form of the matrix K.

The rates of collisional excitations from level n to $n \pm 1$, induced by electrons and protons, are calculated using the impact parameter method of Seaton[7]. Symmetrized binary encounter theory[8] is used for all other collisional transitions and for the ionization rates. Expressions for radiative transition probabilities are given by Baker and Menzel[9], and for radiative recombination rates by Seaton[10]. Accurate Gaunt factors are used throughout[11,12].

This method has several advantages over the differential equation approach[4,5,13]. It avoids having to replace

Table 1. TYPICAL RESULTS ASSUMING CASE B OF BAKER AND MENZEL

	$N_e = 10^4$ cm^{-3}		$N_e = 10^3$ cm^{-3}		$N_e = 10^2$ cm^{-3}		$N_e = 10$ cm^{-3}	
n	b_n	d ln b_n/dn	b_n	d ln b_n/dn	b_n	d ln b_n/dn	b_n	d ln b_n/dn
$T_e = 10^4$ K								
20	0·6248	1·357 (−2)	0·6190	1·291 (−2)	0·6162	1·263 (−2)	0·6147	1·249 (−2)
40	0·7606	1·153 (−2)	0·7152	4·898 (−3)	0·7047	3·896 (−3)	0·7005	3·639 (−3)
60	0·9226	5·513 (−3)	0·8097	7·699 (−3)	0·7512	2·961 (−3)	0·7396	2·037 (−3)
80	0·9782	1·326 (−3)	0·9175	4·190 (−3)	0·8123	4·887 (−3)	0·7667	1·745 (−3)
100	0·9928	3·691 (−4)	0·9674	1·520 (−3)	0·8911	3·857 (−3)	0·8000	2·678 (−3)
120	0·9972	1·232 (−4)	0·9861	5·687 (−4)	0·9437	2·000 (−3)	0·8509	3·227 (−3)
150	0·9991	3·110 (−5)	0·9954	1·553 (−4)	0·9788	6·719 (−4)	0·9223	1·995 (−3)
200	0·9998	5·125 (−6)	0·9990	2·699 (−5)	0·9947	1·325 (−4)	0·9761	5·536 (−4)
250	0·9999	1·253 (−6)	0·9997	6·744 (−6)	0·9983	3·478 (−5)	0·9917	1·638 (−4)
300	1·0000	3·960 (−7)	0·9999	2·152 (−6)	0·9993	1·137 (−5)	0·9966	5·659 (−5)
$T_e = 7·5 \times 10^3$ K								
20	0·5661	1·699 (−2)	0·5592	1·614 (−2)	0·5559	1·578 (−2)	0·5542	1·561 (−2)
40	0·7276	1·457 (−2)	0·6710	6·189 (−3)	0·6582	4·851 (−3)	0·6531	4·521 (−3)
60	0·9153	6·195 (−3)	0·7853	9·542 (−3)	0·7128	3·770 (−3)	0·6983	2·521 (−3)
80	0·9766	1·441 (−3)	0·9095	4·736 (−3)	0·7874	6·068 (−3)	0·7305	2·204 (−3)
100	0·9923	3·965 (−4)	0·9647	1·665 (−3)	0·8796	4·456 (−3)	0·7713	3·421 (−3)
120	0·9970	1·316 (−4)	0·9851	6·145 (−4)	0·9387	2·227 (−3)	0·8327	3·890 (−3)
150	0·9991	3·306 (−5)	0·9951	1·662 (−4)	0·9772	7·303 (−4)	0·9148	2·259 (−3)
200	0·9998	5·424 (−6)	0·9989	2·868 (−5)	0·9944	1·419 (−4)	0·9743	6·032 (−4)
250	0·9999	1·323 (−6)	0·9997	7·142 (−6)	0·9982	3·702 (−5)	0·9911	1·760 (−4)
300	1·0000	4·172 (−7)	0·9999	2·273 (−6)	0·9993	1·206 (−5)	0·9964	6·042 (−5)
$T_e = 5 \times 10^3$ K								
20	0·4853	2·327 (−2)	0·4768	2·208 (−2)	0·4728	2·160 (−2)	0·4707	2·137 (−2)
40	0·6843	1·952 (−2)	0·6098	8·405 (−3)	0·5933	6·437 (−3)	0·5869	5·980 (−3)
60	0·9072	7·024 (−3)	0·7542	1·211 (−2)	0·6598	5·172 (−3)	0·6408	3·312 (−3)
80	0·9749	1·566 (−3)	0·9006	5·412 (−3)	0·7553	7·899 (−3)	0·6803	2·991 (−3)
100	0·9918	4·242 (−4)	0·9620	1·826 (−3)	0·8660	5·243 (−3)	0·7331	4·657 (−3)
120	0·9968	1·398 (−4)	0·9841	6·623 (−4)	0·9332	2·496 (−3)	0·8101	4·850 (−3)
150	0·9990	3·489 (−5)	0·9949	1·770 (−4)	0·9755	7·932 (−4)	0·9062	2·589 (−3)
200	0·9998	5·693 (−6)	0·9988	3·026 (−5)	0·9941	1·512 (−4)	0·9724	6·572 (−4)
250	0·9999	1·384 (−6)	0·9996	7·499 (−6)	0·9981	3·912 (−5)	0·9906	1·884 (−4)
300	1·0000	4·354 (−7)	0·9999	2·380 (−6)	0·9993	1·269 (−5)	0·9962	6·412 (−5)

Table 2. EFFECT OF INCLUDING COLLISIONAL EXCITATION AND DE-EXCITATION

	$T_e = 10^4$ K All transitions		$N_e = 10^4$ cm^{-3} $n \to n \pm 1$ transitions	
n	b_n	d ln b_n/d$_n$	b_n	d ln b_n/d$_n$
100	0·9928	3·691 (− 4)	0·9859	7·317 (− 4)
150	0·9991	3·110 (− 5)	0·9984	5·887 (− 5)
200	0·9998	5·125 (− 6)	0·9997	8·938 (− 6)
250	0·9999	1·253 (− 6)	0·9999	2·028 (− 6)

equation (2) by an approximate, differential form and eliminates the arbitrary lower boundary condition where the solutions are matched to the radiative case. Collisional transitions to any level are included and solutions may be obtained over all n.

Factors b_n and d log b_n/d$_n$ have been computed for a large range of temperatures and densities. Table 1 gives some typical results assuming case B of Baker and Menzel[9]. The important effect of including collisional excitation and de-excitation to any level is illustrated in Table 2. Results obtained allowing for all transitions (columns 2 and 3) are compared with those obtained by the same method assuming *n to n* ± 1 transitions only (columns 4 and 5).

Calculations allowing for all collisional transitions have recently been made by Sejnowski and Hjellming[14], using an iterative method of solution. Our results for the b_n factors are larger than theirs, probably due to differences in the adopted rate coefficients for the collisional processes. I consider that my rate coefficients are more accurate than those of Sejnowski and Hjellming.

A more detailed account and discussion of the results will be published later. I thank Professor M. J. Seaton for introducing me to the problem and for guidance. This work was supported by a Science Research Council grant.

M. BROCKLEHURST

Department of Physics,
University College London.

Received September 2, 1969.

[1] Kardashev, N. S., *Sov. Astron. AJ*, **3**, 813 (1959).
[2] Terzian, Y., *Interstellar Ionized Hydrogen* (Benjamin, New York, 1968).
[3] Goldberg, L., *Astrophys. J.*, **144**, 1225 (1966).
[4] Dyson, J. E., *Astrophys. J.*, **155**, 4 (1969).
[5] Seaton, M. J., *Mon. Not. Roy. Astron. Soc.*, **127**, 177 (1964).
[6] Burgess, A., and Summers, H. P., *Astrophys. J.*, **157**, 1007 (1969).
[7] Seaton, M. J., *Proc. Phys. Soc.*, **79**, 1105 (1962).
[8] Burgess, A., and Percival, I. C., *Adv. Atomic Molecular Phys.*, **4**, 109 (1968).
[9] Baker, J. G., and Menzel, D. H., *Astrophys. J.*, **88**, 52 (1938).
[10] Seaton, M. J., *Mon. Not. Roy. Astron. Soc.*, **119**, 81 (1959).
[11] Menzel, D. H., and Pekeris, C. L., *Mon. Not. Roy. Astron. Soc.*, **96**, 77 (1935).
[12] Menzel, D. H., *Nature*, **218**, 756 (1968).
[13] Hoang-Binh, D., *Astrophys. Lett.*, **2**, 231 (1968).
[14] Sejnowski, T. J., and Hjellming, R. M., *Astrophys. J.*, **156**, 915 (1969).

Long Period Oscillations in Rotating Neutron Stars

THEORETICALLY, the structure of a rotating neutron star can support a variety of oscillations which may coexist with and couple to its surface rotation. Because it seems extremely probable that the pulsar period is kept by the rotating surface of a neutron star, the excitation of such modes would give small periodic modulations in observed pulsar frequencies. Most oscillatory modes have relatively high frequencies. But one does exist, unique to a rotating sphere of neutron superfluid, which has a period of about 4 months—close to the "wobble" reported for the period of the Crab pulsar[1]. The frequency of the oscillation mode of the rotating superfluid is insensitive to unknown parameters describing the stellar interior.

A probable neutron star model consists of a solid outer crust[2] surrounding a quantum fluid of nuclear density the main constituent of which is a BCS-type neutron superfluid[3,4]. Its temperature is estimated to be one or two orders of magnitude below the superfluid transition temperature. The crust is rather weakly coupled to the neutron superfluid; in the Vela pulsar the characteristic time for the exchange of angular momentum between the two seems to be a few years[5]. A highly conducting electron–proton sea (the protons are probably superconducting) contributes a few per cent of the interior density and is strongly coupled to the crust (but not to the neutrons) by the strong magnetic field which connects them. For oscillations involving stellar shape or density changes the period $T \lesssim 10^{-3}$ s. Torsional oscillations of the crust[2] have $T \lesssim 10^{-1}$ s. The magnetic coupling between the conducting crust and the core proton–electron sea gives[5] $T \sim 10^{14} B^{-1}$ s; for the magnetic fields usually assumed for pulsars $B \sim 10^9$–10^{12} G, and $T \lesssim 10^5$ s.

Two other kinds of oscillations are possible when the interior superfluid is rotating. One is the classical torsional type oscillation of a uniformly rotating fluid. The characteristic period for such a mode is $\sim \pi\Omega^{-1}$ where Ω is the fluid angular velocity. For all known pulsars such oscillations have $T \lesssim 0{\cdot}3$ s. The other fluid motion, unique to a superfluid, depends on the quantization of its microscopic motion. A rotating superfluid moves irrotationally: it can mimic rigid body rotation through a paraxial array of moving quantized vortex lines[6–8]. The fluid velocity satisfies $\boldsymbol{\nabla} \times \mathbf{v} = 0$ except within the core of each vortex. For rotating interiors of neutron stars the core radius[9] $\xi \sim 10^{-12}$ cm and the separation between quantized vortex lines is of order 10^{-2} cm. When averaged over many vortex lines the average fluid velocity $\langle \mathbf{v} \rangle$ can satisfy the usual relation for rigid rotation: $\boldsymbol{\nabla} \times \langle \mathbf{v} \rangle = 2\boldsymbol{\Omega}$. In the corotating frame of an axially spinning cylinder the free energy of a contained superfluid is minimized by a regular (triangular) lattice array at rest with respect to the cylinder walls[10]. Such a vortex array admits two kinds of oscillations. In the first the vortex lines bend; for wavelengths large compared with the intervortex spacing, the frequencies of such oscillations $\sim 2\Omega$, just those expected for a classical fluid. The second mode of vibration of the vortex lattice consists of a purely axial movement of the vortex lines[10,11]; each vortex line remains parallel to the cylinder axis, but there is a redistribution of density of vortices and superfluid angular momenta.

The equation of motion of the vortex lattice for such motions (in the corotating frame) is given by[10]

$$\frac{1}{c_V^2}\,\frac{\partial^2}{\partial t^2}\,\mathbf{s} - \nabla^2 \mathbf{s} = 0 \qquad (1)$$

where $\mathbf{s}(\boldsymbol{\rho},t)$ specifies the parallel displacement of that vortex line whose normal position is $\boldsymbol{\rho}$ from the cylinder axis. The wave velocity is

$$c_V = \frac{1}{2}\left(\frac{\hbar\Omega}{\hat{m}}\right)^{\frac{1}{2}} \qquad (2)$$

which is approximately the intervortex spacing times Ω. The mass $\hat{m}$ is that of the equivalent boson in the BCS model of a fermion superfluid. For the neutron star interior $\hat{m}$ equals twice the neutron mass. Dyson (personal communication) has shown that the lattice vibrations must satisfy the condition $\boldsymbol{\nabla} \times \dot{\mathbf{s}} = -2\Omega \boldsymbol{\nabla} . \mathbf{s}$. Then the lowest admissible mode of vortex lattice motion described by equation (1) is

$$\mathbf{s} = A J_1(k\rho)\left[\cos \omega t\, \boldsymbol{\rho} + \frac{2\Omega}{\omega}\sin \omega t\, \hat{\boldsymbol{\varphi}}\right] \qquad (3)$$

with

$$\omega = k c_V \qquad (4)$$

The associated oscillation of averaged fluid motion is azimuthal. In the rotating reference frame

$$\langle \mathbf{v} \rangle = 2A\Omega J_1(k\rho)\cos\omega t\,\hat{\boldsymbol{\phi}} \tag{5}$$

The appropriate boundary condition depends somewhat on the crust-superfluid coupling at the interface. But because typically the crust has about 10^{-2} the moment of inertia of the neutron superfluid contained within it, only a small part of the angular momentum of the core should be transferred to it during an oscillation no matter what the boundary is like. When transfer of angular momentum across the interface is neglected, the wave number k is determined by

$$k\int_0^R r^2 J_1(kr)\mathrm{d}r = R^2 J_2(kR) = 0 \tag{6}$$

Then for the fundamental oscillation mode $kR \sim 5$ and equations (2) and (4) give for the oscillation period

$$T = \frac{4\pi}{k}\left(\frac{\hat{m}}{\hbar\Omega}\right)^{\frac{1}{2}} \sim \frac{140\,R}{(\Omega)^{\frac{1}{2}}}\ \mathrm{s} \tag{7}$$

for R the neutron star radius, in cm and Ω its angular frequency in rad s^{-1}. Almost all models give $R \sim 10^6$ cm. Then for the Crab pulsar, with $\Omega \sim 2\times10^2\ \mathrm{s}^{-1}$, $T \sim 4$ months, near that reported for the "wobble"[1]. A similar mode excited in the Vela pulsar would be expected to have a somewhat longer period (~ 7 months) because its frequency is about three times less than that of the Crab.

The oscillatory superfluid vortex mode will be coupled to the crust by two mechanisms: (*a*) the transfer of angular momentum directly through the crust-superfluid boundary region; (*b*) the interaction, throughout the entire superfluid, between the normal neutrons inside the vortex cores and the degenerate electron sea which pervades the core[5]; this transfers angular momentum between the neutrons and the electrons and thereby to the conducting crust which is strongly coupled to the latter by magnetic fields.

Any sudden (on a time scale of months) movement of the crust will excite the slow superfluid vortex mode (as well as higher ones). In the case of Vela the time scale for crust–core coupling by mechanism (*b*) has been estimated to be a few years[5]. Because it is directly proportional to the number of vortex lines, to Ω that is, and inversely proportional to the square of the core temperature it is presumably much stronger for the faster and younger (hotter) Crab. (The Vela analysis applied to the small jump $\Delta\Omega/\Omega$ of 4×10^{-9} reported for the Crab pulsar by Boynton *et al.* at the Princeton pulsar symposium suggests a time scale of a week.) Mechanism (*b*) chiefly will be effective in sharing any change in angular speed between the core and the crest. It will also damp variations in $\boldsymbol{\nabla}\times\langle\mathbf{v}\rangle$ within the core but probably on a very much longer time scale because of the very large core relative to crust inertia[5]. Thus the period of core rotational oscillation agrees with that reported if the neutron core is superfluid and appropriate mechanisms exist to couple it to the crust motion. For sufficient core–crust coupling a sudden change in crust motion[12,13] will excite core motions the relative amplitude of which is less than that of the crust by the ratio of the magnetically coupled crust—electron–proton sea moment of inertia to that of the core, typically $\sim 10^{-2}$. Because the reported Crab period "wobble" gives a periodic variation in crust Ω of 1 in 10^{10}, a small starquake which causes a sudden jump in crust Ω of 1 in 10^8 (ref. 9) may be sufficient to leave a fundamental core vortex mode oscillation of the required magnitude after other transients have damped.

A more definitive analysis should substitute spherical for cylindrical geometry, include radial density variation of neutron stars, and offer a quantitative description of core–crust coupling and damping.

I thank Professor E. Spiegel for his interest and Dr P. Hohenberg and Professors J. Luttinger, D. Pines and L. Woltjer for conversations. I also thank Professor Freeman Dyson for his generous counsel. This research was supported in part by the US National Science Foundation.

MALVIN RUDERMAN

Department of Physics,
Columbia University,
New York, NY.

Received November 17, 1969.

[1] Richards, D., Pettengill, G., Counselman, C., and Rankin, J., *IAU Circ. No. 2180* (1969).

[2] Ruderman, M., *Nature*, **218**, 1128 (1968).

[3] Ginzburg, V., and Kirzhnits, D., *Zh. Exp. Theor. Fiz.*, **47**, 2006 (1964); *Sov. Phys. JETP*, **20**, 1346 (1965).

[4] Migdal, A., *Zh. Exp. Theor. Fiz.*, **37**, 249 (1959).

[5] Baym, G., Pethick, C., Pines, D., and Ruderman, M., *Nature*, **224**, 872 (1969).

[6] Feynman, R., *Prog. Low Temp. Phys.*, **1**, 17 (North-Holland Pub. Co., 1955).

[7] Onsager, L., *Nuovo Cimento*, **6**, Suppl., **2**, 249 (1959).

[8] Andronikashvili, E., and Mamaladze, Yu., *Rev. Mod. Phys.*, **38**, 567 (1966).

[9] Baym, G., Pethick, C., and Pines, D., *Nature*, **224**, 673 (1969).

[10] Tkachenko, K., *Zh. Exp. Theor. Fiz.*, **50**, 1573 (1966) *Sov. Phys. JETP*, **23**, 1049 (1966).

[11] Fetter, A., *Phys. Rev.*, **162**, 143 (1967).

[12] Dyson, F., *Nature*, **223**, 486 (1969).

[13] Ruderman, M., *Nature*, **223**, 597 (1969).

Apparent Periodicities in the Redshifts of Quasi-stellar Objects

COWAN[1] has applied the method of power spectrum analysis to the distribution of redshifts of quasi-stellar objects and has found several peaks in the power spectrum which, if real, would correspond to periodicities in the distribution of QSO redshifts. This letter points out that the distribution of power levels in his Fig. 1 is not significantly different from what would be expected from the analysis of purely random noise.

If a power spectrum analysis is applied to purely random (white) noise, the distribution of power levels is expected to be a χ^2 distribution with two degrees of freedom. A proof of this is given by Jenkins and Watts[2]. It can also be seen from the fact that the real and imaginary parts of a Fourier transform are linear functions of the data. If the data consist of random fluctuations, then these linear functions will also show random fluctuations which are very nearly normal, by virtue of the central limit theorem. The power, which is the sum of the squares of the real and imaginary parts, has the distribution of the sum of the squares of two normal variates, that is, the χ^2 distribution with two degrees of freedom. The analytic expression for this distribution, $F(\chi^2)$, is

$$F(\chi^2)\,\mathrm{d}\chi^2 = \tfrac{1}{2}\,\mathrm{e}^{-\frac{1}{2}\chi^2}\,\mathrm{d}\chi^2$$

which is normalized so that its integral is unity. The mean value is 2. This distribution function is plotted as the solid line in Fig. 1. Clearly it has quite a long "tail" at large values of χ^2. It would not be surprising, therefore, to find a few points corresponding to quite high power levels in the spectrum of random noise.

I have taken Cowan's Fig. 1 and have attempted to count the relative numbers of points in the power ranges (0, 0·1), (0·1, 0·2), (0·2, 0·3), (0·3, 0·4), (0·4, 0·5). In doing this, I have been guided by the intrinsic width of his peaks as regards the total number of points to count. In the ranges listed I found 14, 9, 5, 3 and 0 points, respectively. To enter these points on Fig. 1 it is necessary to establish the scale of the abscissa, that is, to find the constant a such that $P = a\chi^2$, where P is the measured power level. There are at least three possible ways of doing this: (i) we could equate the mean power level with the mean, 2, of the χ^2 distribution; (ii) we could fit the fraction of power in the low power levels to the theoretical curve; (iii) we could fit the logarithmic derivative of the distribution to the theoretical slope $\mathrm{d}\ln F/\mathrm{d}\chi^2 = -\frac{1}{2}$. Because of the long tail of the χ^2 distribution, it is possible that we may have missed some points at even

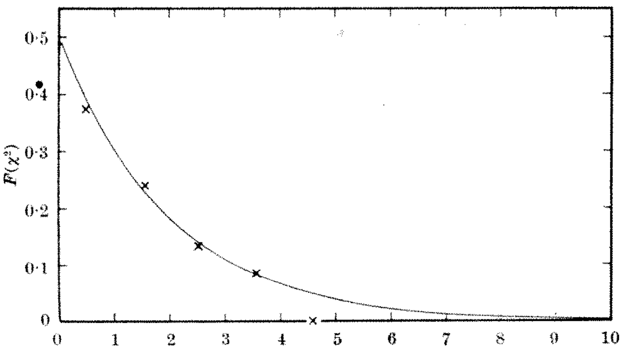


Fig. 1. Theoretical (line) and observed (crosses) distribution of power levels in analysis of QSO redshift periodicities.

higher powers than those calculated. Such an incompleteness in the data will affect both the estimate of mean power and the calculation of the fraction of the total power at low power levels. It does not affect the logarithmic derivative, however. For this reason I have used method (iii) to find a mean value of $a = 0{\cdot}098 \pm 0{\cdot}014$. With this value of a, we can calculate that the fraction of points lost at power values greater than 0·5 should be 0·078, so that, whereas only thirty-one power points were counted, there should have been thirty-eight. Hence the counted points represent fractions 0·37, 0·24, 0·13, 0·08, 0·00 at abscissae 0·51, 1·53, 2·55, 3·57, 4·60, respectively. These points are plotted as crosses on Fig. 1.

There seems to be no significant departure from the distribution expected for a random distribution of data points. Of course, like any statistical test of significance, this is one-way; it allows us to accept the hypothesis that the original data were random, but does not necessarily reject the hypothesis that the data were non-random.

If the original data were random, then probably seven or so more points could be discovered in the power spectrum at still higher power levels.

Since this note was prepared, the article by Plagemann, Feldman and Gribbin[3] has appeared, reaching essentially the same conclusions. I believe that the presentation of the expected power levels from random noise in terms of the χ^2 distribution in Fig. 1 is an additional point of interest which is not immediately clear from their results.

T. J. DEEMING

Department of Astronomy,
University of Texas at Austin,
Austin, Texas.

Received December 9, 1969.

[1] Cowan, C. L., *Nature*, **224**, 655 (1969).
[2] Jenkins, G. M., and Watts, D. G., *Spectral Analysis* (Holden-Day, San Francisco, California, 1968).
[3] Plagemann, S. M., Feldman, P. A., and Gribbin, J. R., *Nature*, **224**, 875 (1969).

Origin of the Optical Polarization in the Nucleus of NGC 1068

MEASUREMENTS of the degree of polarization of the nuclear region of NGC 1068 by Kruszewski[1] and Visvanathan and Oke[2] show that the degree of polarization increases with decreasing diaphragm size, which suggests that the polarization is produced in and close to the nucleus and that the radiation contributed by other parts of the galaxy is unpolarized. In order to find what process or processes are responsible for the optical radiation from the nucleus it is important to determine the intrinsic polarization of the nucleus. To do this we require the dilution factor, D, which is the ratio of the integrated surface brightness of all the radiation from the galaxy within the diaphragm to that of the nucleus. The distribution of surface brightness across the galaxy has been measured with a resolution of 2·6 square seconds by van Houten[3]; he has corrected the data for the smoothing introduced by finite angular resolution and by atmospheric dispersion to give a resolution of 1 square second. At this resolution the nucleus has a surface brightness $S = 12\overset{\mathrm{m}}{.}77$ (square s)$^{-1}$; at $r = 1$ s, $S = 16\overset{\mathrm{m}}{.}35$ (square s)$^{-1}$. Walker[4] places an upper limit of 1·5 s on the nuclear diameter, $2r_{\mathrm{N}}$, corresponding to a nuclear area of 1·8 square s; we shall assume these values in what follows.

We assume circular symmetry and $S(r)$ as the surface brightness at distance r s from the nucleus. The degree of polarization is P_{N} at $r < r_{\mathrm{N}}$, where r_{N} defines the edge of the nucleus, and at $r > r_{\mathrm{N}}$ the degree of polarization is zero. The observed flux measured with a diaphragm of radius r_{D} s is then

$$\begin{aligned} F &= \int_0^{r_{\mathrm{D}}} S(r)\ 2\pi r dr \\ &= \pi r_{\mathrm{N}}^2 < S_{\mathrm{N}}(r) > + (\pi r_{\mathrm{D}}^2 - \pi r_{\mathrm{N}}^2) < S_{\mathrm{G}}(r) > \end{aligned}$$

where suffices N and G refer to the nucleus and the remaining part of the galaxy respectively. The fraction of the observed flux which is plane polarized is

$$\begin{aligned} P_{\mathrm{OBS}}\ F &= \int_0^{r_{\mathrm{D}}} p(r)\ S(r)\ 2\pi r dr \\ &= \pi r_{\mathrm{N}}^2\ P_{\mathrm{N}} < S_{\mathrm{N}}(r) > \end{aligned}$$

Thus

$$P_{\mathrm{N}} = D\ P_{\mathrm{OBS}}$$

where the dilution factor is

$$D = 1 + \frac{r_{\mathrm{D}}^2 - r_{\mathrm{N}}^2}{r_{\mathrm{N}}^2}\ \frac{< S_{\mathrm{G}}(r) >}{< S_{\mathrm{N}}(r) >}$$

We shall compute D for the recent observations of Kruszewski[1] who used $r_{\mathrm{D}} = 4{\cdot}6$ s. Because $r_{\mathrm{D}}^2 \gg r_{\mathrm{N}}^2$ we find, using van Houten's data, that

$$D = 1 + \frac{\int_{r_{\mathrm{N}}}^{r_{\mathrm{D}}} r\ S(r)\ \mathrm{d}r}{\int_0^{r_{\mathrm{N}}} r\ S(r)\ \mathrm{d}r} = 16{\cdot}9$$

We have used an upper limit to r_{N} so that strictly $D \geq 16{\cdot}9$.

The photometric results of van Houten for NGC 1068 are given in the international photographic magnitude system which is close to the B system. Thus the dilution factor is relevant to the polarization observations in B. Kruszewski finds $P_{\mathrm{OBS}}(B) = 2{\cdot}36$ per cent and thus

$$P_{\mathrm{N}}(B) = 39{\cdot}9 \text{ per cent}$$

It is interesting to note that if this dilution factor were relevant to the observations in U for which Kruszewski finds $P_{\mathrm{OBS}}(U) = 5{\cdot}12$ per cent, the $P_{\mathrm{N}}(U) = 86{\cdot}5$ per cent and the polarization in U would be almost complete. Further note that $P_{\mathrm{N}}(B)$ cannot exceed 100 per cent and thus we can determine the limiting value $(r_{\mathrm{N}})_{\mathrm{MIN}}$ which produces $P_{\mathrm{N}}(B) = 100$ per cent: we find $(r_{\mathrm{N}})_{\mathrm{MIN}} = 0{\cdot}46$ s corresponding to a linear radius of about 25 pc.

The clues to the origin of the optical radiation from the nucleus are (*a*) $P_{\mathrm{N}}(B) \sim 40$ per cent is very high, (*b*) P_{N} depends strongly on wavelength[1] and (*c*) the radiation is very blue[4]. Before allowance was made for the dilution factor it seemed perfectly reasonable to suggest[4] that a degree of polarization ~ 0 to 5 per cent should be produced by optical synchrotron radiation. To explain 40 per cent polarization is much more difficult, however: not only is a strong magnetic field ($> \sim 10^{-3}$ G) required but also it must be well ordered over dimensions ~ 50 pc, the minimum diameter of the nucleus. Moreover, to obtain maximum linear polarization the magnetic field should be normal to the line of sight, but this would require that the nuclear magnetic field is approximately in the plane of the galaxy because the line of sight is nearly normal to the plane. If the required strong magnetic field were produced by dynamo action in a rotating nucleus[5], we would require that the axis of rotation of the nucleus be in the plane of the galaxy, an unlikely occurrence. An alternative possibility is that the dust surrounding the nucleus, which is required to explain the infrared radiation[6], could be strongly aligned and could produce the strong polarization. For perfectly aligned grains either of iron or graphite[7] a maximum polarization of only 25 to 30 per cent can be obtained, however.

A further possibility deserves consideration. If the nucleus is surrounded by a dense shell of grains, as suggested by Rees *et al.*[6], then much of the light from the nuclear region may be radiation scattered by the grains. Given a model of the dust shell in which the grain density decreases outwards so that most of the scattering is at phase angles of 90° ± 35° and given very small grains which scatter according to Rayleigh–Gans theory, so that the degree of polarization of the first order scattered light lies between 50 and 100 per cent, it is conceivable that $P_N \sim 40$ per cent might be explained. The observed blueness and the observed increase of $p_{\rm OBS}$ with decreasing wavelength are both consistent with this explanation.

K. NANDY

Royal Observatory, Edinburgh.

R. D. WOLSTENCROFT

Institute for Astronomy,
University of Hawaii.

Received December 1, 1969.

[1] Kruszewski, A., *Astron. J.*, **73**, 852 (1968).
[2] Visvanathan, N., and Oke, J. B., *Astrophys. J. Lett.*, **152**, L165 (1968).
[3] Van Houten, C. J., *Bull. Astron. Insts. Netherlands*, **16**, 1 (1961).
[4] Walker, M. F., *Astrophys. J.*, **151**, 71 (1968).
[5] Hoyle, F., *Nature*, **223**, 936 (1969).
[6] Rees, M. J., Silk, J. I., Werner, M. W., and Wickramasinghe, N. C., *Nature*, **223**, 788 (1969).
[7] Hoyle, F., and Wickramasinghe, N. C., *Nature*, **218**, 1126 (1968).

Cassiopeia A Absorption Spectrum and the Interarm Medium

A MEASUREMENT of the neutral hydrogen absorption spectrum of the Cassiopeia A radio source can in principle give information about the interarm regions of the Milky Way because this source lies in (and probably on the far side of) the Perseus arm. Neutral hydrogen lying in the region between the local arm and the Perseus arm may therefore be detectable in absorption as well as in emission. A combination of the emission and absorption data can give the spin temperature of the interarm gas (for example, see ref. 1). During the Conference of Young European Radio Astronomers at Jodrell Bank in June 1969, I was encouraged to make these measurements in discussions with Dr U. Mebold. This report describes measurements of the Cassiopeia A spectrum made with higher precision than those already published[2–4].

Observations were made on August 16, 1969, with the 250-foot radio telescope which had a primary halfpower beamwidth for this experiment of 13′ × 18′ arc. It has a further response in a beam 30′ arc in radius which contains about 30 per cent of the power. The new 256 channel digital autocorrelation spectrometer was used to measure neutral hydrogen spectra on and near Cassiopeia A. A 1·25 MHz overall bandwidth was chosen to give adequate base level in the spectra on either side of the neutral hydrogen features. The effective halfpower resolution bandwidth was 9 kHz.

A calculation of the spin temperature of neutral hydrogen by this method requires first a good estimate of the emission spectrum interpolated to the position of the source. Spectra were obtained on a grid of points surrounding Cassiopeia A and the results of averaging spectra at different distances from Cassiopeia A are shown in Fig. 1. Those at 39′ arc (Fig. 1*a*) varied by ± 2·5 K at the minimum near 110 kHz, while those at 19′ arc (Fig. 1*b*) varied by ± 1·1 K, indicating the improvement in accuracy of the interpolation over smaller distances. It should be noted that the spectra obtained at 19′ arc contained 1·2 per cent of the Cassiopeia A absorption spectrum; this has a negligible effect on the part of the spectrum near 110 kHz—the actual "expected" emission profile at the position of Cassiopeia A when corrected for this effect is 0·1 K higher than shown in Fig. 1*b*. The similarity of Figs. 1*a* and 1*b* in the frequency interval 90 to 130 kHz relative to the l.s.r. would indicate that the expected profile in the range is accurate to better than 2 K.

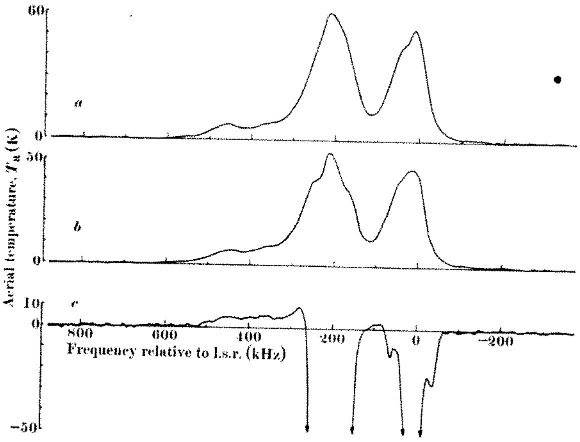


Fig. 1. *a*, Average of four spectra taken on a grid spaced 39′ arc from Cassiopeia A; resolution is 9 kHz; *b*, average of four spectra taken on a grid spaced 19′ arc from Cassiopeia A; note that 1·2 per cent of the absorption spectrum is included; *c*, spectrum observed on the position of Cassiopeia A.

The true absorption spectrum of Cassiopeia A is obtained by subtracting the expected emission profile from the profile observed at the source position (Fig. 1*c*). The expected profile was that shown in Fig. 1*b*, and corrected for the small amount of absorption profile contained in it. Fig. 2*b* gives our best estimate of the true absorption spectrum as obtained with a frequency resolution of 9 kHz. With this resolution the narrow absorption features are not completely resolved although the interarm region which we are interested in as represented by the frequency interval 90 to 130 kHz is completely resolved. Fig. 2*c*, which gives the central part of the spectrum with a resolution of 4·5 kHz, illustrates this point. No attempt has been made at an absolute determination of the optical depth scale, for it is difficult and not necessary for the present purpose. I have taken the absorption at the deep feature in the Perseus arm to be 99·9 per cent (optical depth = 7·0) as given by Shuter and Verschuur.

If we consider that the part of the spectrum near the position of the emission minimum between the local and Perseus arms is representative of the interarm medium, we can derive the spin temperature T_s of this medium from the ratio $T_s = T_b/\tau$, where T_b is the brightness temperature of the emission taken as 1·2 T_a and τ is the optical depth in absorption. The minimum values of T_b and τ are 12·1 ± 2·5 K and 0·022 ± 0·008 respectively. The errors given take account of the interpolation in position for T_b and the assessment of the baseline for the spectra; the error contribution by noise fluctuations in the receiver is small in comparison with these values. It will be noted that the T_B and τ curves are not the same shape in the frequency interval 90 to 130 kHz and in particular the minima are displaced relative to one another by about 15 kHz. This will mean that our derived T_s varies with

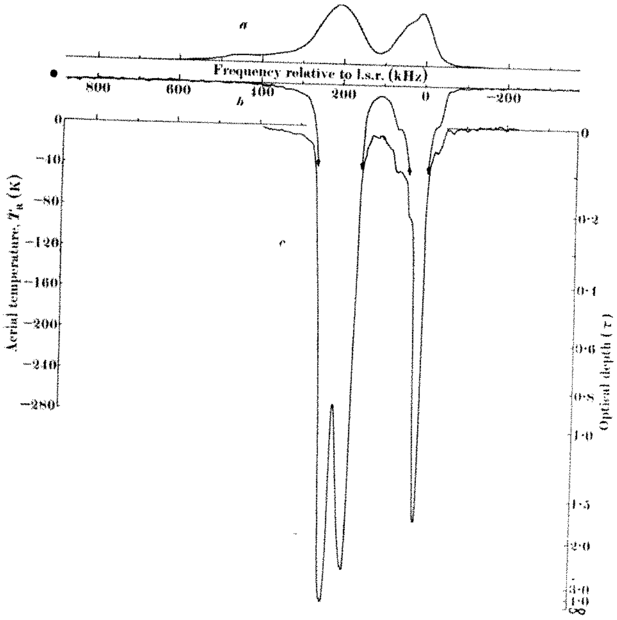


Fig. 2. *a*, The "expected" emission spectrum of Cassiopeia A. Resolution is 9 kHz; *b*, true absorption spectrum of Cassiopeia A with a resolution of 9 kHz; *c*, true absorption spectrum of Cassiopeia A with a resolution of 4·5 kHz.

frequency. Our estimate of T_s including the uncertainties given is $T_s = 540 \pm 230$ K.

An independent assessment of both the emission and absorption spectra was made from the data obtained during observations[5] of the Zeeman splitting of the Cassiopeia A absorption spectrum using the Mark II telescope with a beam of half intensity width 36′ arc and low sidelobes (less than 1 per cent). Although the beamwidth is more than twice that of the 250 ft telescope its sidelobe structure is very much cleaner and will have less effect on the emission spectrum (the true absorption spectrum is not affected by the sidelobe structure). The minimum T_B in the range 90 to 130 kHz was 10·8 K, which is within the uncertainty assigned from data from the 250 ft telescope. τ was estimated to be $0{\cdot}014 \pm 0{\cdot}010$, again within the errors given.

I conclude that it is possible with the present techniques to measure the "interarm" absorption spectrum of Cassiopeia A to an accuracy rather better than 1 per cent when account is taken of all the likely sources of error. A marginally significant absorption was found at frequencies which might reasonably be assigned to the interarm medium and this leads to an estimate of the spin temperature of 540 ± 230 K on certain simplifying assumptions. The first assumption is that this part of the spectrum is indeed the result of interarm gas rather than the high dispersion of wings of the local and Perseus arm gas. And the second is that the interarm gas is uniformly distributed; if this is not so, the value of T_s derived from the observed values of T_b and τ could be uncertain by a factor of at least two either way, for example if the scale size was comparable with the angular diameter of Cassiopeia A.

G. DE JAGER

University of Manchester,
Nuffield Radio Astronomy Laboratories,
Jodrell Bank, Cheshire.

Received November 28, 1969.

[1] Mebold, U., *Beiträge z. Radioastronomie*, **1**, 97 (1969).
[2] Muller, C. A., *Astrophys. J.*, **125**, 830 (1957).
[3] Shuter, W. L. H., and Verschuur, G. L., *Mon. Not. Roy. Astron. Soc.*, **127**, 387 (1964).
[4] Clark, B. G., *Astrophys. J.*, **142**, 1398 (1965).
[5] Davies, R. D., Booth, R. S., and Wilson, A. J., *Nature*, **220**, 1207 (1969).

Thermal History of the Moon

I HAVE already used[1] the creep behaviour of the mascons in my model of their origin and morphology[2] to set limits on the internal temperature of the Moon at present, former and primordial epochs. Results for the present are consistent with the upper limit obtained by Ness[3] from the electrical conductivity of the Moon implied by magnetometry in space. The purpose of this article is to use the approximation to the central temperature set by the result of Ness in order to correct previous solutions[4–7] of the heat-conduction equation, to infer possible thermal histories consistent with the present thermal regime.

The thermal history of a planetary body after full accretion is controlled by the internal heat arising from radioactive decay, as specified by the equation of heat conduction. For simplicity, the total rate Q of production of heat per unit mass will be assumed uniform throughout the body at a particular time. The thermal parameters of the body will be taken as constant.

Under the last restrictions, the equation of heat conduction has been solved by Lowan[8]. The solution consists of two parts, $T = T_{\text{par}} + T_{\text{hom}}$, where T_{par} is a particular solution of the full equation involving Q and T_{hom} is the solution of the homogeneous equation dependent on the initial temperature distribution. As a simplification, it will be assumed that the initial temperature at the epoch of origin 4·5 Gyr ago equals 0° C throughout the body. In this case T_{hom} vanishes for all time and the solution becomes $T = T_{\text{par}}$.

Let $T^*(r)$ be such a solution at the present epoch as a function of the radial distance r in the body for particular values μ_i^* of the present abundances of isotopes (designated by i). For another choice $\mu_i = f\mu_i^*$ of abundances, where f is a constant, the ratios of abundances of different isotopes remain invariant. From the equation of heat conduction, one verifies that the solution $T(r)$ corresponding to the values of μ_i is $T = fT^*$, for the particular solutions. One has

$$f = \mu_i/\mu_i^* = T_c/T_c^* \tag{1}$$

immediately in terms of the central temperatures T_c and T_c^* for values of μ_i and μ_i^*, respectively.

The current density J of heat flow from the body is $J = -K(\partial T/\partial r)_0$ in terms of the thermal conductivity and gradient at the boundary. Under the assumption of an equilibrium condition in which all the heat generated at the present time reaches the surface, J is related to the heat production Q by

$$SJ = MQ \tag{2}$$

where S is the surface area and M the mass of the body. While the values of μ_i for the significant radioactive species show large variations for rocks found on the Earth's surface, the Wasserburg ratios Th/U and K/U of the concentrations are relatively constant for different rocks[9]. For average values of these ratios, the abundance μ_U of uranium in the Wasserburg model is fixed by demanding that Q in equation (2) yield the observed average heat flow J over the Earth. This procedure fixes the value of μ_i for ^{238}U, ^{235}U, ^{232}Th and ^{40}K for the Wasserburg model of terrestrial abundances. In what follows, the isotope ^{235}U will be ignored, in view of its relatively small contribution to the heat budget.

The abundances of radioactive elements for a homogeneous Earth on the Wasserburg model[9] are given in Table 1, as determined by MacDonald[10] for the Wasserburg ratios[9] Th/U = 3·7 and K/U = 1×10^4. In Fig. 1, the corresponding solution of the equation of heat conduction for the temperature is shown as a function of depth at the present time from results of Fricker *et el.*[6] for a Moon initially at 0° C with uniformly distributed radioactivities. Also shown is the curve of MacDonald[5] for an initially cold Moon heated by chondritic radioactivity.

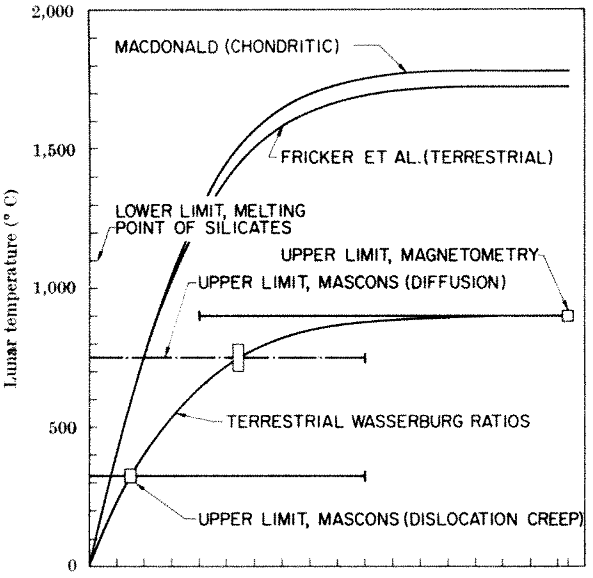


Fig. 1. Present temperature of the Moon as a function of depth for terrestrial Wasserburg ratios, scaled from the solution of the equation of heat conduction by Fricker *et al.* for a terrestrial composition (as shown) to correspond to the central temperature set by the magnetometric result of Ness. Coordinates of the lunar asthenosphere on the hypotheses of dislocation and diffusion creep are indicated by rectangles of error.

The upper limit 900° C on the internal temperature from magnetometry in space (indicated by the horizontal bar in Fig. 1) will be taken as an approximation to the central temperature of the Moon. By means of equation (1), one can then correct the curve of Fricker *et al.* as shown to this central temperature for the same Wasserburg ratios (for $f = 0{\cdot}46$). The lunar temperature on this basis for terrestrial Wasserburg ratios at the present time appears as a function of depth in Fig. 1; values are approximately one-half the corresponding ones of Fricker *et al.* for radioactivity specified by MacDonald[10]. In view of the lower limit on the melting point of silicates indicated, melting at any depth in the Moon is not permitted at the present time by this result.

Lunar temperatures in the past for terrestrial Wasserburg ratios are shown as a function of time from the epoch of origin 4·5 Gyr ago by the curves in Fig. 2 for different depths, computed in the same manner as that of Fig. 1 from data of Fricker *et al.* for a Moon initially at 0° C with uniform radioactivity. Obviously, these results do not permit melting in the lunar interior at any epoch. On the other hand, the curves of Fricker *et al.* for the Wasserburg terrestrial model, as shown, demand internal melting for an extended period in the past (over 1 Gyr at 1 to 3 Gyr ago) for depths up to 150 km from the surface.

The abundances of the radioactive elements in the Moon implied by equation (1) from the curve of Fig. 1 for terrestrial Wasserburg ratios are shown in Table 1; they are somewhat less than one-half the corresponding values of MacDonald[10]. Recently Hanks and Anderson have emphasized that the procedure of MacDonald in using equation (2) to fix the concentration of U in the Earth by means of the average heat flow probably results in a serious overestimate, because he suppressed such other sources of heat in the calibration as terrestrial core formation and high initial temperature[11]. The determination of radioactivity in the continental crust and oceanic rocks by Heir and Rogers[12], the recent measurements on oceanic lherzolites by Wakita *et al.*[13], and new evidence from continental heat flow by Roy *et al.*[14] all point to radioactive concentrations considerably lower than the values of MacDonald in Table 1. From these data, Hanks and Anderson have constructed a preferred model (their Model IV) for averaged continental and oceanic radioactivity in the Earth, as shown in Table 1. These values are roughly one-half those of MacDonald, and the values of this work for the Moon are in fairly good agreement with them.

Table 1. ABUNDANCES OF RADIOACTIVE ELEMENTS FOR A HOMOGENEOUS EARTH AND MOON IN g/g

Nuclide	Earth (MacDonald)	Moon (this work)	Earth (Hanks and Anderson)
^{238}U	$3{\cdot}52 \times 10^{-8}$	$1{\cdot}5 \times 10^{-8}$	$1{\cdot}87 \times 10^{-8}$
^{232}Th	13×10^{-8}	6×10^{-8}	$5{\cdot}91 \times 10^{-8}$
^{40}K	$4{\cdot}2 \times 10^{-8}$	2×10^{-8}	$1{\cdot}14 \times 10^{-8}$

I have inferred upper limits to the internal temperature of the Moon in the layer of low strength (the asthenosphere) from the creep behaviour of the mascons[1]. For the two possibilities of diffusion and dislocation creep, the intersections in Fig. 1 of the horizontal bars for the temperature limits with the curve of lunar temperature against depth permit one to specify approximately the pertinent asthenosphere depths at present, as indicated by the rectangles delineating limits of error. It is obvious from Fig. 2 that the creep of the mascons could be controlled by diffusion only if they were formed less than about 1 to 1·5 Gyr ago, in which case the asthenosphere would lie near or above the Moon's centre. Such a young age seems unreasonable in view of my prior results[1,2]. Hence one must infer that mascon creep most likely is controlled by dislocation motion—contrary to the conclusion of my earlier paper[1].

Table 2 shows values of the surface temperature gradient, the corresponding heat flow J for a mean conductivity[15] and the heat production Q from equation (2) for the Moon at the present time, for terrestrial Wasserburg ratios. For the Earth, the surface temperature gradient is an average for continental areas[16], reflecting the high central temperature of about 6,000° C, as estimated by me[17] and others[18]. Values from MacDonald[10] are shown for J and Q; the latter exceeds the tabulated lunar value by a factor roughly two, in agreement with the contention of Hanks and Anderson[11] that it is too high for the Earth by about this factor.

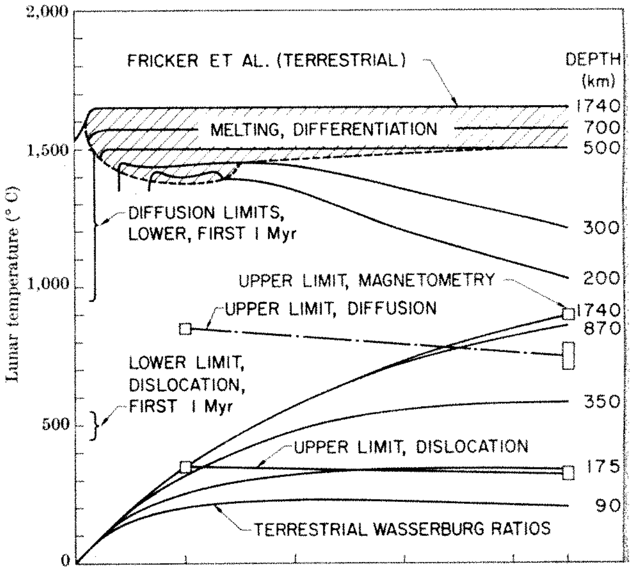


Fig. 2. Temperatures of the Moon at different depths as a function of time in the past for terrestrial Wasserburg ratios, scaled from solutions of the equation of heat conduction by Fricker *et al.* for a terrestrial composition to correspond to the central temperature set by the magnetometric result of Ness. Results of Fricker *et al.* for an initially hot Moon, including effects of melting and differentiation, are shown for comparison. Upper limits on the asthenosphere temperature at the present and former epochs are indicated by lines, and lower limits for the primordial epoch by brackets.

Estimates of the initial temperature near the surface toward the end of the main phase of accretion for bodies of lunar and terrestrial size vary from about 100 K to over 1,000° C. This procedure of adopting an initial temperature as a boundary value on the equation of heat conduction may be merely an ingenuous artifice which can mislead, as emphasized recently by Hanks and Anderson[11] in a discussion of the possibly very significant effects of the early accretion stage on the later thermal evolution of the Earth.

Table 2. PHYSICAL PARAMETERS AT THE PRESENT TIME ASSOCIATED WITH THE THERMAL HISTORY OF THE MOON, COMPARED WITH THOSE FOR THE EARTH

Body	Model	Surface gradient $-(\partial T/\partial r)_0$ (°C km^{-1})	Heat flow J (erg cm^{-2} s^{-1})	Heat production Q (erg g^{-1} yr^{-1})
Moon	Earth Wasserburg ratios	2·1	6·5	1·1
Earth	Observational	17·9	63·9	2·55

My theory of a former atmosphere and hydrosphere of the Moon presupposes a relatively brief episode of moderate internal heating near the epoch of origin to release the volatiles, by the accretion mechanism or by decay of nuclides of short lifetime[2,19]. Thus it is a satisfying feature of my theory of creep behaviour of the mascons[1] that it requires an elevated initial temperature near the surface to permit isostatic compensation within a time in the range 1 kyr to 1 Myr from the Moon's origin. The range of the lower limits to the temperature in the lunar asthenosphere at this pristine epoch is indicated by a bracket in Fig. 2, as demanded by dislocation creep of the mascons.

Lowan's solution[8] T_{hom} specifying the decay of an initial temperature distribution cannot be used directly with temperature data from the primordial mascon creep to obtain a realistic description of the early thermal history. First, the mascon data yield a lower limit only to the temperature[1]. Second, they refer to the unknown depth of the primordial asthenosphere, on which the only datum is that it probably lay close to the pristine level of mean density[2]. Finally, this procedure would ignore the possibility of significant heating by short-lived radioactive nuclides[2,19] (such as ^{26}Al).

The most one can assert is that the temperature for the primordial asthenosphere lay in the range of about 500° C to the melting point of silicates, from Fig. 2. Thus the mascon data yield primarily clues to the early thermal history.

In view of the possible thermal history found, the conclusion of my earlier paper[1] stands, that any extensive lava flow present on the lunar surface must have erupted in the early history, at least 3·5 Gyr ago, roughly. The potassium–argon ages ($3 \cdot 0 \pm 0 \cdot 7$ Gyr) found in Apollo 11 samples for the igneous crystalline rocks are consistent with this estimate[20] (because they represent the time from last melting).

The basic procedure used here is to calibrate the average abundances of radioactive isotopes throughout the Moon by means of the central temperature inferred from the result of Ness. The method of Wasserburg *et al.*[9] and of MacDonald[10] for the Earth based on equation (2) yields abundances far lower than correspond to common surface rocks like basalt.

A similar situation seems to prevail for the Moon[20]. Mean abundances of U and Th in the lunar surface are $5 \cdot 4 \times 10^{-7}$ and $2 \cdot 2 \times 10^{-6}$ g/g, respectively—considerably higher than those appearing in Table 1 and within the range for a terrestrial basalt. The value 4·1 of Th/U is close to the terrestrial Wasserburg ratio, but the lunar ratio K/U is about 3×10^3, roughly one-third the terrestrial value. If lunar Wasserburg ratios show the same constancy as terrestrial ones, they require lowering of potassium abundance relative to the model considered here.

The present results make it abundantly clear that a relatively cold thermal regime in the Moon over roughly the last 3·5 Gyr can be justified consistently with observed levels of radioactivity in terrestrial rocks, without resort to the drastic suggestion by Anderson and Phinney[7] of Moon formation antedating that of the Earth by several milliard years. The mascon data seem to require a relatively hot Moon initially, contrary to Urey's idea of a Moon cold at origin[4].

Apollo 13 or 14 is scheduled to measure the heat flow J of the Moon, as predicted by Table 2.

I thank B. W. Augenstein, S. H. Dole, and Professors D. L. Anderson and D. T. Griggs.

J. J. GILVARRY*

Space Science Laboratory,
General Dynamics/Astronautics,
San Diego, California.

Received November 28, 1969; revised January 13, 1970.

* Present address: The Rand Corporation, Santa Monica, California.

[1] Gilvarry, J. J., *Nature*, **224**, 968 (1969).
[2] Gilvarry, J. J., *Nature*, **221**, 732 (1969); *ibid.*, **223**, 255 (1969).
[3] Ness, N. F., *Proc. Twelfth COSPAR Meeting, Prague* (in the press).
[4] Urey, H. C., in *Physics and Astronomy of the Moon* (edit. by Kopal, Z.), 502 (Academic Press, New York, 1962).
[5] MacDonald, G. J. F., *J. Geophys. Res.*, **64**, 1967 (1959).
[6] Fricker, P. E., Reynolds, R. T., and Summers, A. L., *J. Geophys. Res.*, **72**, 2649 (1967).
[7] Anderson, D. L., and Phinney, R. A., in *Mantles of the Earth and Terrestrial Planets* (edit. by Runcorn, S. K.), 113 (Interscience, London, 1967).
[8] Lowan, A. N., *Phys. Rev.*, **44**, 769 (1933).
[9] Wasserburg, G. J., MacDonald, G. J. F., Hoyle, F., and Fowler, W. A., *Science*, **143**, 465 (1964).
[10] MacDonald, G. J. F., *J. Geophys. Res.*, **69**, 2933 (1964).
[11] Hanks, T. C., and Anderson, D. L., *Phys. Earth Planet. Interiors*, **2**, 19 (1969).
[12] Heir, K. S., and Rogers, J. J. W., *Geochim. Cosmochim. Acta*, **27**, 137 (1963).
[13] Wakita, H., Nagasawa, H., Uyeda, S., and Kuno, H., *Geochim. J.*, **1**, 183 (1967).
[14] Roy, R. F., Blackwell, D. D., and Birch, F., *Earth Planet. Sci. Lett.*, **5**, 4 (1968).
[15] Clark, S. P., in *Handbook of Physical Constants* (edit. by Clark, S. P.), 461 (Geological Society of America, New York, 1954).
[16] Birch, F., in *Nuclear Geology* (edit. by Faul, H.), 163 (Wiley, New York, 1954).
[17] Gilvarry, J. J., *Nature*, **178**, 1249 (1956); *J. Atmos. Terr. Phys.*, **10**, 84 (1957).
[18] Kennedy, G. C., *Trans. Amer. Geophys. Un.*, **47**, 173 (1966); Gilvarry, J. J., *Phys. Rev. Lett.*, **16**, 1089 (1966).
[19] Gilvarry, J. J., *Nature*, **188**, 804 (1960).
[20] Lunar Sample Preliminary Examination Team, *Science*, **165**, 1211 (1969).

Silicon Tetrahedral Sheet in Stilpnomelane

A CONTINUOUS hexagonal sheet of silicon tetrahedra (as in kaolinite) has lateral dimensions too small to permit articulation with a trioctahedral sheet in which the octahedral cations are larger than those of magnesium. In micas and chlorites where iron may occur in the octahedral sheet, the tetrahedral sheet is expanded by the substitution of aluminium for silicon; in astrophyllite[1] insertion of TiO_6 octahedra in the tetrahedra sheet permits coordination to the iron-bearing octahedral sheet. Expansion by inversion of tetrahedra has been found in the iron silicate zussmanite[2]. Stilpnomelane is a trioctahedral layer silicate with a layer thickness of about 12 Å. The octahedral cations are largely iron, which can range in oxidation state from all ferrous to almost all ferric[3], whereas the tetrahedral cations are nearly all silicon. In this paper the tetrahedral sheet of stilpnomelane is described.

The stilpnomelane used in this study is from Crystal Falls, Minnesota. Details of this sample and of the subcell structure of stilpnomelane have previously been described[4]. The unit cell of stilpnomelane is made up of sixteen subcells, and is triclinic with dimensions

$$a=21\cdot7\ \text{Å},\ b=21\cdot7\ \text{Å},\ c=17\cdot7\ \text{Å},\ \alpha=124°,\ \beta=96°,\ \gamma=120°$$

The X-ray reflexions that distinguish the unit cell from the subcell (so-called superlattice reflexions) are weak and are somewhat streaked parallel to c^*, indicating stacking disorder of the stilpnomelane layers. The superlattice reflexions are trigonally pseudosymmetric, and this property has been used to derive the Fourier transform of a single tetrahedral sheet. A distinction between the transform of the tetrahedral sheet and of the whole crystal is possible because only the tetrahedral sheet contributes to the superlattice reflexions.

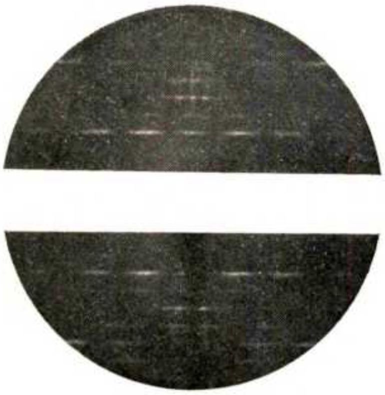


Fig. 1. Precession photograph of stilpnomelane, $h0l$, $0kl$, $\bar{h}hl$ triple exposure, superlattice reflexions only. Rows are parallel to c^*.

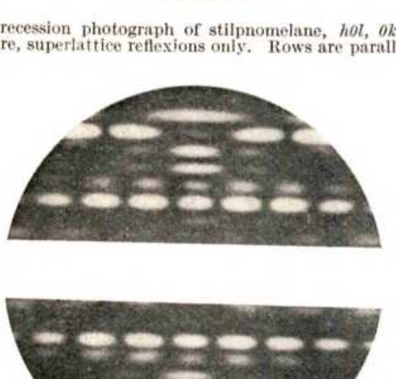


Fig. 2. Optical diffraction pattern (above) produced from the mask (below).

The tetrahedral sheet has three-fold symmetry about c^* but the triclinic reciprocal lattice does not. Superposition of reciprocal lattice planes spaced at 120° around c^* thus triples the number of points at which the Fourier transform can be observed and shows the transform of a single tetrahedral sheet[5]. The structure of the tetrahedral sheet was deduced by simulating the X-ray diffraction pattern (shown in Fig. 1) using an optical diffractometer[6] illuminated with an He–Ne laser (Fig. 2).

Based on this tetrahedral arrangement the full unit cell contains 48 octahedral cations, 72 tetrahedral cations, and 216 anions, and may be written

$$[(\mathrm{Al},\mathrm{Fe}^{+3})_x(\mathrm{Fe}^{+2},\mathrm{Mg},\mathrm{Mn})_{48-x}(\mathrm{OH})_{48-x}\mathrm{O}_{48+x}]$$
$$[\mathrm{Si}_{30\text{ to }36}\,\mathrm{Al}_{6\text{ to }0}\,\mathrm{O}_{60}]_2$$

Chemical analyses in the literature[3] show that up to five alkali atoms may be incorporated in the structure, as well as a considerable quantity of water.

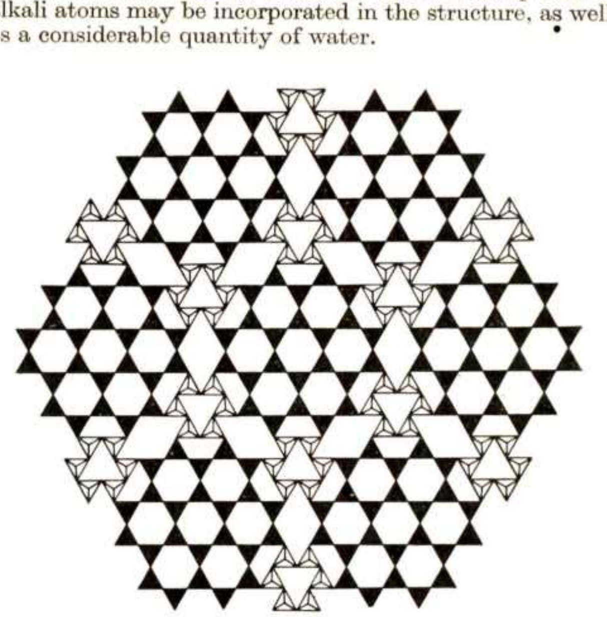


Fig. 3. Plan view of the stilpnomelane tetrahedral sheet. Black triangles represent tetrahedra coordinated to the octahedra; outline tetrahedra coordinate at their apices to a like sheet above.

The diagram of the tetrahedral sheet (Fig. 3) shows that articulation of tetrahedra to octahedra is only achieved over a short distance, producing "islands" of 24 tetrahedra linked by trigonal rings of six tetrahedra. Each tetrahedron of the trigonal ring is joined by its apical oxygen to a like but inverted ring above, which in turn serves to link further "islands" of tetrahedra connected to the next octahedral sheet. The tetrahedral sheet figured is that of a ferri-stilpnomelane. The larger octahedral sheet of ferro-stilpnomelane ($a=22\cdot0$ Å) can easily be accommodated by expanding the trigonal rings of tetrahedra to a more nearly hexagonal shape. There is ample space between the "islands" of tetrahedra and within the paired trigonal rings of tetrahedra to accommodate the alkali atoms and water recorded in most analyses of stilpnomelane.

R. A. EGGLETON

Department of Geology,
Australian National University,
Canberra, Australia, 2600.

Received November 24, 1969.

---

[1] Woodrow, P. J., *Acta Cryst.*, **22**, 673 (1967).

[2] Lopes-Vieira, A., and Zussman, J., *Mineral. Mag.*, **37**, 49 (1969).

[3] Hutton, C. O., *Mineral. Mag.*, **25**, 172 (1938).

[4] Eggleton, R. A., and Bailey, S. W., *Clays, Clay Miner. Proc. Nat. Conf.*, **13**, 49 (Pergamon Press, 1965).

[5] Eggleton, R. A., *Acta Cryst.*, **A25**, 543 (1969).

[6] Taylor, C. A., and Lipson, H., *Optical Transforms* (G. Bell and Sons Ltd., London, 1964).

Crystalline Structures of Alginic Acids

WE have recently obtained X-ray diffraction photographs from orientated samples of alginic acid preparations in which the ratio of mannuronic acid to guluronic acid was known. The purified unorientated material was supplied by Dr A. Haug, and our results form the basis for structure determinations of polymannuronic acid and polyguluronic acid respectively.

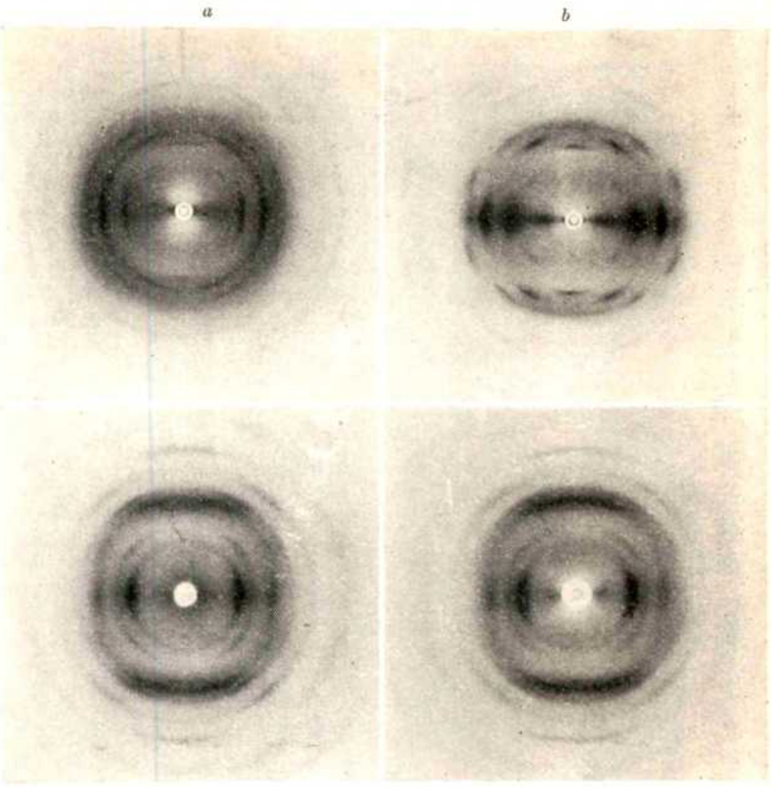


Fig. 1. X-ray fibre diffraction photographs of alginic acids. *a*, 96 per cent mannuronic acid; *b*, specimen isolated from *Fucus serratus*; *c*, commercial alginic acid; *d*, 73 per cent guluronic acid.

The discovery that alginic acid preparations of different composition could give rise to different X-ray powder photographs was made originally by Frei and Preston[1]. Although they recognized that the analyses of their alginic acids had to be interpreted with caution, they nevertheless concluded then that there were two types of X-ray powder photograph and they correlated these with polymannuronic acid and polyguluronic acid.

Fig. 1*a* shows the X-ray fibre diffraction photograph obtained from a sample of alginic acid consisting of 96 per cent mannuronic acid. To our knowledge this is the first authentic standard for polymannuronic acid. Fig. 1*b* is the X-ray fibre diffraction photograph of a specimen of alginic acid isolated by us from the brown alga, *Fucus serratus*. This is clearly the same X-ray fibre diagram as Fig. 1*a* and therefore must refer to polymannuronic acid. Fig. 1*c* shows the "usual" alginic acid X-ray fibre diagram as obtained by Astbury[2] in the 1940s from samples of commercial alginic acid and supposed, by him, to refer to polymannuronic acid. Fig. 1*d* shows the X-ray fibre diffraction photograph we have obtained from a sample of alginic acid containing 73 per cent guluronic acid and 27 per cent mannuronic acid. (This sample was the richest in guluronic acid from which orientated specimens could be prepared.) This latter substance gave the same X-ray powder diagram (see Fig. 2) as a degraded[3] sample of alginic acid containing 92 per cent guluronic acid. It therefore appears that the mannuronic acid units in the 73 per cent guluronic acid sample do not contribute to the X-ray diffraction pattern (as suggested previously[1]). We therefore conclude that the X-ray fibre diagram shown in Figs. 1*c* and 1*d* is indeed that of polyguluronic acid.

We are able to give the following preliminary details of the structures of these two substances.

Polymannuronic acid: The X-ray fibre diffraction photograph (Fig. 1*b*) has been indexed to an orthorhombic unit cell in which $a = 7{\cdot}58$ Å, $b = 10{\cdot}35$ Å (fibre axis) and $c = 8{\cdot}58$ Å, the unit cell containing two disaccharide chain segments with probable space group $P2_12_12_1$. Chemical evidence indicates that the D-mannuronic acid units are β-1→4 linked, and it is therefore satisfying that the fibre repeating distance of 10·35 Å is the same as that observed in the three other β-1→4 linked hexosans, cellulose chitin and mannan[4,5]. In models of these substances, the required repeating distance can be obtained by constructing chains in which the monosaccharide units are in the energetically favourable C1 chair conformation, while simultaneously maintaining an *intra* residue hydrogen bond between the $—O_3H$ and the ring oxygen atom (O_5) of the next sugar unit in the chain (that is, $O_3 \ldots O_5$ distance of 2·7–2·8 Å). A chain built in this way automatically contains di-equatorially (1e, 4e) (ref. 6) linked sugar units (without regard to the nature of the sugar units itself). This is the basic structural form which we have adopted in model building studies of polymannuronic acid.

Polyguluronic acid. The X-ray fibre diffraction photograph of this substance (Fig. 1*d*) has been indexed to an orthorhombic unit cell also containing two disaccharide chain segments and in which $a = 8{\cdot}6$ Å, $b = 8{\cdot}72$ Å (fibre axis) and $c = 10{\cdot}74$ Å with probable space group $P2_12_12_1$ (see also ref. 2). The most significant difference from the polymannuronic acid structure is the fibre repeat of 8·72 Å. This value can be obtained in molecular models by maintaining the 1→4 linked L-guluronic acid units in the *1C* conformation[7], the glycosidic linkages having the α-configuration. Thus the building units are di-axially (1*a*,4*a*) linked. When the sugar units are arranged in this way, it is found that several possibilities exist for the formation of *intra* residue hydrogen bonds between the equatorial hydroxyl group of the C_2 atom $(—O_2H)$ and either oxygen atom in the carboxyl group of the adjacent sugar unit in the chain. It is likely that other examples of 1*a*,4*a* linked structures exist. For example, poorly orientated fibre diffraction photographs of pectic acid (α-1→4 linked poly D-galacturonic acid) show the same fibre repeating distance of 8·7 Å (see also refs. 2 and 8), and this can be understood if the D-galacturonic acid units are in the *C*1 conformation.

Attention is currently being directed to the arrangement of the chains of polymannuronic and polyguluronic acid in the unit cells and the nature and extent of inter-chain bondings. Trial structure factors have been calculated and refinement of the models is in progress.

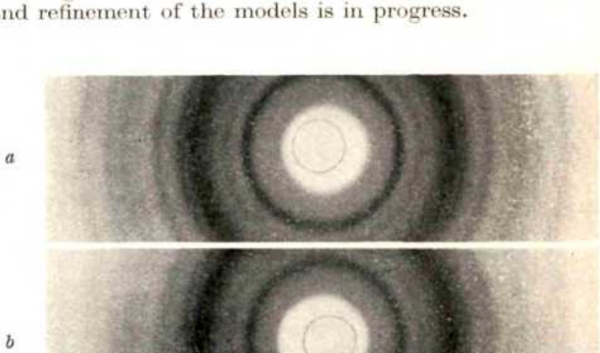


Fig. 2. X-ray powder photographs of (*a*) 92 per cent guluronic acid and (*b*) 73 per cent guluronic acid.

It is expected that the structural determinations of these substances will provide information which will be helpful in reaching an understanding of the physical and biological properties of these molecules.

We thank Professor R. D. Preston and Dr Eva Frei for useful discussions and Dr K. M. Rudall for providing the photograph in Fig. 1*c*. One of us (E. E. S.) acknowledges an award from the International Atomic Energy Agency.

E. D. T. Atkins*
W. Mackie
E. E. Smolko

Astbury Department of Biophysics,
University of Leeds.

Received October 2; revised December 11, 1969.

* Present address: H. H. Wills Physics Laboratory, University of Bristol.

[1] Frei, Eva, and Preston, R. D., *Nature*, **196**, 130 (1962).
[2] Astbury, W. T., *Nature*, **155**, 667 (1945).
[3] Haug, A., Larson, B., and Smidsrod, O., *Acta Chem. Scand.*, **20**, 183 (1966).
[4] Marchessault, R. H., and Sarko, A., *Advances in Carbohydrate Chemistry*, **22**, 421 (1967).
[5] Frei, Eva, and Preston, R. D., *Proc. Roy. Soc.*, B, **169**, 127 (1968).
[6] Sundaralingam, M., *Biopolymers*, **6**, 189 (1968).
[7] Percival, E., and McDowell, R. H., *Chemistry and Enzymology of Marine Algal Polysaccharides*, 107 (Academic Press, 1967).
[8] Sterling, C., *Biochim. Biophys. Acta*, **26**, 186 (1957).

Stored Energy in Creep Deformed Graphite

Graphite which has been strained in tensile creep releases a very large amount of stored energy when the creep stress is reduced, as happens in the dip test[1]. The very large amount of negative creep (actual shrinkage of the specimen while it is still under an appreciable applied tensile force) that takes place after a stress reduction is shown graphically in Fig. 1. Three individual stress reduction tests are shown. The work the specimen does against gravity in lifting the hanging weights is measured by the distance the specimen shrinks in negative creep multiplied by the remaining applied force. In a series of tests on ZTA graphite, the largest measured amount of energy released was $1{\cdot}1\times10^6$ ergs/cm^3 (0·17 calories/g atom). This was released when one specimen was strained almost to fracture (20 per cent elongation) at 3,200 pounds inch^{-2}, at which point the applied stress was reduced to 1,600 pounds inch^{-2}. The negative creep strain after the load reduction was 1·2 per cent. In time the negative creep period ended, but additional negative creep, some four times greater than that already measured, then occurred after complete removal of the applied stress. If the total energy release were proportional to that measured after the initial stress reduction, it would have been about $5{\cdot}5\times10^6$ ergs/cm^3. It was probably less than this. For further calculations, however, let us assume that the released energy was somewhere between $1{\cdot}1\times10^6$ and $5{\cdot}5\times10^6$ ergs/cm^3.

The total work done on the sample per unit volume during creep was simply the product of the creep stress and the creep strain. Dividing this into the energy recovered puts the recoverable percentage of the total expended energy at between 3 and 15 per cent.

We now compute from the appropriate surface and line energies the crack area or dislocation line length per unit volume which, if annihilated during recovery, would release $1{\cdot}1\times10^6$ to $5{\cdot}5\times10^6$ ergs/cm^3.

The energy per unit area for a basal plane cleavage crack is about 150 ergs per cm^2, according to theory[2,3]. The average crack opened up during creep is coin shaped and is approximately 0·01 cm in diameter[4]. Such a crack, if it is smooth, has a surface area of $1{\cdot}6\times10^{-4}$ cm^2. The number of such cracks per cm^3 necessary to release the required energy on their annihilation is between $0{\cdot}7\times10^{10}$ and 3×10^{10}; their spacing, if arranged in a rectangular grid, is between 0·003 and 0·005 cm.

The line energy per unit length, Γ, of a dislocation is

$$\Gamma = \frac{kb^2 \ln (\overline{R}/R_0)}{4\pi} \tag{1}$$

and in graphite is $1{\cdot}1\times10^{-4}$ ergs per cm length (here b is the Burgers vector, 2·46 Å; $\overline{R}$ the dislocation spacing, about 1000 Å; R_0 the core diameter, 1 Å; and k an elastic constant term, 4×10^{11} dynes per cm^2). The method of Hirth and Lothe[5] was used to compute k. Their equations (13–84 and 13–137) show that for anisotropic elastic media k, the so-called energy factor, must replace G, the shear modulus, which is normally used in equations to calculate line energy. If this modification for the anisotropic elastic behaviour of graphite is not made and the interlayer shear constant is used instead, Γ is two orders of magnitude smaller. An unacceptably great length of annihilating dislocation results. Annihilation of 1×10^{10} to 5×10^{10} cm per cm^3 length of dislocation would account for the observed release of stored energy.

From these calculations, either form of imperfection could account for the observed energy release because the calculated crack and dislocation densities do not seem unrealistically large. Almost all of the elongation observed in tensile creep of graphite has been shown[4] to be associated with the opening of cracks. With the layered structure of graphite, however, the opening of a crack must involve not only forming new surface but also bending the surrounding material, in which dislocations are certainly involved. Either surface area or bending deformation could dominate in the storage of energy, and at present it is not possible to favour one mechanism over the other. Perhaps transmission electron microscopy of the dislocation structure and crack surface area measurement (by the B.E.T. gas adsorption method, for example) could be used to determine whether either imperfection density changes during recovery in the required way.

Very little quantitative information has been published on the amount of energy stored in materials deformed by creep, but it seems likely that most materials would not store as much as does graphite. In a metal with many slip systems, all grains in a polycrystalline sample can find suitable slip systems. As a result, all grains deform once the flow stress is reached. But there are not enough active slip systems in the hexagonal structure of highly anisotropic graphite. Graphite can therefore sustain higher stresses than a metal and can thereby store more energy. The amount of recoverable energy, between 3 and 15 per cent of the work done on the graphite sample

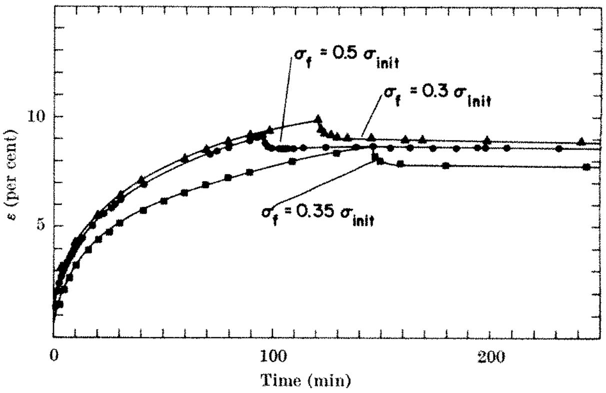


Fig. 1. Creep tests at 2,500° C on ZTA graphite in which the initial stresses were 3,200 pounds inch^{-2} and the final stresses were at the indicated values. Note the negative creep period that follows the stress drops; the sample does work against gravity by lifting the weights hung on the sample. The energy released per unit volume is the product of the final stress and the negative creep strain.

during creep, is comparable with that reported for cold worked metals, but the structures of creep-deformed metals do not contain the large density of dislocations normally associated with metals in the cold-worked state. The fact that graphite lacks slip systems capable of plastically deforming any given crystal along the *c*-axis must, in some way, be responsible for the large build-up of stored energy.

This work was carried out under the auspices of the US Atomic Energy Commission and with the support of the US National Aeronautics and Space Administration. We thank Dr R. B. Gibney and Mr M. C. Smith, under whose leadership this work was performed, and Mr J. J. Medina for assistance in the laboratory.

W. V. GREEN
E. G. ZUKAS

University of California,
Los Alamos Scientific Laboratory,
Los Alamos, New Mexico 87544.

Received August 21; revised December 1, 1969.

[1] Nix, W. D., and Barrett, C. R., *ASM Trans.*, **61**, 695 (1968).
[2] Bruce, R. H. J., *Metal. Club, R.C.S. Glasgow 10*, 41 (1958–9).
[3] Knibbs, R. H., *J. Nuc. Mat.*, **24**, 174 (1967).
[4] Green, W. V., Levinson, L. S., Reiswig, R. D., and Zukas, E. G., *Carbon*, **5**, 583 (1967).
[5] Hirth, J. P., and Lothe, J., *Theory of Dislocations*, 398 (McGraw-Hill, NY, 1968).

Catalytic Oxidation of SO_2 on Carbon Films studied by Electrical Resistance Measurements

ELECTRICAL resistance measurements have been used for investigating the mechanism of chemisorption and catalysis on metals and oxides, but comparable data for carbon are scarce. We have demonstrated[1] that the resistance of vacuum evaporated carbon films increases during oxygen chemisorption. We have also used this technique, which rapidly provides information on chemisorption by carbon, to study the kinetics and energetics of oxygen chemisorption[1].

We have now measured the effect of various adsorbates on the resistance of carbon films. Particularly striking effects were observed with (SO_2+O_2) mixtures and we report here some results for this system. The experimental details have been described[1]. Films were transferred in air to the resistance-measurement apparatus, where vacuum heat treatments and gas admission were carried out in the absence of air. The resistance of evaporated carbon films decreases[1-4] on vacuum heat treatment with an annealing process which has an activation energy increasing from 20 to 90 kcalories/mole[5,6], according to the extent of annealing. Heat treatment also removes surface oxygen, and room temperature resistance is sensitive to oxygen only for heat treatment temperatures above about 200° C (ref. 1). The present experiments involved room temperature measurements on films initially heat treated for 6 h under vacuum at 350° C.

Admission of SO_2 decreased film resistance, typically by 5 per cent for pressures of 20 mm Hg. The decrease was completed within 2 s and was rapidly reversed by evacuation at room temperature. All heat treated films, not previously exposed to SO_2, showed the usual increases in resistance under O_2 and these were completely reversed by evacuation only at the temperature of the initial heat treatment[1].

The effect of (SO_2+O_2) mixtures was strikingly different from the effects of each gas alone. Such mixtures caused large decreases in resistance and these were not reversed at all by evacuation at room temperature. It took about one thousand minutes to attain essentially constant resistance under (SO_2+O_2), but a large fraction of the change took place within a few minutes (Fig. 1). The overall decrease amounted in some cases to over 70 per cent. Evacuation at higher temperatures caused a progressive reversal which was complete at 200°–250° C (Fig. 1). Both the rate and extent of the decreases in resistance increased with the partial pressure of each gas (Figs. 2 and 3). It is remarkable that O_2 partial pressures below 10^{-1} mm Hg produced significant irreversible decreases; in the absence of SO_2 the resistance increases brought about by O_2 took place only at pressures above several mm. The decreases in resistance, brought about by (SO_2+O_2), followed Elovich kinetics[1,7-9] during the later stages of the process. The initial kinetics may be complicated by a contribution from the rapid reversible effect of SO_2 alone, but it is interesting that none of the (SO_2+O_2) effects are reversed by evacuation at room temperature.

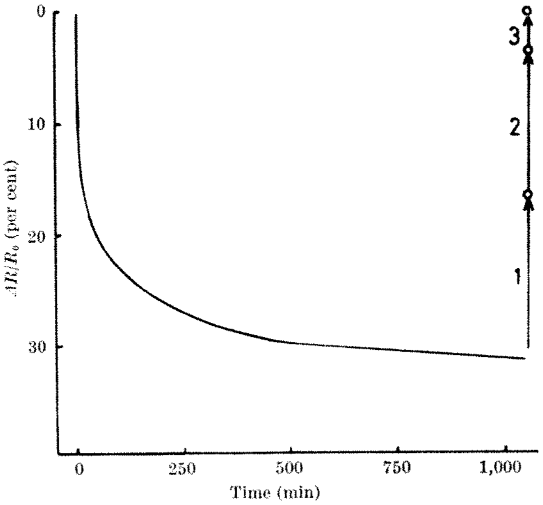


Fig. 1. Percentage decrease in resistance ($\Delta R/R_0$ per cent) with time following admission of an (SO_2+O_2) mixture (16 mm SO_2 and 25·5 mm O_2) to a film heat treated at 350° C. $R_0=1{\cdot}62\times10^7\,\Omega$. Also shown is the reversibility of the effect on evacuation to constant resistance at temperatures up to 230° C. 1, Evacuation at 100° C; 2, evacuation at 200° C; 3, evacuation at 230° C.

Reversal of the (SO_2+O_2) effect could also be achieved by evacuation at room temperature (half an hour),

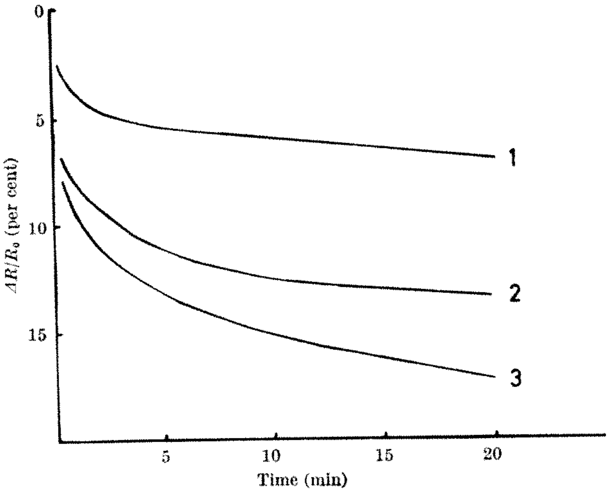


Fig. 2. Resistance decreases under (SO_2+O_2) at an SO_2 partial pressure of 16 mm and varying O_2 partial pressures. Original resistance ($1{\cdot}57\times10^7$ Ω) recovered between each experiment by 1 h evacuation at 300° C. Film previously heat treated at 350° C. Curve 1, $8{\cdot}5\times10^{-2}$ mm O_2; decrease after 1,000 min 14·1 per cent. Curve 2, 1·5 mm O_2; decrease after 1,000 min 21·2 per cent. Curve 3, 6·0 mm O_2; decrease after 1,000 min 31·6 per cent.

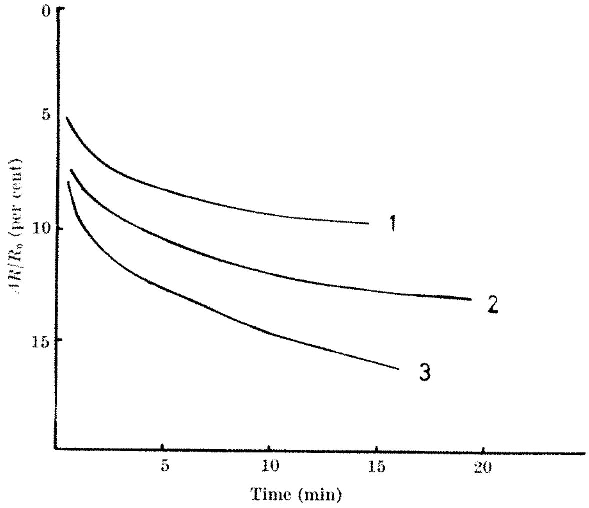


Fig. 3. Resistance decreases under (SO_2+O_2) at an O_2 partial pressure of 16 mm and varying SO_2 partial pressures, for the film of Fig. 2. Original resistance recovered between each experiment by 1 h evacuation at 300° C. Curve 1, 1·0 mm SO_2; decrease after 1,000 min 12·7 per cent. Curve 2, 5·0 mm SO_2; decrease after 1,000 min 20·4 per cent. Curve 3, 16·0 mm SO_2; decrease after 1,000 min 34·6 per cent.

followed by exposure to water vapour (3–15 mm Hg) at room temperature. The resistance increased when water vapour was admitted, and after several hours a constant value corresponding to a complete reversal of the (SO_2+O_2) effect was attained. No further resistance changes took place on subsequent evacuation at room temperature. Alternate treatments with (SO_2+O_2) and water vapour could be carried out indefinitely with identical cycles of resistance changes. Resistance increases were observed on admission of water vapour to films before treatment with (SO_2+O_2) but these were small (< 5 per cent) and were partly reversed by room temperature evacuation (compare with ref. 1). When water vapour was present in (SO_2+O_2) mixtures, their effect on resistance was largely inhibited.

Conventional measurements have shown[10–15] that SO_2 chemisorption on carbons is enhanced by the presence of O_2 in the gas phase. Weakly chemisorbed O_2 is believed[11,12] to react with adsorbed SO_2 to produce surface (SO_3). Our results confirm the promotion of SO_2 chemisorption by O_2. We assume that $(SO_3)_{ads}$ is responsible for the resistance decreases which are reversed by evacuation at 200°–250° C. It is relevant that desorption of SO_3 from sugar chars is virtually complete at 250° C (ref. 16).

The resistance technique can provide direct evidence about the form of chemisorbed species (see references in ref. 1). The large changes in resistance, observed under (SO_2+O_2), suggest that formation of surface SO_3 involves charge transfer between the carbon surface and the adsorbed complex. This will involve electron transfer from carbon to $(SO_3)_{ads}$. Possible negatively charged forms of the adsorbed complex include either the sulphite ion $(SO_3)^{2-}$ or $(R{-}SO_3)^-$, an anion analogous to that of a sulphonic acid $(R{-}SO_3)^-$. Carbon films consist[2,17,18] of graphitic regions some 10 Å in diameter separated by tetrahedrally bonded carbon. The structure involving $(R{-}SO_3)^-$ could represent an SO_3 group covalently bonded to a graphitic region (R) to form a macro anion, with an adjacent region acting as the electron donor and forming a macro cation. Decreases in resistance could result from an increase in the number of positive charges responsible for conduction. Thermoelectric[3] and field effect[19] data indicate that conduction in these films does involve positive carriers. Alternatively, charged chemisorbed species may alter resistance by affecting carrier mobility through modification of potential barriers between surface regions[4].

A more pronounced enhancement of SO_2 chemisorption occurs[12–15] in the presence of both O_2 and water vapour; adsorbed H_2SO_4 is formed in these conditions. Our results on the inhibition of the (SO_2+O_2) effect by water vapour may result from the reaction—

$$SO_{3ads}+H_2O \longrightarrow H_2SO_{4ads}$$

if resistance is decreased by SO_{3ads} but not by H_2SO_{4ads}. The formation of H_2SO_4 from negatively charged $(SO_3)_{ads}$ and H_2O would return the charge on the $(SO_3)_{ads}$ to the carbon. The repetitive cycling experiments, involving alternate treatment with (SO_2+O_2) and water vapour, suggest that H_2SO_4 vacates the sites at which it is formed from $(SO_3)_{ads}$, making them available for further reaction between SO_2 and O_2.

Large decreases in resistance were observed only when both SO_2 and O_2 were present in the gas phase. This suggests the presence of a reactive, weakly adsorbed intermediate in equilibrium with molecules in the gas phase. Likely candidates for such an intermediate are the adsorbed SO_2 which reversibly decreases resistance, or the types of weakly chemisorbed O_2 which reversibly decrease[5] or increase (our unpublished work) the spin concentration of carbon films.

We are further investigating these resistance effects. Radioactively labelled SO_2 is being used to correlate the number of chemisorbed SO_2 molecules with the resistance changes.

I. S. McLintock
J. C. Orr

Isotopes Laboratory,
University of Dundee.

Received November 14, 1969.

[1] McLintock, I. S., and Orr, J. C., *Carbon*, **6**, 309 (1968).
[2] Kakinoki, J., *Proc. Fifth Carbon Conference*, **2**, 499 (Pergamon Press, Oxford, 1963).
[3] Blue, M. D., and Danielson, G. C., *J. Appl. Phys.*, **28**, 583 (1957).
[4] Hirabayashi, H., and Toyada, H., *Proc. Fourth Carbon Conf.*, 227 (Pergamon Press, Oxford, 1961).
[5] McLintock, I. S., and Orr, J. C., *Carbon*, **5**, 291 (1967).
[6] McLintock, I. S., and Orr, J. C., *Carbon*, **6**, 744 (1968).
[7] Low, M. J. D., *Chem. Rev.*, **60**, 267 (1960).
[8] McLintock, I. S., *Nature*, **216**, 1204 (1967).
[9] McLintock, I. S., *J. Catalysis* (in the press).
[10] Beebe, R. A., and Dell, R. M., *J. Phys. Chem.*, **59**, 746 (1955).
[11] Davtyan, O. K., and Ovchinnikova, E. N., *Dokl. Akad. Nauk. SSSR*, **104**, 857 (1955).
[12] Ovchinnikova, E. N., and Davtyan, O. K., *Zh. Fiz. Khim.*, **30**, 1735 (1956).
[13] Ovchinnikova, E. N., and Davtyan, O. K., *Zh. Fiz. Khim.*, **35**, 713 (1961).
[14] Ovchinnikova, E. N., and Tkach Yu., A., *Zh. Fiz. Khim.*, **35**, 992 (1961).
[15] Billinge, B. H. M., *Second Conf. Industrial Carbon and Graphite*, 398 (Society of Chemical Industry, London, 1966).
[16] Siedlewski, J., *Roczniki Chemii*, **38**, 1151 (1964).
[17] Bell, A. E., Pritchard, J., and Sykes, K. W., *Second Conf. Industrial Carbon and Graphite* (Society of Chemical Industry, London, 1966).
[18] Kakinoki, J., Karada, K., Hanawa, T., and Ino, T., *Acta Crystallogr.*, **13**, 171 (1960).
[19] Hanawa, T., and Kakinoki, J., *Proc. Sixth Amer. Carbon Conference*, Pittsburgh (1963); abstracted in *Carbon*, **1**, 403 (1964).

Iodine as a Boundary Lubricant: A Contrary View

Roberts and Owens reported[1] that charge-transfer complexes of iodine and *n*-butylbenzene were effective boundary lubricants for titanium sliding on titanium. The discovery is interesting because titanium and its alloys are being used more widely and are difficult to machine and lubricate.

According to Roberts and Owens[2], charge–transfer complexes of iodine and aromatic compounds greatly reduce friction and wear with titanium or steel sliding on titanium. Similar results were obtained with a mixture of these complexes and a straight-chain hydrocarbon oil. The suggested lubrication mechanism was the formation of the low shear strength laminar solid TiI_2 at the wearing interface; the hydrophobic nature of the lubricant was believed to prevent the titanium diiodide from reacting with water in the air. The idea is attractive, and indeed G. W. Rowe had shown earlier (see description in ref. 3) that the exposure of clean titanium to iodine vapour resulted in lower friction and the formation of TiI_2.

Roberts and Owens have also reported that iodine–anisole complexes are effective for the boundary lubrication of chrome steel[4]. They suggested that the mechanism may be the same as that for the lubrication of titanium, because FeI_2 is a lamellar solid with the CdI_2 structure.

The results of friction, wear and metalworking tests confirm that the iodine-containing fluids are effective as boundary lubricants. These findings and the diiodide layer-lattice mechanism have been widely publicized[5-8]. But I find the data in support of this theory far from convincing. For example, each of the metals studied by Roberts and Owens (titanium, steel and chrome steel) is capable of forming a diiodide (TiI_2, FeI_2 and even CrI_2). What about substances that do not form diiodides ?

I here summarize findings almost completely at odds with Roberts and Owens's theory.

(1) The formation of a diiodide layer is not essential for an antifriction or antiwear effect of iodine as a lubricant additive. (2) The iodine need not be in the form of a charge–transfer complex with an aromatic compound in order to function. (3) The mechanism, although not fully understood, is apparently simpler and does not depend greatly on the characteristics of the rubbing solids. (4) One of the most striking effects of iodine is its action in producing extremely low friction, particularly in steel systems.

I first discovered that minor amounts of elemental iodine in hydrocarbons such as mineral oils are unusually effective in reducing friction with steel-on-steel[9,10]. The primary system was one of pure sliding and relatively high contact stress—a ball-on-cylinder device described in ref. 11. As little as 0·0075 per cent iodine, in either solvated or diatomic form in a mineral oil, reduced friction in this system by well over 90 per cent. Coefficients of friction were reduced from the usual value of about 0·1 to the unusually small value of 0·01; in several cases, to values as low as 0·001. Furthermore, the extent of metallic contact was greatly reduced. Conventional lubricant additives (such as fatty acids, phosphates and halogen compounds) usually reduce friction by no more than 20 to 30 per cent in these conditions. In a few cases, friction reductions of 50 per cent have been observed with ~1 per cent additive; but the large effect of such a low concentration of additive had never been observed. Iodine was much more effective than other halogens, even though FeI_2, $FeBr_2$ and $FeCl_2$ all have layer structures.

Similar effects occurred in other steel systems, including engine friction, gear scoring, and conventional extreme pressure tests. In the high-load gear and extreme pressure tests I believe that the iodine, rather than acting as a classical antiweld agent, may have prevented scuffing by keeping the surface temperatures low through a great reduction in friction.

Solutions of iodine in pure aliphatic hydrocarbons (such as hexadecane) were equally effective; thus aromatic compounds, and therefore charge–transfer complexes, need not be present.

If the layer-lattice theory is correct, iodine should not work with aluminium because the reaction product, AlI_3, is a three-dimensional, non-layer complex. Furthermore, iodine would not be effective with substrates such as gold, glass or polymers, for they do not readily react with iodine and, even if they did, would not be expected to yield layer-lattice structures. For example, although gold does not easily react with iodine, the compounds AuI and AuI_3 do exist; but neither has a layer structure. I therefore studied the action of iodine with other rubbing solids, using the same ball-on-cylinder device.

With aluminium-on-aluminium, the addition of minor amounts (such as 0·075 per cent) of iodine to a white mineral oil usually produced a marked decrease in friction and wear, particularly at higher loads. For example, at a load of 4,000 g, iodine reduced the coefficient of friction from about 0·12 to 0·04 and the wear scar diameter by about 40 per cent.

With gold-on-gold, iodine usually reduced friction by about 85–90 per cent; at greater loads, it increased wear by approximately 15 per cent. But the increase in wear was not responsible for the lower friction through a polishing or geometry effect.

With glass-on-glass, iodine reduced friction by a factor of about three. Furthermore, there was an enormous reduction in wear (see Table 1).

Table 1. INFLUENCE OF I_2 ON FRICTION AND WEAR WITH GLASS-ON-GLASS : BALL-ON-CYLINDER TESTS, 16 MIN AT 120 R.P.M.

Load (g)	I_2 in white oil (per cent)	Coefficient of friction	Wear scar diameter (mm)
30	0	0·060	0·79
	0·75	0·020	0·0*
60	0	0·042	1·08
	0·75	0·015	0·0*
240	0	0·125	2·05
	0·75	0·046	0·0*

* No wear could be detected at × 20 magnification.

I also observed that iodine leads to the formation of an unusual pattern of deposits, consisting of many small dark droplets near the contact region on a ball; the pattern resembles lines of force in an electric or magnetic field. The chemical composition of the droplets has not been determined, but the resinous nature of the deposit may mean that the hydrocarbon is also involved in some way with the film-forming process. Iodine is well known as an effective catalyst for a variety of chemical reactions. One possibility, unproven, is that a gross protective polymeric substance or viscous film is formed in the contact zone.

Sliding contact tests have also been carried out with a polymer-on-polymer (polymethylmethacrylate) system. The effect was basically the same: minor amounts of iodine dissolved in a white oil markedly reduced both friction and wear.

The action of iodine thus seems not to be simply that of a classical halogen-containing extreme-pressure additive. (Most people think that these rather complex organic EP compounds function by decomposing and reacting with rubbing metal surfaces at high temperatures to form metal halides, not necessarily of the layer-lattice type.) The data obtained with elemental iodine dissolved in a hydrocarbon suggest that a less specific and possibly simpler lubrication mechanism may exist. For example, the adsorption of the rather large iodine molecule from an organic solution could conceivably lead to a significant repulsive force between the solids. I am now studying this idea and trying to elucidate the mechanism by which iodine functions as a boundary lubricant.

MICHAEL J. FUREY

Department of Mechanical Engineering,
Virginia Polytechnic Institute,
Blacksburg, Virginia.

Received December 1, 1969.

[1] Roberts, R. W., and Owens, R. S., *Nature*, **200**, 357 (1963).
[2] Roberts, R. W., and Owens, R. S., *Wear*, **6**, 444 (1963).
[3] Bowden, F. P., and Tabor, D., *The Friction and Lubrication of Solids: Part II*, 211 (Oxford University Press, London, 1964).
[4] Roberts, R. W., and Owens, R. S., *Wear*, **7**, 513 (1964).
[5] *Chem. Eng. News*, 53 (Nov. 29, 1965).
[6] Owens, R. S., and Roberts, R. W., *S.A.E. Journal*, **74**, 34 (1966).
[7] *Scientific Lubrication*, **18**, 13 (1966).
[8] Owens, R. S., and Roberts, R. W., *Wear*, **9**, 79 (1966).
[9] Furey, M. J., *Wear*, **9**, 369 (1966).
[10] Furey, M. J., *Hydrocarbon Compositions containing Iodine as an Antifriction Agent*, US Patent, 3,184,409 (filed Jan. 31, 1962; issued May 18, 1965).
[11] Furey, M. J., *A.S.L.E. Trans.*, **4**, 1 (1961).

Charge Transfer Complexes of Tropylium and *N*-Heterocyclic Cations with Aromatic Amines

THE tropylium[1-3] and *N*-heterocyclic cations[4-6] have been shown to act as electron acceptors in forming complexes of the electron–donor–acceptor type with halide ions and aromatic hydrocarbons as donors. Aliphatic amines form adducts of the σ-complex type with *N*-heterocyclic cations[7].

Addition of an aromatic amine to a solution of tropylium fluoroborate, *N*-methylquinolinium or *N*-methylacridinium perchlorate led to the observation of a new, featureless absorption band in the visible. With *p*-phenylenediamine and *p*-toluidine, this band appeared only transitorily, decaying too rapidly for establishment of its position, while *NNN*¹*N*¹-tetramethyl-*p*-phenylenediamine gave immediately the characteristic absorption and electron spin resonance spectrum of Wurster's blue radical cation. Other amines gave rise to more stable absorption bands, the positions of these being given in Table 1. Plotting $\bar{\nu}_{\max}$ for these bands against the ionization potential of the donor for both amine and aromatic hydrocarbon complexes of the tropylium ion and against $\bar{\nu}_{CT}$, the charge transfer (CT) band maximum for complexes of chloranil with the same amines gave good linearity in both plots, thus establishing the CT nature of the transition and the complexes as the electron–donor–acceptor type.

Table 1. CHARGE TRANSFER BAND MAXIMA (NM) FOR AROMATIC AMINE/CATION COMPLEXES

Acceptor Donor	Tropylium	*N*-methyl-quinolinium	*N*-methyl-acridinium
Aniline	520^a		
N-methylaniline	552^b	397^c	500^a (shoulder)
NN-dimethylaniline	571^b	408^d	$537{\cdot}5^a$
NN-diethylaniline	578^b	430^c	570^a
σ-toluidine	$533{\cdot}5^b$		500^a (shoulder)

a, In dichloromethane; *b*, in 50/50 dichloromethane/acetonitrile; *c*, in acetonitrile; *d*, in ethanol.

The CT bands decayed over a period of time, being less stable in solvents of high polarity, which is in agreement with the observation[8] that carbazole complexes of the tropylium ion are stable in methyl cyanide, the addition of methanol or water leading to formation of carbazole radical cations. Preliminary investigation showed that tropilidene is one of the major products of decomposition of the tropylium complexes. Tropylium is known to abstract hydrogen from tertiary aliphatic amines[9] and from methyltropilidene[10], to form tropilidene. The mechanism in these reactions is postulated as hydride transfer. Kosower[11], however, has suggested that where intermediate CT complex formation occurs, hydride transfer, that is, a two electron transfer process, might alternatively be interpreted as two one electron transfers, electron transfer being followed by hydrogen atom transfer. Observation of electron transfer between tropylium and *NNN*¹*N*¹-tetramethyl-*p*-phenylenediamine and the carbazoles to form the corresponding radical cations lends support to a mechanism for the decomposition of tropylium–amine complexes via the electron transferred form of the CT complex.

The strongest evidence for an electron transfer mechanism was found for complexes of *NN*-dimethylaniline (DMA) and *NN*-diethylaniline with the *N*-methylquinolinium ion. Decay of the CT band in these systems was accompanied by the growth of a characteristic spectrum with three bands in the 440–480 mμ region and three bands in the 900–1,100 nm region. This is identical with the spectrum reported[12] for *NNN*¹*N*¹-tetramethylbenzedine (TMB) radical cation. The growth of this spectrum was accompanied by the growth of an electron spin resonance absorption, both being identical to those obtained by bromine oxidation of TMB. DMA also yields TMB and its radical cation on anodic oxidation[13] and we believe that a similar mechanism is probably operative in the decomposition of the complex—that is, formation of DMA (or DEA) radical cation by electron transfer within the complex, followed by tail-to-tail coupling of the radical cation and elimination of two protons to give TMB. The radical cation of TMB is formed by further electron transfer to the acceptor.

$$\mathrm{PhNMe_2 + A^+ \rightarrow \underset{CT\ complex}{PhNMe_2.A^+} \rightarrow Ph\overset{+}{N}Me_2}$$

$$\mathrm{Ph\overset{+}{N}Me_2 \rightarrow Me_2NPhPhNMe_2 + 2H^+ \xrightarrow{A^+} TMB^+}$$

Decomposition of the tropylium complexes may occur by a slightly different route, hydrogen atom transfer from the *N*-alkyl group of the amine to the intermediate tropyl radical giving tropilidene. No electron spin resonance evidence was found for the intermediate arylamine radicals or for the tropyl radical, but this is in accord with the lack of electron spin resonance evidence for these intermediates in anodic oxidation or oxidative dimerization of aromatic amines[14]. We feel that the evidence, although of a preliminary nature, places these reactions in the group where a CT complex plays a definite part as intermediate in the chemical reactions.

T. G. BEAUMONT
K. M. C. DAVIS

Department of Chemistry,
University of Leicester,
Leicester LE1 7RH.

Received December 9, 1969.

[1] Feldman, M., and Winstein, S., *J. Amer. Chem. Soc.*, **83**, 3338 (1961).
[2] LeGoff, E., and LaCount, R. B., *J. Amer. Chem. Soc.*, **85**, 1354 (1963).
[3] Cummings, F. E., Davis, D. A., Diestler, D. J., and Harmon, K. M., *J. Amer. Chem. Soc.*, **84**, 120 (1962).
[4] Mason, S. F., *J. Chem. Soc.*, 2437 (1960).
[5] Nasielski, J., and Van der Donckt, E., *Theor. Chim. Acta*, **2**, 22 (1964).
[6] Briegleb, G., Jung, W., and Herre, W., *Z. Phys. Chem.*, **38**, 253 (1963).
[7] Briegleb, G., Liptay, W., and Jung, W., *Z. Naturforsch.*, **196**, 97 (1964).
[8] Ledwith, A., and Sambhi, M., *Chem. Commun.*, 64 (1965).
[9] McGeachin, S. G., *Canad. J. Chem.*, **47**, 151 (1969).
[10] Conrow, K., *J. Amer. Chem. Soc.*, **83**, 2343 (1961).
[11] Kosower, E., *Progress in Physical Organic Chemistry*, **3**, 81 (1965).
[12] Takemoto, K., Matsusaka, H., Nakayama, S., and Suzuki, K., *Bull. Chem. Soc. Japan*, **41**, 764 (1968).
[13] Bacon, J., and Adams, R. N., *J. Amer. Chem. Soc.*, **90**, 6596 (1968).
[14] Effenberger, F., Stohrer, W. D., and Steinbach, A., *Angew. Chemie Internat. Edn.*, **8**, 280 (1969).

Effects due to Non-equilibrium *p*H Conditions in the Radiolysis of Aqueous Systems

DURING studies of the pulse radiolysis of aqueous solutions of pyrimidines and related substances, we have observed the formation of short-lived organic species which are formed by reactions other than those of the free radicals H, OH and e^-_{aq} produced by the radiolysis of the solvent. On the basis of the evidence presented here, we believe that these molecular transient species are the result of acid-base reactions between the pyrimidines and the radiolytic yield of hydroxide ions. These processes, although unlikely to lead to permanent products, are important in the pulse technique where the correct interpretation of a transient absorption is vital.

Nitrous oxide saturated solutions of thymine (T) (10–600 μM) were irradiated with 0·1–2 μs pulses of electrons (2–4 MeV), typical doses being 1 krad/pulse. In these conditions, the primarily produced solvated electrons are converted to OH radicals according to $N_2O+e^-_{aq}\xrightarrow{H_2O}N_2+OH+OH^-$, thus effectively producing a one-radical system containing OH. The absorption spectrum of the OH adduct to thymine (represented as TOH) was studied between *p*H 5·5 and *p*H 7·5, usually in the presence of Sørensen phosphate buffer. The transient absorption in solutions at *p*H 5·5 (Fig. 1) decays by a second-order process, attributable to the reaction $2TOH\rightarrow$products. On increasing the *p*H of the solutions, however, an additional component became evident; it absorbed in the region 270–320 nm and decayed rapidly in an apparently first order process ($\tau_{\frac{1}{2}}\approx 3$ μs); the residual absorption then decayed by the second order process observed at lower *p*H. The initial decay appears as a "spike" on the oscillogram (Fig. 2). A plot of the absorption at 300 nm and at 400 nm as a function of *p*H is given in Fig. 3. No spike was observed in solutions of $pH > pK$ of the base (~ 10).

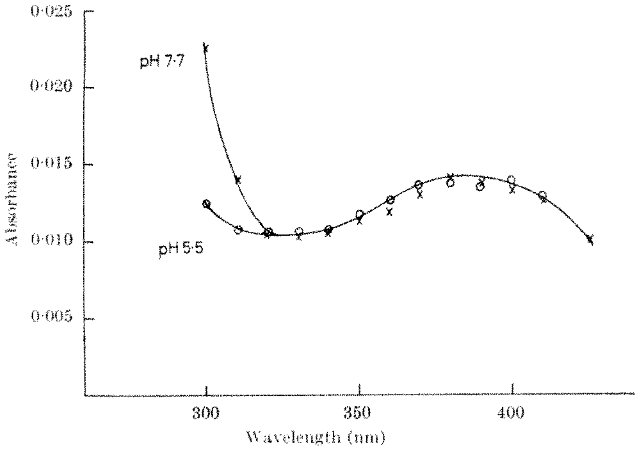


Fig. 1. Transient absorption spectra produced at *p*H 7·7 and 5·5 in 6×10^{-4} M thymine (N_2O saturated) with 4×10^{-3} M phosphate buffer by a 1·6 krad pulse of radiation.

A similar phenomenon was observed in uracil–N_2O solutions, but not in systems containing either thymidine, thymidylic acid, 1:3 dimethyl uracil, 1 methyl thymine or 2,4-dimethoxy uracil. Addition to thymine–N_2O solutions of excess of the OH radical scavenger diethyl ether, so that all the OH radicals reacted with the ether, resulted in a suppression of TOH (at 400 nm) but left the spike unaffected. It follows that the spike is not the result of OH radical reaction with the pyrimidine. Similarly, H atoms are not responsible because their rates of reaction with pyrimidines are at least one order of magnitude less than those of OH.

These experimental facts can be adequately accounted for by ionization of the pyrimidines on reaction with the radiolytically produced hydroxide ions. After a 0·2 μs pulse delivering a dose of 1·5 krad to a solution saturated with N_2O, the ion yields are: $[OH^-]=[H_3O^+]\simeq 5\times 10^{-6}$ M (ref. 1). The rate of reaction of the hydroxyl ion with pyrimidines is fast[2], for example, $k_{OH^-+\text{uracil}}=9{\cdot}9\times 10^9$ $M^{-1}\ s^{-1}$. In the competition (PyH = pyrimidine)

$$OH^-+PyH\rightarrow Py^-+H_2O \qquad (1)$$

$$OH^-+H_3O^+\rightarrow 2H_2O \qquad (2)$$

and given[3] that $k_2=1{\cdot}4\times 10^{11}\ M^{-1}\ s^{-1}$, therefore, it follows that a pyrimidine at a concentration of 10^{-4} M can compete equally with hydrogen ions for a hydroxyl ion. Re-establishment of the equilibrium will occur through the reverse of reaction (1); for uracil ($pK=9{\cdot}3$) this pseudo first-order process should have a half life of 3 μs. Thus if reaction (1) leads to a change in the spectral characteristics of the pyrimidine, the transient existence of P_y^- should be directly observable in the above pulse radiolysis experiments. Such spectral changes around 300 nm are known to occur in the ionization of thymine and uracil[4]; on the other hand, negligible changes occur with thymidine, thymidylic acid, 1 : 3 dimethyl uracil, 1 methyl thymine and 2,4-dimethoxy uracil. Assuming that the spikes in the irradiated thymine and uracil solutions are due solely to Py^-, then from known spectra[4] one can predict the spectral changes in the range 280–320 nm. Fig. 4 compares the predicted spectrum in the pulsed thymine solutions with the experimental points for the spectrum of the spike; the agreement is excellent.

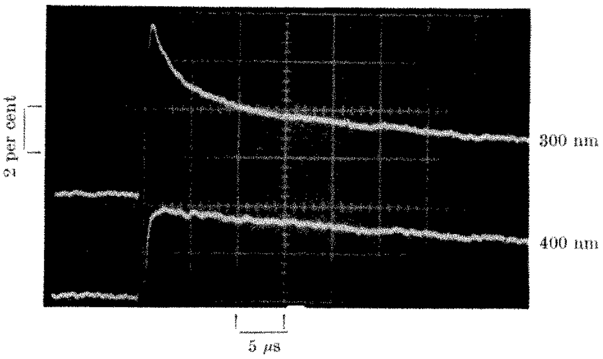


Fig. 2. Oscillograms of the transient absorption produced at *p*H 7 in 5×10^{-4} M thymine (N_2O saturated) by a 0·2 μs pulse of 2 krads. Upper trace at 300 nm showing the "spike"; lower trace at 400 nm.

In solutions containing phosphate buffer the following reactions of OH^- can also occur

$$OH^-+H_2PO^-{}_4\rightarrow HPO_4^{=}+H_2O \qquad (3)$$

$$OH^-+HPO_4^{--}\rightarrow PO_4^{\equiv}+H_2O \qquad (4)$$

Reaction (4) has a rate constant of $2\times 10^9\ M^{-1}\ s^{-1}$ (ref. 2). It is reasonable to assume that $k_3>k_4$, so that the observed suppression of the spike by phosphate buffer should be more marked at lower *p*Hs. This would explain the suppression of the spike on lowering the *p*H to 6·5 in the presence of 4×10^{-3} M phosphate buffer shown in Fig. 3.

The reaction of radiation-induced non-equilibrium quantities of hydroxyl ions with pyrimidines has been

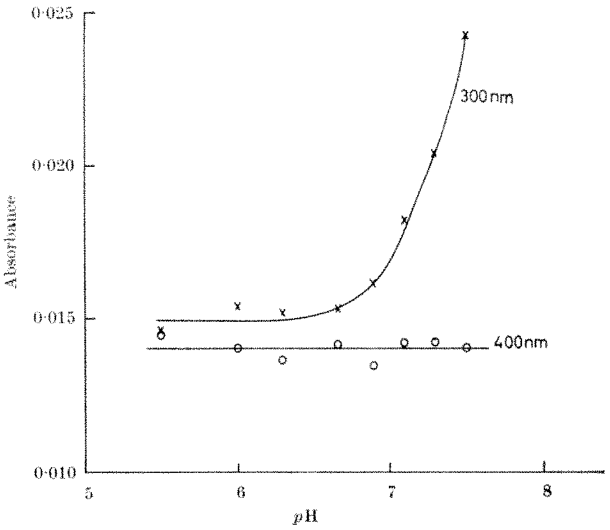


Fig. 3. Variation in transient absorption at 300 and 400 nm with *p*H. Solution irradiated as for Fig. 1.

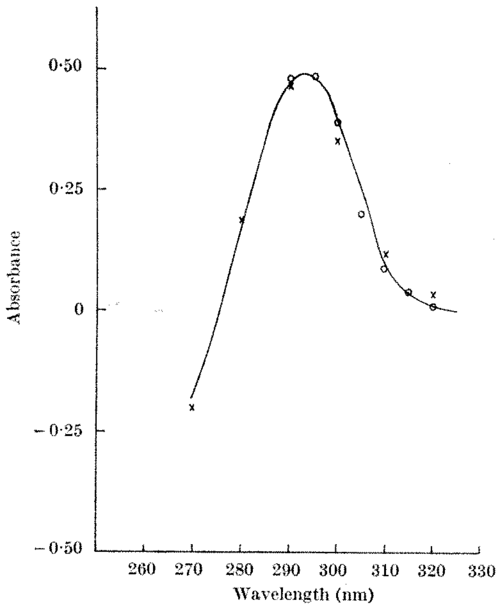


Fig. 4. Observed spectrum of the "spike" (experimental points) obtained in thymine solutions and the difference spectrum between equal quantities of thymine and its ionized form (solid curve). The curve was normalized to the experimental data at 290 nm.

clearly demonstrated. Such a phenomenon should be quite general and should occur wherever the ionization of an acid (or protonation of a base) occurs fast enough, and this will be observable spectrophotometrically where there are appreciable spectral differences between the conjugate acid and base forms of the solutes. Further, the possibility also exists for the occurrence of analogous reactions in non-aqueous systems.

E. M. Fielden
G. C. Stevens

Department of Physics,
Institute of Cancer Research,
Belmont, Sutton,
Surrey.

J. M. Phillips
G. Scholes

Laboratory of Radiation Chemistry,
University of Newcastle upon Tyne.

R. L. Willson

BECC Unit of Radiobiology,
Mount Vernon Hospital,
Northwood,
Middlesex.

Received November 5; revised December 17, 1969.

[1] Schmidt, K. H., and Ander, S. M., *J. Phys. Chem.*, **73**, 2846 (1969).

[2] Eigen, M., Kruse, W., Maass, G., and DeMaeyer, L., *Prog. Reaction Kinetics*, **2**, 285 (1964).

[3] Eigen, M., and DeMaeyer, L., *Z. Elektrochem.*, **59**, 986 (1955).

[4] Shugar, D., and Fox, J., *J. Biochem. Biophys. Acta*, **9**, 199 (1952).

Radiation Grafting of Polysulphides to Wool

Successful attempts to graft polysulphide polymers to wool have recently been made at this laboratory.

Interest in this kind of grafting was originally aroused by certain similarities in structure between the two—namely, disulphide bonds and, to a lesser extent, thiol terminal groups. It was hoped that a radiation-induced wool–polymer graft might show better mechanical properties than untreated wool. The mechanical properties we examined were load-extension and felting. Felting is the progressive fibre entanglement in wool resulting from mechanical agitation, particularly in the presence of water.

Polysulphide polymers are produced by reacting organic dihalides with sodium polysulphide[1-3]. The high molecular weight polysulphides formed can be converted to low molecular weight liquid polymers with thiol terminals by reductive cleavage of some of the disulphide bonds. The liquid product can be reconverted to rubbers of high molecular weight by a wide variety of methods.

The polysulphide polymers we used were manufactured by Thiokol Chemical Corporation of Trenton, New Jersey. The polymers chosen as representative of the range available were Thiokol LP-8 (ref. 4), Thiokol LP-33 (ref. 5) and Thiokol LP-32 (ref. 6), which have average molecular weights of 600, 1,000 and 4,000 respectively. These polymers are composed of 98 per cent (mole) dichloroethyl formal and 2 per cent (mole) trichloropropane. The finally cured product thus consists of long linear chains held together by the small amount of trifunctionality placed in the polymer by the trichloropropane.

Samples of 64s quality (denoting a mean fibre thickness of 20·6–22·0 µm) merino fleece and woollen cloth woven from 64s quality merino wool were irradiated with gamma rays from cobalt-60. The wool was immersed in a solution consisting of polymer, solvent and swelling agent during the irradiation. This is similar to the system of Stannett *et al.*[7] except that we used polymer instead of monomer. The concentrations were: polymer 3 to 5 per cent by volume; water, as swelling agent, up to 10 per cent by volume. Irradiations were carried out under nitrogen and in the presence of air at dose rates up to 100 krads/h. Solvents included dioxan, pyridine and dimethylsulphoxide, which dissolve the polymers and at the same time make it possible to add a certain amount of water before polymer is precipitated.

In each case it was found that the amount of polymer grafted to the wool was smaller when the irradiations were carried out under nitrogen than in the presence of air. With pyridine as solvent, no polymer at all was grafted under nitrogen. For absorbed doses of 20 to 30 Mrads and water concentrations of 10 per cent, maximum weight increases of 2 per cent and 12·7 per cent resulted for irradiations carried out under nitrogen and in air respectively.

Load-extension curves up to 30 per cent wet extension were obtained for untreated single fibres. Polymer was then grafted to these calibrated single fibres and the load-extension characteristics were re-examined. A steady decline in mechanical strength had taken place as the radiation dose and the grafted polymer had accumulated. Similarly calibrated fibres, however, irradiated in the same solvent–water solution but without polymer, showed a much sharper decline in mechanical strength, implying that the added polymer had contributed towards improving the mechanical strength or had partially protected the fibre from radiation damage. Thiols are effective protective agents in irradiations of chemical systems.

The felting tendency of the polymer grafted wool was greater than that of untreated wool, increasing with the radiation dose.

We found that the added polymer was chemically bonded to the treated wool, using Simpson's method[8]: the wool–polymer combination was reduced and alkylated[9], and then digested in pronase[10], a protease from *Streptomyces griseus*. If the polymer were chemically bonded to the wool, polymer with amino-acid residues attached would be likely to remain. Solvent extraction of this material and subsequent hydrolysis release some or all of these residues for amino-acid analysis. All radiation grafted samples so treated gave evidence of the presence of amino-acids in these hydrolysates, showing that there was chemical combination of polymer with the wool.

C. SAMWAYS
G. W. HASTINGS

Department of Polymer Science,
School of Chemical Technology,
University of New South Wales,
Kensington,
New South Wales, Australia.

Received September 9; revised December 8, 1969.

[1] Martin, jun., S. M., and Patrick, J. C., *Ind. Eng. Chem.*, **28**, 1145 (1936).
[2] Patrick, J. C., *Trans. Faraday Soc.*, **32**, 347 (1936).
[3] Fettes, E. M., and Jorczak, J. S., *Ind. Eng. Chem.*, **42**, 11 (1950).
[4] Thiokol Chemical Corp., *Bulletin No. LP-5* (May 1961).
[5] Thiokol Chemical Corp., *Bulletin No. LP-4* (May 1961).
[6] Thiokol Chemical Corp., *Bulletin No. LP-3* (July 1961).
[7] Stannett, V., Araki, K., Gervasi, J. A., and McLeskey, S. W., *J. Polymer Sci.*, *A***3**, 3763 (1965).
[8] Simpson, I., thesis (1968).
[9] Maclaren, J. A., *Austral. J. Chem.*, **15**, 824 (1962).
[10] Springell, P. H., *Austral. J. Biol. Sci.*, **16**, 727 (1963).

Piezoelectric Resonances in Amino-acids

CRYSTALS of amino-acids may exhibit the property of piezoelectricity[1-4] and, like other crystals, may belong to one of the twenty-one so-called piezoelectric crystalline classes[5]. Our particular interest in investigating piezoelectric resonances in amino-acids was in connexion with our current study of nuclear quadrupole resonances (NQR). For this purpose, it is preferable to choose non-piezoelectric amino-acids so that there is no interference in the detection of the quadrupole spectra[6,7].

The results of our measurements on amino-acids[8-11] are given in Table 1. Experiments are performed on powders, using a superregenerative spectrograph[12] of NQR type[13] specially adapted for this work[14]. We have studied principally the L isomers of amino-acids, the only ones occurring in proteins.

Table 1. COMPARISON OF EXPERIMENTAL AND THEORETICAL PIEZOELECTRICITY FOR AMINO-ACIDS

Amino-acid	Spatial group	Crystalline class	Theoretical piezo-electricity[16-19]	Experimental piezo-electricity
L-Alanine	$P2_12_12_1$	222	+	+
D-Alanine				+
DL-Alanine	Pna	mm2	+	+
β-Alanine	Pbca	mmm	–	–
L-Arginine·2H$_2$O	$P2_12_12_1$	222	+	–
D-Arginine·HCl				+
L-Asparagine·H$_2$O	$P2_12_12_1$	222	+	+
L-Aspartic acid	$P2_1$	2	+	+
DL-Aspartic acid	I2/a	2/m	–	–
L-Cysteine	$P2_1$	2	+	+
L-Cystine	$P6_122$	622	+	+
DL-Cystine				+
L-Glutamine	$P2_12_12_1$	222	+	–
L-Glutamic acid	$P2_12_12_1$	222	+	–
D-Glutamic acid				–
Glycine	See Table 2			+
L-Histidine				+
DL-Histidine				–
L-Hydroxyproline	$P2_12_12_1$	222	+	+
L-Isoleucine				+
D-Isoleucine				+
L-Leucine				+
D-Leucine	$P2_12_12_1$	222	+	–
DL-Leucine	P1	1	+	–
L-Lysine·HCl·2H$_2$O	$P2_1$	2	+	+
L-Methionine				–
DL-Methionine	See Table 2			–
L-Phenylalanine				–
D-Phenylalanine				–
L-Proline	$P2_12_12_1$	222	+	+
DL-Proline				–
L-Serine				+
DL-Serine	$P2_1/a$	2/m	–	+
L-Threonine	$P2_12_12_1$	222	+	+
DL-Threonine		mm2	+	+
L-Tryptophan				–
DL-Tryptophan				–
L-Tyrosine				+
DL-Tyrosine				+
L-Valine	$P2_1$	2	+	–
D-Valine				+
DL-Valine	P1 or $P\bar{1}$	1 or $\bar{1}$	+ or –	–

Table 1 gives for each crystalline compound the spatial group and the corresponding crystalline class in the Hermann–Maugin notation as stated by Gurskaya[16], Derissen *et al.*[17], Harding and Long[18] and Albrecht *et al.*[19]. In the fourth column, a plus sign indicates a piezoelectric class; a minus, a non-piezoelectric one. The last column records our experimental results, a plus sign being assigned for the samples in which piezoelectricity has been identified. Glycine is a special case: it has three crystalline forms (Table 2). Piezoelectricity is detected in the powder[3,8,15], but not in single crystals, in agreement with Guibé's experiments (personal communication). Because the γ-form is the most stable at room temperature[16], it is reasonable to think that glycine powder is in the γ-form and single crystals are in the non-piezoelectric α-form.

Table 2. COMPARISON OF EXPERIMENTAL AND THEORETICAL PIEZOELECTRICITY FOR GLYCINE AND DL-METHIONINE

Amino-acid	Spatial group	Crystalline class	Theoretical piezo-electricity[16]	Experimental piezo-electricity
α-Glycine	$P2_1/n$	2/m	–	–
β-Glycine	$P2_1$	2	+	+ (β or γ)
γ-Glycine	$P3_1$ or $P3_2$	3	+	
α-DL-Methionine	$P2_1/a$	2/m	–	+ (α or β)
β-DL-Methionine	I2/a	2/m	–	

Our experimental results agree well with crystalline studies. In spite of the weakness of the piezoelectric resonances, it was generally possible to confirm this effect in those crystalline classes for which it would be expected. Recent crystallographic results[17,18] confirm our experiments. For DL-methionine, which crystallizes in two forms (Table 2), and DL-serine, experimental piezoelectricity is exhibited in contradiction with the findings on crystal structure. This discrepancy needs to be resolved. In about twenty cases—for lack of crystal data—it is impossible to make a comparison, but we believe our results will be of importance in the assignment of crystal structure. It is interesting to note that of the twenty L-amino-acids we have studied (glycine included), nineteen exhibit theoretical and/or experimental piezoelectricity.

D. VASILESCU
R. CORNILLON
G. MALLET

Laboratoire de Biophysique,
Faculté des Sciences,
Université de Nice.

Received July 17; revised September 3, 1969.

[1] Duchesne, J., and Monfils, A., *CR Acad. Sci.*, **241**, 749 (1955).
[2] Duchesne, J., Depireux, J., Bertinchamps, A., Cornet, N., and Van der Kaa, J., *Nature*, **188**, 405 (1960).
[3] Toulsky, S., and Read, M., *CR Acad. Sci.*, **260**, 7030 (1965).
[4] Toulsky, S., Kukushkin, A., Blyumenfeld, L., and Lomonosov, M., *Molekul. Biophys., Akad. Nauk., SSSR, Inst. Biol. Fiz., Sb. Statei*, 41 (1965).
[5] Cady, W., *Piezoelectricity* (Dover, New York, 1962).
[6] Livingston, R., *Ann. NY Acad. Sci.*, **55**, 800 (1952).
[7] Guibé, L., *CR du 9ème Colloque Ampère, Pisa*, 657 (1960).
[8] Mesnard, G., and Vasilescu, D., *CR Acad. Sci.*, **260**, 4843 (1965).
[9] Vasilescu, D., Mesnard, G., Moussiegt, J., and Cornillon, R., *Abstr. Second Intern. Cong. Biophys.*, Vienna (1966).
[10] Vasilescu, D., Cornillon, R., and Mallet, G., *CR Acad. Sci.*, **265**, 631 (1967).
[11] Cornillon, R., thesis (University of Nice, 1968).
[12] Whitehead, J., *Superregenerative Receivers* (Cambridge University Press, 1950).
[13] Dean, C., *Rev. Sci. Instrum.*, **31**, 934 (1960).
[14] Vasilescu, D., Moussiegt, J., Cornillon, R., Farges, J-P., and Mallet, G., *CR 92ème Congrès des Sociétés Savantes*, Strasbourg (1967).
[15] Mason, W., *Piezoelectric Crystals and their Application to Ultrasonics* (Van Nostrand, New York, 1950).
[16] Gurskaya, G. V., *The Molecular Structure of Amino Acids; Determination by X-Ray Diffraction Analysis* (Consultants Bureau, New York, 1968).
[17] Derissen, J. L., Endeman, H. J., and Peerdeman, A. F., *Acta Cryst.*, **B24**, 1349 (1968).
[18] Harding, M. M., and Long, H. A., *Acta Cryst.*, **B24**, 1096 (1968).
[19] Albrecht, G., Schnakenberg, G., Dunn, M., and McCullough, J., *J. Phys. Chem.*, **47**, 24 (1943).

"Evolution"—"Development"—Anatomical and Cerebral Features and the Pathological Consequences

We recently published papers in the oceanographic field which contained in their titles the words "development"[1], "evolution"[2], "triple junctions"[2] and "fingers"[3]. These key words were dispatched by computers to thousands of child psychiatrists, biologists, neurologists and medical practitioners. Hundreds have requested reprints. We are curious to know how many are going to request a reprint of the present communication on the same basis.

D. Davies*
D. P. McKenzie
Department of Geodesy and Geophysics,
Madingley Rise, Madingley Road, Cambridge.
J. S. Turner
Department of Applied Mathematics and
Theoretical Physics, Silver Street, Cambridge.

Received January 2, 1970.

* Present address: Lincoln Laboratory, Group 22, 42 Carleton Street, Cambridge, Massachusetts 02142, USA.

[1] Davies, D., *Sci. J.*, **5A**, 2, 39 (1969).

[2] McKenzie, D. P., and Morgan, W. J., *Nature*, **224**, 125 (1969).

[3] Turner, J. S., *Deep Sea Res.*, **14**, 599 (1967).

BIOLOGICAL SCIENCES

Prostaglandins—an Experiment

I am writing not about prostaglandins but about requests for reprints of a *Nature* article[1] about them. In the first two months after this was published, I received 615 reprint requests of which 53 per cent came from the USA or Canada. Thirty-eight per cent of these, and 16 per cent of those from other countries, bore the name of the sender only in typewritten or rubber-stamped form. Of the handwritten names, internal evidence suggested that many were not personal signatures.

This kind of experience is doubtless very common. How can one assess the probability that a rubber-stamped card (probably sent by a technician who has scanned *Current Contents* for key-words) indicates anything more than a general interest in a subject and a disinclination to go to the library ? The answer may partially be provided by the response to the present letter. Anyone who reads this is asked not to request a reprint.

V. R. Pickles
Department of Physiology, University College, Cardiff.

Received January 2, 1970.

[1] Pickles, V. R., *Nature*, **224**, 221 (1969).

Absorptivity and Quantum Yield of Bleaching in Bovine Visual Pigment$_{500}$

Visual pigments are conjugated proteins containing either retinal or 3-dehydroretinal as the chromophoric prosthetic group. The molar absorptivity (ε) of the pigments at their absorption maximum in the visible spectrum has been determined either by measuring the retinal or the apoprotein content in a given amount of visual pigment. Knowledge of the molar absorptivity of visual pigments is of practical importance because concentrations are most easily determined by spectrophotometric methods. In addition, this parameter has a theoretical interest in that it enables one to calculate the quantum yield (φ) of bleaching.

Wald and Brown[1] determined the amount of all-*trans* retinal oxime formed from a given amount of illuminated visual pigment after reaction with hydroxylamine and found an $\varepsilon_{500\text{ nm}}$ of 40,600, assuming one chromophore per molecule. A similar value was found by Shichi *et al.*[2] using a chemical determination of the retinal liberated from illuminated visual pigment. The determination[3] of the molar absorptivity ($\varepsilon_{500\text{ nm}}$), however, performed by measuring the amount of protein in purified samples of visual pigment, gave a value of 23,100. A new attempt was therefore made to determine the molar absorptivity of visual pigment by at least two different methods to find the cause of the disagreement.

Table 1. MOLAR ABSORPTIVITY OF BOVINE VISUAL PIGMENT$_{500}$ DETERMINED BY AMINO-ACID ANALYSIS

	$A_{500\text{ nm}}$	μmole protein	$\varepsilon_{500\text{ nm}}$
Sample I	0·417	0·01914	21,786
		0·01924	21,673
Sample II	0·350	0·01638	21,367
		0·01646	21,263
Average			21,524

Native visual pigment$_{500}$ was purified as the cetyltrimethylammonium bromide complex[3]. Duplicate samples of known absorptions obtained from two separately purified visual pigment$_{500}$ preparations were hydrolysed with 6 M HCl for 24 h at 110° in sealed, evacuated tubes and were then analysed on the amino-acid analyser. Assuming a molecular weight of 26,400 for the visual pigment apoprotein, the stable amino-acid residues aspartic acid, glutamic acid, glycine, alanine, leucine and phenylalanine were used to calculate the amount of protein present on the basis of the previously known composition[4]. Independent measurements in several laboratories have led to the conclusion that the molecular weight of visual pigment is indeed around 28,000 (refs. 2–4). The molar absorptivity of the sample could then be calculated in a direct way and the results are shown in Table 1.

Attempts were then made to determine the molar absorptivity as described by Shichi *et al.*[2], who determined the retinal content of visual pigment using the method of Futterman and Saslaw[5]. This method is based on the colorimetric reaction of the aldehyde group of retinal with thiobarbituric acid. Although excellent reproducibility and accuracy were obtained with standard all-*trans* retinal, only very erratic and nonreproducible results were obtained with visual pigment. The $\varepsilon_{500\text{ nm}}$ values determined by this method, using the same pigment samples as were used for amino-acid analysis, ranged from 35,000 to more than 50,000. The variability and low yield of the reaction probably arise because other amino groups of the protein compete for the potential aldehyde group of retinal, as was shown by Ball *et al.*[6] for a series of interactions between amino groups and retinal, and possibly as a result of other effects such as oxidation or reduction of the aldehyde group. All the competing reactions would tend to reduce the colour yield with thiobarbituric acid and thus produce an apparent increase in $\varepsilon_{500\text{ nm}}$. Similar considerations may apply to the oxime method[1] of determining the retinal content of visual pigment.

The photosensitivity of visual pigments is defined as the product of the molar absorptivity and the quantum yield of change (εφ). Dartnall *et al.*[7,8] found the photosensitivity of frog visual pigment to be 23,000 and Dartnall[9] later reported that the photosensitivity of a series of visual pigments ranges from 26,300 to 28,500. With the $\varepsilon_{500\text{ nm}}$ value obtained above and with the knowledge that the $\varepsilon_{500\text{ nm}}$ of bovine and frog visual pigment are practically identical[10], it is possible to calculate the quantum yield of bleaching as 1·07 to 1·32 depending on the value of photosensitivity chosen (φ = photosensitivity/ε). Because it seems improbable that the quantum yield is larger than 1·0 in this system, either the molar absorptivity reported here is somewhat too low or the calculated photosensitivity is somewhat too high, or both. Nevertheless, the conclusion derived from these experiments is that the quantum yield of visual pigment$_{500}$ bleaching is either 1·0 or very close to 1·0 and that $\varepsilon_{500\ \mathrm{nm}}$ is between 21,500 and 23,000.

JORAM HELLER

Jules Stein Eye Institute,
UCLA School of Medicine,
Los Angeles, California 90024.

Received August 19; revised October 20, 1969.

[1] Wald, G., and Brown, P. K., *J. Gen. Physiol.*, **37**, 189 (1953).
[2] Shichi, H., Lewis, M. S., Irreverre, F., and Stone, A. L., *J. Biol. Chem.*, **244**, 529 (1969).
[3] Heller, J., *Biochemistry*, **7**, 2906 (1968).
[4] Shields, J. E., Dinovo, E. C., Henricksen, R. A., Kimbel, R. L., and Millar, P. D., *Biochim. Biophys. Acta*, **147**, 238 (1967).
[5] Futterman, S., and Saslaw, L. D., *J. Biol. Chem.*, **236**, 1652 (1961).
[6] Ball, S., Collins, F. D., Dalvi, P. D., and Morton, R. A., *Biochem. J.*, **45**, 305 (1949).
[7] Dartnall, H. J. A., Goodeve, C. F., and Lythgoe, R. J., *Proc. Roy. Soc.*, A, **156**, 158 (1936).
[8] Dartnall, H. J. A., Goodeve, C. F., and Lythgoe, R. J., *Proc. Roy. Soc.*, A, **164**, 216 (1938).
[9] Dartnall, H. J. A., *Vision Res.*, **8**, 339 (1968).
[10] Heller, J., *Biochemistry*, **8**, 675 (1969).

Linkage of Retinal to Opsin and Absence of Phospholipids in Purified Frog Visual Pigment$_{500}$

VISUAL pigments are components of the membranous disks of the rod and cone outer segments of vertebrates[1]. The traditional picture of visual pigment$_{500}$ (rhodopsin) is a chromophore (retinal) bound to opsin through a Schiff base linkage[2,3]. A role has long been sought for the large quantities of phospholipids present in outer segment disks, however, and it has been proposed that, in native visual pigment, retinal is bound by a Schiff base linkage to phosphatidyl ethanolamine (PE) rather than to opsin[4-6]. When exposed to light, retinal is considered to migrate from PE to the ε-amino group of a lysine residue in opsin, but Heller[7] has found that purified visual pigment contains little or no phospholipid (< 2 per cent). The following experiments are intended to resolve this contradiction; we have used the recent observation that visual pigment in the outer segment disks is continually being renewed[8,9], to show that purified visual pigment contains no phospholipid.

Two groups of fourteen frogs (*Rana pipiens*) were injected intravenously with 8·3 μCi/g body weight of sodium ^{32}P-orthophosphate. Group 1 was killed three days after injection and group 2 six days after. The rod outer segments were prepared under dim red light by the method of Heller[7], and the final pellet from both groups was treated identically. One third of the rod outer segment pellet was extracted with cetyltrimethylammonium bromide and the visual pigment was purified in the dark on an agarose column[7,8]. The absorptivity of the effluent was monitored at 280 nm and 500 nm. Radioactivity in each tube was measured and corrected for decay and quenching. The results are shown in Figs. 1*a* and *b*. The remaining two-thirds of the pellet was exposed to light, homogenized in 2 : 1 chloroform–methanol mixture and used for extraction of total lipids. The residue was extracted successively with 5 ml. aliquots of the following solvents: chloroform–methanol, 2 : 1 (twice); ethanol (twice); chloroform–methanol–conc. HCl, 200 : 100 : 1 (twice); and chloroform–methanol, 2 : 1 (three times). The extracts were combined, taken to dryness and dissolved in 3 ml. of chloroform–methanol, 2 : 1. This solution was extracted three times with 2 ml. aliquots of 0·73 per cent NaCl. The organic phase was dried under nitrogen and then under vacuum overnight. The dried residue was weighed (Table 1) and dissolved in 2 ml. of chloroform. Phospholipids in the extract were separated by DEAE-cellulose chromatography[10]. The purity of the PE and phosphatidyl choline (PC) fractions was verified on thin layer chromatograms (silica gel G)[11]. Radioactivity on the strips was located using a chromatogram scanner. The PE and PC samples prepared by this method showed no contamination by other phospholipids, or by other radioactive compounds. The purified samples of PE and PC were taken to dryness under nitrogen and dissolved in 1 ml. of chloroform. Duplicate aliquots were taken for the measurement of radioactivity and for phosphorus analysis[12]. No inorganic orthophosphate was present in these phospholipid samples. After correction for decay and quenching, the specific activity (d.p.m./μmole P) of the phospholipid phosphorus could be calculated for the PE and PC. These results are shown in Table 1.

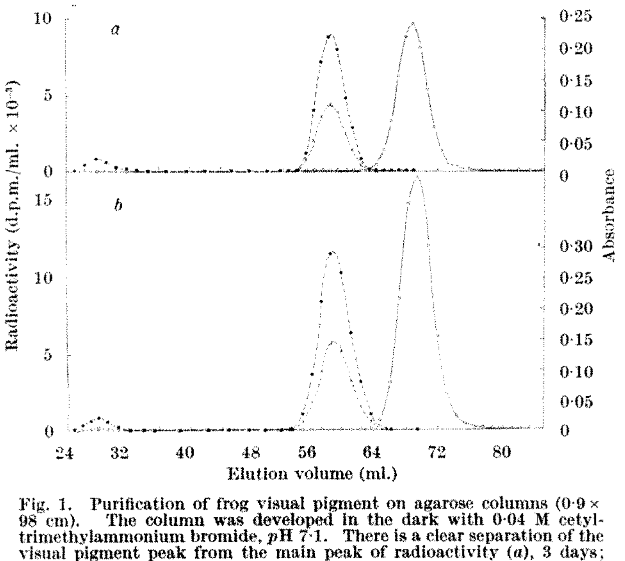


Fig. 1. Purification of frog visual pigment on agarose columns (0·9 × 98 cm). The column was developed in the dark with 0·04 M cetyltrimethylammonium bromide, *p*H 7·1. There is a clear separation of the visual pigment peak from the main peak of radioactivity (*a*), 3 days; (*b*), 6 days. ● - - - ●, A_{280}; × - - - ×, A_{500}; ○—○, d.p.m.

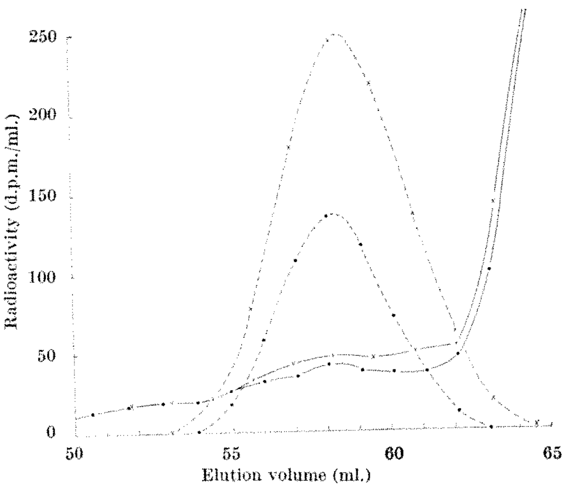


Fig. 2. Expanded scale plot of a portion of Fig. 1*a* and *b*, showing the comparison between observed d.p.m./ml. (———) and theoretical d.p.m./ml. (- - -) across the visual pigment peaks after agarose column purification. ●, Group 1; ×, group 2.

Figs. 1*a* and *b* show that agarose gel filtration completely separates the visual pigment and the main peak of radioactivity. The small amount of radioactive contamination of the visual pigment is shown in Fig. 2, and is discussed below.

From the known ε_{500} of frog visual pigment (23,100) (ref. 13) and the measured A_{500} nm of the purified visual

Table 1. SPECIFIC ACTIVITIES OF PE AND PC ISOLATED FROM FROG RETINAL ROD OUTER SEGMENTS

	Dry wt of total lipid	Specific activity (d.p.m./μmole P)	
		PE	PC
Group 1	7·85 mg	27,800	26,200
Group 2	8·13 mg	39,000	39,400

pigment fractions, it is possible to calculate the μmoles of visual pigment/ml. in each tube across the visual pigment peak (Figs. 1*a* and *b*). It has recently been directly demonstrated that one mole of retinal is bound per mole of visual pigment[14]. If retinal is bound to PE in native visual pigment, as proposed by Poincelot *et al.*[4], there must be a minimum of one mole of PE phosphorus/mole of visual pigment across the chromatographic peak. Because the specific activity of the PE phosphorus is known (Table 1), it is possible to calculate the theoretical d.p.m./ml. in each tube across the visual pigment peak.

In Fig. 2, elution profiles of the visual pigment isolated from both groups of frogs are plotted on an expanded scale to show the observed d.p.m./ml. across the respective visual pigment peaks and the theoretical d.p.m./ml. are plotted on the same scale. It can be seen that only 19 per cent (group 2) or 31 per cent (group 1) of the expected disintegrations are actually present in the visual pigment at the elution peaks: if the radioactivity found in the visual pigment fraction is not all due to PE, the discrepancy would be even greater. (Using 40,600 as the ε_{500} of frog visual pigment[15], then only 33 per cent (group 2) or 54 per cent (group 1) of the expected disintegrations are found.) To determine whether the low levels of radioactivity in the visual pigment were actually due to phospholipid, the tubes across the visual pigment peaks, from both groups of animals, were combined and lyophilized. The residue was exhaustively extracted for lipids, RNA and DNA[16]. The percentage of the total radioactivity extracted into each fraction was: lipid extract, 0 per cent; RNA extract, 12 per cent; DNA extract, 9 per cent; residue, 74 per cent.

The absorptivity of each nucleic acid extract was measured at 260 nm and 280 nm. Because these extracts showed no absorption at either wavelength, it is clear that the contaminating radioactivity was not caused by nucleic acids. The remaining 74 per cent of the radioactivity present in the residue is probably ^{32}P adsorbed onto the protein. Because no radioactivity was found in the lipid extract, it can be concluded that the small amount of radioactivity present in the purified visual pigment was not due to phospholipid.

It has been demonstrated that purified visual pigment contains no phospholipid and that retinal cannot be bound to PE in native visual pigment. The chromophore must therefore be bound covalently to opsin—probably to the ε-amino group of a lysine residue, as suggested by Bownds[2].

We thank Mr Dennis Muehlenbach for expert technical assistance. This investigation has been supported by a research grant from the National Institutes of Health, US Public Health Service, and a Fight-For-Sight grant-in-aid from the National Council to Combat Blindness, Inc., New York, NY.

MICHAEL O. HALL
A. D. E. BACHARACH

Department of Ophthalmology,
Jules Stein Eye Institute,
UCLA School of Medicine,
Los Angeles, California.

Received September 2; revised October 20, 1969.

[1] Dewey, M. M., Davis, P. K., Blasie, J. K., and Barr, L., *J. Mol. Biol.*, **39**, 395 (1969).
[2] Bownds, D., *Nature*, **216**, 1178 (1967).
[3] Akhtar, M., Blosse, P. T., and Dewhurst, P. B., *Biochem. J.*, **110**, 693 (1968).
[4] Poincelot, R. P., Millar, P. G., Kimbel, R. L., and Abrahamson, E. W., *Nature*, **221**, 256 (1969).
[5] Daemen, F. J. M., and Bonting, S. W., *Nature*, **222**, 879 (1969).
[6] Fukami, I., and Fukami, Y., *Nature*, **223**, 63 (1969).
[7] Heller, J., *Biochemistry*, **7**, 2906 (1968).
[8] Hall, M. O., Bok, D., and Bacharach, A. D. E., *Science*, **161**, 787 (1968).
[9] Hall, M. O., Bok, D., and Bacharach, A. D. E., *J. Mol. Biol.* (in the press).
[10] Rouser, G., Galli, C., Lieber, E., Blank, M. L., and Privett, O. S., *J. Amer. Oil Chem. Soc.*, **41**, 836 (1965).
[11] Wagner, H., Horhammer, L., and Wolff, P., *Biochem. Z.*, **334**, 175 (1961).
[12] Bartlett, G. R., *J. Biol. Chem.*, **234**, 466 (1959).
[13] Heller, J., *Biochemistry*, **8**, 675 (1969).
[14] Heller, J., *Biochemistry*, **7**, 2914 (1968).
[15] Wald, G., and Brown, P. K., *J. Gen. Physiol.*, **38**, 189 (1953–54).
[16] Hess, H. H., and Thalheimer, C., *J. Neurochem.*, **12**, 193 (1965).

Relation of Intracellular Ca^{2+} to Retention of K^+ by Liver Slices

CALCIUM ions are necessary for the maintenance of the normal, low permeability of cell plasma membranes[1-3]: if animal cells are incubated in media free of Ca^{2+} the passive permeability of the membranes increases, and the cells are unable to maintain their normal concentration gradients of K^+ and Na^+ (refs. 4, 5). Recent work has suggested, however, that a raised level of intracellular Ca^{2+} specifically increases the passive permeability of erythrocyte membranes to K^+. Liver cells possess an energy dependent mechanism which can bring about the net extrusion of Ca^{2+} that has accumulated during incubation at 1° C (refs. 7, 8). It was therefore of interest to examine the relationship between the lowering of intracellular Ca^{2+} thus produced and the accompanying recovery of the K^+ and Na^+ composition of the cells.

Liver slices were prepared from well fed, male albino rats and incubated for 90 min at 1° C in phosphate or bicarbonate buffered media. They were then transferred to Warburg manometric vessels which, after being flushed with O_2 or 95 per cent O_2–5 per cent CO_2, were placed in a bath maintained at 38° C. Slices were removed at intervals and their ionic contents determined by atomic absorption spectrophotometry (for further details, see refs. 8, 9). Results are related to the fat-free dry weight of the slices, and are expressed as mean ± *S.E.*

The time-course of the net, active movements of cations in slices incubated at 38° C in bicarbonate medium (Fig. 1) indicates that a net extrusion of Na^+ and Ca^{2+} began within the first sampling period (3 min) but, as noted previously[9,10], accumulation of K^+ was delayed by 10–15 min. In view of the coupling between Na^+ and K^+ transport in rat liver[9,11], the failure to observe an immediate K^+ accumulation seems to be the result of an inability of the cells to retain the K^+ transported inwards in exchange for Na^+, rather than a failure of the transport itself. There was a coincidence between the time at which the

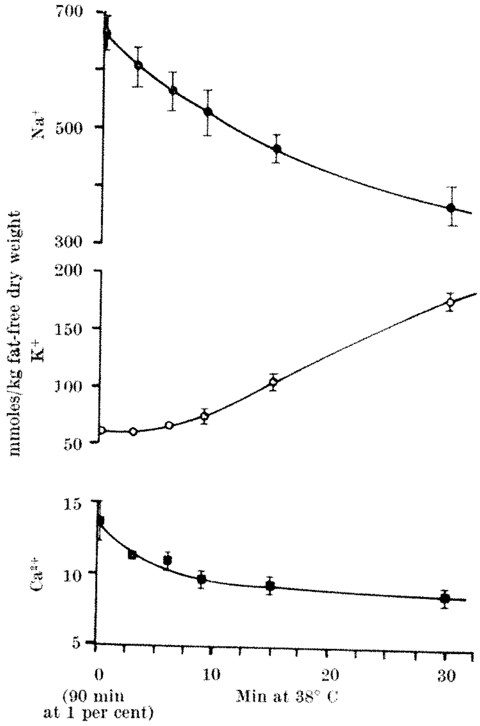


Fig. 1. Changes in cation content of liver slices during incubation at 38° C. Slices were incubated in bicarbonate medium at 1° C for 90 min, followed by incubation at 38° C for the times indicated. The medium contained (mM): Na^+ 161·5, K^+ 5·0, Ca^{2+} 1·3, Mg^{2+} 1·0, Cl^- 147·5, HCO_3^- 25·0, SO_4^{2-} 1·0, phosphate 2·0; it was gassed with 95 per cent O_2–5 per cent CO_2; *p*H was 7·4. Each point is the mean of five–seven observations.

net extrusion of Ca^{2+} was largely completed, and that at which net accumulation of K^+ started to occur. One possible explanation for this, which is suggested by Whittam's results[6], is that the initial high intracellular Ca^{2+} concentration was the cause of the failure of K^+ retention, K^+ becoming more efficiently retained as the Ca^{2+} concentration fell.

If this explanation is correct, the lag in K^+ accumulation should not be seen in slices which have been allowed to complete the extrusion of Ca^{2+} before the coupled Na^+–K^+ transport system is activated. It should be noted that the mechanisms for Ca^{2+} transport and for Na^+–K^+ transport seem to be independent in this tissue[8]. Slices were incubated at 1° C in K^+-free bicarbonate medium which was changed several times during the 90 min incubation to remove as much endogenous K^+ as possible from the system. To some flasks (controls), K^+ was tipped from the side-arms at the moment of being transferred to the bath at 38° C, to activate the Na^+–K^+ transport; its concentration in the medium was 5 mM. In other flasks (experimental), this transport was only activated by tipping K^+ after incubation at 38° C had proceeded for 20 min, that is, after Ca^{2+} extrusion was complete. Fig. 2 shows that the experimental slices accumulated a considerable amount of K^+ within 5 min after K^+ addition, and there was no indication of a lag period. At 10 min the net accumulation of intracellular K^+ in these slices (67 ± 14 mmoles/kg fat-free dry weight) was significantly higher than it was in the controls 10 min after the simultaneous addition of K^+ and start of incubation at 38° C (29 ± 2 mmoles/kg, $P < 0.05$; in calculating these values, an allowance was made for extracellular K^+ on the basis of the inulin-space measurements of Elshove and myself).

By contrast with these experiments, in which the tissue Ca^{2+} was lowered by active transport, the more drastic depletion of Ca^{2+} caused by incubation in a Ca^{2+}-free medium, especially with the addition of 2 mM EDTA, resulted in a failure of the liver slices to restore the maximal gradients of Na^+ and K^+ after much longer times (70 min) at 38° C (see also ref. 4). This effect must be largely the result of an increased passive permeability of the cells in the absence of medium Ca^{2+} (ref. 5).

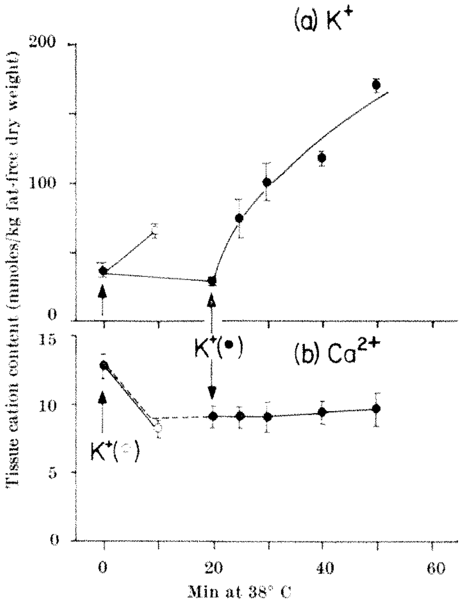


Fig. 2. Effect of K^+ addition on active movements of K^+ and Ca^{2+} in liver slices incubated at 38° C. Conditions as for Fig. 1, except that the incubation was carried out in K^+-free bicarbonate medium until the addition of K^+ (5 mM) at the times indicated by the arrows. Each point is the mean of six–eight observations. ○, K^+ added to medium after 0 min at 38° C; ●, K^+ added to medium after 20 min at 38° C.

The temporal relationship between Ca^{2+} extrusion and the onset of K^+ accumulation (Figs. 1 and 2) suggests that the initial failure to retain K^+ may be attributed in some way to the high intracellular Ca^{2+} concentration. This suggestion is further supported by the work of Judah and Ahmed[4], who found that the accumulation of K^+ in 20 min by slices recovering from pre-incubation in low-Na^+ media varied inversely as the concentration of Ca^{2+} in the pre-incubation medium (and so, presumably, as the initial intracellular Ca^{2+}). By contrast, however, the failure of K^+ retention in the presence of high intracellular Ca^{2+} does not result from the inhibition of the Na^+–K^+ transport mechanism by Ca^{2+}, for in the present work Na^+ extrusion at 38° C proceeded without a delay period. For the same reason, an inhibitory effect of Ca^{2+} on the energy supply for alkali-cation transport (for example, by uncoupling oxidative phosphorylation) can be excluded in these conditions (but see ref. 12). The effect of intracelluar Ca^{2+} would therefore seem to arise either from some action at a K^+-retaining site within the cell (for example, neutralization by Ca^{2+} of anionic binding sites for K^+), or from a Ca^{2+} induced increase in the passive permeability of the plasma membrane towards K^+ efflux (as indicated by Whittam[6]). The mechanism is not clear at present, but because a general deprivation of tissue Ca^{2+} (as in Table 1) leads to increased passive permeability, the Ca^{2+} the removal of which by active transport would result in a decreased passive permeability must be a rather specific fraction of the total cellular content of this cation.

Table 1. EFFECT OF Ca^{2+}-FREE MEDIUM ON THE CATION COMPOSITION OF RAT LIVER SLICES INCUBATED AT 38° C

Medium (mM)		Tissue content (mmoles/kg fat-free dry weight)					
		Ca^{2+}		K^+		Na^+	
Ca^{2+}	EDTA	1°	38°	1°	38°	1°	38°
1·3	0·0	13·5 ± 0·3 (8)	10·7 ± 0·5 (15)	73 ± 6	305 ± 5	939 ± 34	474 ± 19
0·0	0·0	—	2·6 ± 0·2 (8)	—	147 ± 17	—	606 ± 44
0·0	2·0	2·8 ± 0·5 (3)	1·6 ± 0·2 (8)	61 ± 6	106 ± 5	—	844 ± 22

The slices were incubated for 90 min at 1° C in phosphate buffered medium containing Ca^{2+} or EDTA as indicated. Vessels were gassed with O_2 before transfer to a bath at 38° C for a further 70 min incubation. The complete phosphate medium used in line 1 contained (mM): Na^+ 161·5, K^+ 5·0, Ca^{2+} 1·3, Mg^{2+} 1·0, Cl^- 151·5, SO_4^{2-} 1·0, phosphate (pH 7·4) 10·0. The number of experiments is indicated in parentheses.

I thank Dr B. Chance for his interest. The work was supported by a grant from the US Public Health Service.

G. D. V. van Rossum*

Johnson Research Foundation,
University of Pennsylvania,
Philadelphia, Pennsylvania.

Received August 11, 1969.

* Present address: Department of Pharmacology, Temple University, Philadelphia.

[1] Shanes, A. M., *Pharmacol. Rev.*, **10**, 59 (1958).
[2] Whittembury, G., Sugino, N., and Solomon, A. K., *Nature*, **187**, 699 (1960).
[3] Curran, P. F., Herrera, F. C., and Flanigan, W. J., *J. Gen. Physiol.*, **46**, 1011 (1963).
[4] Judah, J. D., and Ahmed, K., *Biochim. Biophys. Acta*, **71**, 34 (1963).
[5] Kleinzeller, A., Knotková, A., and Nedvídková, J., *J. Gen. Physiol.*, **51**, 326 S (1968).
[6] Whittam, R., *Nature*, **219**, 610 (1968).
[7] Judah, J. D., and Ahmed, K., *Biol. Rev.*, **39**, 160 (1964).
[8] van Rossum, G. D. V. (in the press).
[9] Elshove, A., and van Rossum, G. D. V., *J. Physiol.*, **168**, 531 (1963).
[10] Judah, J. D., and McLean, A. E. M., *Biochem. Pharmacol.*, **11**, 593 (1962).
[11] van Rossum, G. D. V., *Biochim. Biophys. Acta*, **122**, 323 (1966).
[12] van Rossum, G. D. V., *Arch. Biochem. Biophys.* (in the press).

NMR Investigation of Deuterated Collagen

KNOWLEDGE of the structures of biological macromolecules stems almost exclusively from X-ray diffraction studies. In conjunction with molecular models, the diffraction patterns may be used to assign the positions of atoms other than hydrogen, and ultimately their relative angular

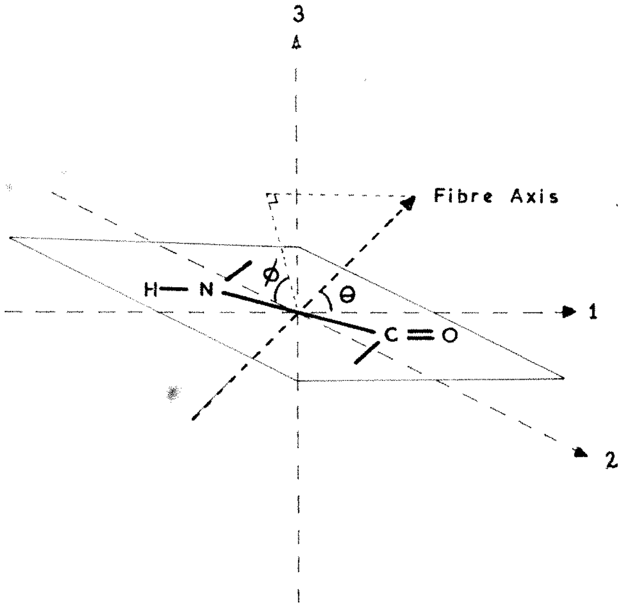


Fig. 1. The axis system for the planar peptide group and the definition of the spherical polar angles θ and φ.

spacing. We may describe the information as translational in character and this can only be interpreted in rotational, or angular, terms with the help of model building. Further, the assignment of any hydrogen bonds is not possible. We demonstrate here that the orientation of peptide groups may be determined relative to a fibre axis directly from nuclear magnetic resonance (NMR) measurements of the quadrupole couplings experienced by deuterium nuclei substituted for the peptide hydrogens. The couplings observed are sensitive to the type of hydrogen bond present and may become diagnostic for them.

When the electronic charge distribution around a spin 1 nucleus does not have spherical symmetry, the interaction between the nuclear quadrupole moment and the electric field gradients lifts the degeneracy of the $-1 \rightarrow 0$ and $0 \rightarrow 1$ transitions in the NMR spectrum. The Hamiltonian for this interaction is[1]:

$$\frac{(e^2 q_{zz}\, Q)}{4I\,(2I-1)}\,(3I_z^2 - I\,(I+1))$$

where e is the electronic charge, Q is the nuclear quadrupole moment and q_{zz} is the component of the field gradient tensor in the magnetic field direction. In the first order case, where the Zeeman interaction is much greater than the quadrupole one, this gives rise to a doublet in the NMR spectrum with a frequency splitting of $(3/2)e^2q_{zz}\,Q/h$, where h is Planck's constant. When the perturbation is expanded to second order, the doublet splitting does not alter, though the doublet components are slightly shifted. The field gradient tensor is traceless and of second rank. This means that q_{zz} can be expressed as

$$q_{zz} = q_{11}[\cos^2 \alpha_1 - \tfrac{1}{2}(1-\eta)\cos^2 \alpha_2 - \tfrac{1}{2}(1+\eta)\cos^2 \alpha_3]$$

where q_{11} is the quadrupole coupling constant in the 1-direction, α_n is the angle between the nth principal axis of the tensor and the z axis, and η is the asymmetry parameter—a measure of the departure of the tensor from axial symmetry about the 1 axis.

The essence of the application to fibrous proteins is that if the magnetic field direction is made coincident with the fibre axis, all equivalent spin 1 nuclei in the structure will give identical quadrupole splittings in the NMR spectrum. Deuterium has a spin of 1, and can be exchanged for hydrogen in the peptide groups quite easily. There are other easily exchangeable hydrogens in proteins, but they are usually well out in the residue side chains, and it will be assumed here that these have little regularity in their orientation. As a result, they will give a very weak background spectrum, out of which the relatively sharp doublet components from the deuteriums of the peptide groups will appear.

The problem now is to decide the magnitudes and directions of the principal components of the field gradient tensor for deuterium in a peptide group. The parameters chosen were those for the *trans* deuteriums in $^2H_4$-urea[2]. These are: $q_{11} = 210{\cdot}8$ kHz, $q_{22} = -90{\cdot}8$ kHz, $q_{33} = -120{\cdot}0$ kHz, that is $\eta = 0{\cdot}139$. The 1 axis is parallel to the C–O axis, the 3 axis is perpendicular to the plane of the peptide group, and the 2 axis is mutually perpendicular to these. This choice was considered to be justified because the field gradients are sensitive only to the charge distribution near the nucleus[3], which is expected to be very similar for urea and the peptide group.

The angles α_n can now be expressed in terms of spherical polar angles θ, φ, which are defined in Fig. 1. Thus $\cos \alpha_1 = \cos \theta$, $\cos \alpha_2 = \sin \theta \cdot \cos \varphi$, $\cos \alpha_3 = \sin \theta \cdot \sin \varphi$. In this way, the method gives the angles describing the orientation of the peptide group with respect to the fibre axis.

The measurement of the deuterium splittings alone does not give the complete information, because this corresponds to one function of two unknowns. A second piece of information that can be extracted from the deuterium spectrum is the linewidths of the doublet components. The linewidths of the components arising from magnetic dipolar coupling should be only of the order of about 1 kHz, but if the fibrous sample is not perfectly oriented, or if the fibre axis is not perfectly aligned with the magnetic field, the doublet components will be broadened. This is because in this case a distribution of q_{zz} values will be sampled, instead of a single value, for equivalent deuteriums. The extent of this broadening is proportional to

$$\left[\left(\frac{\mathrm{d}q_{zz}}{\mathrm{d}\theta}\right)_{\varphi}^2 + \left(\frac{\mathrm{d}q_{zz}}{\mathrm{d}\varphi}\right)_{\theta}^2\right]^{\frac{1}{2}}$$

A contour map of this function is given in Fig. 2, in terms of q_{11}/radian, for the field gradient tensor of the urea *trans* deuterium.

We performed the first measurements on an α-helix, because the geometry is well known and the spectra would be expected to provide a test of the above assumptions. Working from the coordinates of Fraser *et al.*[4], the orientation of the peptide groups (which are all equivalent) is given by: $\theta = 14{\cdot}1°$, $\varphi = 0°$. This gives a predicted splitting of 289·4 kHz.

The second molecule chosen for study was the collagen molecule. A number of proposals have been put forward for the secondary structure of collagen, the ones most

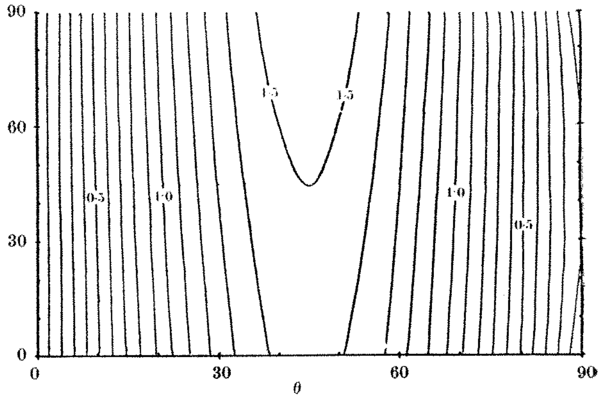


Fig. 2. A contour map showing the variation of the field gradient tensor gradient with θ and φ. The variation in the linewidth is directly proportional to this.

favoured being the collagen II structure[5], the Ramachandran two-bonded structure[6] and the new one-bonded structure[7], which we shall call collagen IIa. A complication now arises: in these three models there are three distinct hydrogen bonded states for the peptide hydrogens. One is the short hydrogen bond, as in the α-helix, which we shall designate state A. The second is a long hydrogen bond, state B. The third is where the deuterium is not formally hydrogen bonded in the structure, state C. For states B and C, there will almost certainly be an increase in the quadrupole coupling constant from the urea value, as is noticed with ^2H_2O when the hydrogen bonding is reduced[8]. We estimate the maximum possible limit of this increase to be 10 per cent for state B and 50 per cent for state C. Thus, working from the model coordinates, the predicted splittings and relative linewidths are as in Table 1.

Table 1. PREDICTED SPLITTINGS AND RELATIVE LINEWIDTHS

State	θ	φ	Splitting (kHz)	Relative broadening
		Collagen II		
C	71	36	102–153	0·90–1·35
C	81	30	136–204	0·47–0·71
A	83	53	157	0·39
		Ramachandran collagen		
B	83	53	157–173	0·39–0·43
B	86	39	151–166	0·25–0·27
C	73	38	107–161	0·84–1·26
		Collagen IIa		
C	70	42	101–152	0·97–1·45
C	70	27	91–137	0·96–1·44
B	85	38	147–162	0·29–0·32

The α-helix sample was a liquid crystalline solution of poly-γ-benzyl-L-glutamate of 300,000 molecular weight in dichloromethane. This phase is known to orient in strong magnetic fields such that the helices are parallel to the field[9]. The polypeptide had been deuterated previously by treatment with anhydrous ^{2}H-trifluoroacetic acid. The collagen sample was a cylindrical piece of well oriented bovine achilles tendon that had been immersed in acidified D_2O for three months. It was then removed and partially dried.

All spectra were recorded on a superconducting magnet spectrometer operating at about 50 kG (ref. 10). The frequency was swept by digitally advancing a frequency synthesizer, and simultaneously advancing a time-averaging computer, into which the spectrometer output was fed. Magnetic field modulation of about 2 G at 70 Hz was used, so the derivative of the spectrum was recorded. About 300 spectra were accumulated in the time-averaging computer on each run, because the signals were expected to be very weak.

The accumulated spectrum for the α-helix sample is shown in Fig. 3*a*. The poor phasing of the lines arises from problems with the bandwidth of the tuned probe assembly. In spite of this, however, it can be seen that there is a well defined doublet with a splitting of about 288·3 kHz. This is in very close agreement with the predicted value of 289·4 kHz, and we consider that this supports the identification of the field gradient tensor at the deuterium atom in the peptide group with that of the *trans* deuterium in urea, in magnitude, orientation and symmetry.

The accumulated spectrum for the collagen sample is shown in Fig. 3*b*, as the derivative of the dispersion mode. There is a doublet with fairly narrow components, with a splitting of about 157 kHz. In addition, there are two doublets with much wider components with splittings of about 117 kHz and 127 kHz. These doublets are consistent only with the collagen IIa model. Most, if not all, of the collagen molecule must therefore have this conformation.

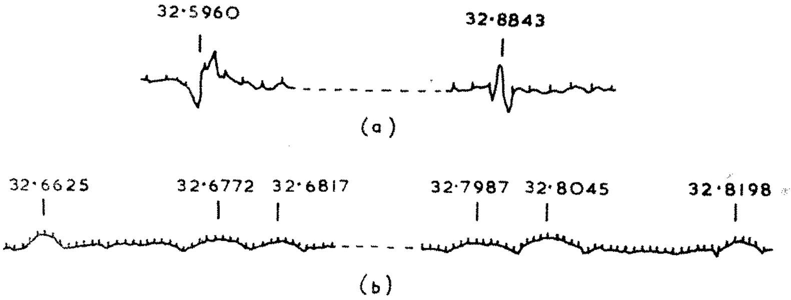


Fig. 3. The observed deuteron spectra of (*a*) the α-helical form of deuterated poly-γ-benzyl-L-glutamate and (*b*) deuterated bovine achilles tendon. In (*a*) the frequency markers are 1·6 kHz apart and in (*b*) 640 Hz apart. The measurements are in MHz.

This conclusion could not have been reached from the X-ray diffraction data and we suggest that the method may be particularly useful in investigating the structures of fibrous proteins which cannot be crystallized. The signal to noise ratio of the NMR spectra is unsatisfactory and work is in progress to improve this aspect of the technique. Attempts are also being made to use collagen samples with better orientations, in order to establish whether or not there is a second type of conformation present in collagen in small proportions.

We thank Professor R. E. Richards for the use of the spectrometer, Dr A. Miller for helpful discussions and Dr E. M. Bradbury for a generous gift of poly-γ-benzyl-L-glutamate. G. E. C. thanks the Medical Research Council for a maintenance award.

G. E. CHAPMAN
I. D. CAMPBELL
K. A. MCLAUCHLAN

Physical Chemical Laboratory,
South Parks Road,
Oxford.

Received September 15, 1969.

[1] Abragam, A., *Principles of Nuclear Magnetism* (Oxford University Press, 1961).

[2] Chiba, T., *Bull. Chem. Soc. Japan*, **38**, 259 (1965).

[3] Slichter, C. P., *Principles of Magnetic Resonance* (Harper, 1963).

[4] Fraser, R. D. B., MacRae, T. P., and Miller, A., *Acta Cryst.*, **17**, 813 (1964).

[5] Rich, A., and Crick, F. H. C., *J. Mol. Biol.*, **3**, 483 (1961).

[6] Ramachandran, G. N., *Treatise on Collagen*, **1**, 103 (Academic Press, New York, 1967).

[7] Traub, W., Yonath, A., and Segal, D. M., *Nature*, **221**, 914 (1969).

[8] Waldstein, P., Rabideau, S. W., and Jackson, J. A., *J. Chem. Phys.*, **41**, 3401 (1964).

[9] Sobajima, S., *J. Phys. Soc. Japan*, **23**, 1070 (1967).

[10] Halliday, J. D., Hill, H. D. W., and Richards, R. E., *J. Phys.* E2 (1969).

Anti-Rh Activity of Human γG4 Proteins

THE subclasses of human IgG, which differ antigenically and structurally, can also be differentiated by functional criteria. Analysis of functional similarities and differences among the immunoglobulins may provide some clue to the biological survival value of these proteins and to the evolutionary mechanisms which have determined the development and maintenance of the various forms.

Examples of functional differences among the subclasses of human IgG are: (1) γG1, γG3 and γG4 sensitize guinea-pig skin for passive, cutaneous anaphylaxis but γG2 does not[1]; (2) γG1 and γG3 interact well with complement components, but γG2 interacts poorly and γG4 does not interact at all[2]; and (3) the metabolic survival of γG3 molecules is shorter than that of the other three subclasses[3].

Consideration of antibody activity also suggests some functional differentiation: (1) antibodies to simple carbohydrate antigens tend to be of the γG2 subclass[4]; (2) all antibodies to coagulation factor VIII investigated so far

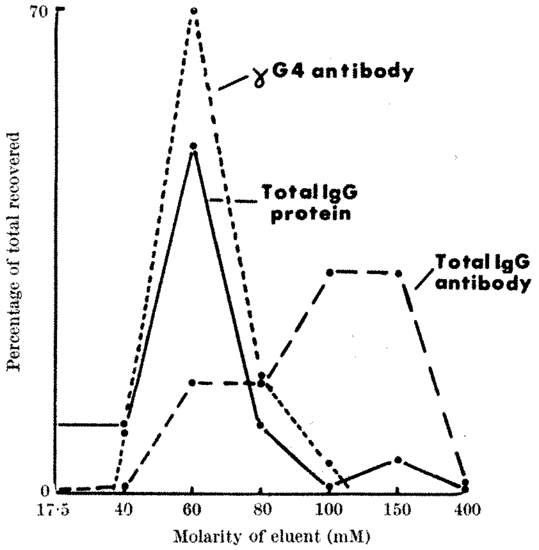


Fig. 1. Distribution of γG4 anti-D, total IgG anti-D and total IgG protein in CM–cellulose fractions of serum Ric.

are of the γG4 subclass[5]; and (3) Rh antibodies are of the γG1 and γG3 subclasses and lack the γG2 and γG4 subclasses[6].

In a search for additional examples of antibody specificity related to IgG subclass, human blood group antibodies were studied. Human red cells were sensitized with various blood group antibodies and the sensitized cells were then tested with antisera prepared against each of the four γG subclasses. Preliminary results strongly suggested that the anti Rh activity of two of the sera was attributable partly to γG4 molecules. γG4 anti Rh had not previously been described and further work was therefore undertaken.

Human sera containing antibodies with the following specificities and titres were tested: thirty-five examples of anti D, titres 64–4096 (no distinction was made between anti CD and anti D); 5 anti-c, titres 16–512 ; 1 anti e, titre 128; 11 anti K, titres 16–256; 1 anti Fy^a, titre 64; and 1 anti Jk^a, titre 64. Four volumes of undiluted serum were incubated with one volume of a 20 per cent suspension of appropriate red cells; after 1·5 h at 37° C, the cells were washed three times and tested, both with a potent anti human IgG and with an anti γG4 serum. The latter serum had been prepared by immunizing a rhesus monkey with a γG4 myeloma protein; the resulting antiserum was extensively absorbed with γG1, γG2 and γG3 myeloma proteins as well as hypogammaglobulinaemia serum. At dilutions greater than 1 in 10 (the optimal dilution was 1 in 800), this serum reacted specifically; that is, cells sensitized with only four of the thirty-five anti D sera were clearly agglutinated. Cells sensitized with three other anti D sera gave weakly positive reactions, while the remaining twenty-eight anti D sera and all other blood group antisera tested gave negative reactions. Among the antibodies which seemed to lack γG4 molecules were some particularly potent examples of anti D including the well known serum "Ripley"[7].

The specificity of the reactions observed with the "γG4 positive" sera was confirmed by inhibition tests; myeloma proteins of known γG subclass were added in a range of dilutions to the anti γG4 serum, and the mixtures were then tested with sensitized red cells. In tests with red cells sensitized with two of the "γG4-positive" anti D sera, the agglutination produced by the anti γG4 serum was completely inhibited by six different γG4 myeloma proteins at concentrations between 6×10^{-5} and $2·4 \times 10^{-4}$ g/100 ml. (slightly higher concentrations being required in tests with more strongly sensitized red cells). On the other hand, γG1, γG2 and γG3 myeloma proteins inhibited agglutination only at concentrations between 0·25 and 1·0 g/100 ml., that is, there was a thousand-fold difference between the inhibition produced by γG4 and by the other three subclasses (Table 1).

The anti Rh molecules in the four "γG4 positive" sera were not all of the γG4 subclass. When serial dilutions of the four "anti γG4 positive" sera were incubated with Rh positive red cells, and the cells were then washed and tested with anti IgG and anti γG4, respectively, three of the four titres with anti γG4 were much lower (Table 2).

Table 1. REACTIONS BETWEEN R_2R_2 CELLS SENSITIZED WITH A 1 IN 20 DILUTION OF SERUM Jud AND ANTI-γG4 SERUM (1 IN 400) TO WHICH AN EQUAL VOLUME OF VARIOUS CONCENTRATIONS OF DIFFERENT MYELOMA PROTEINS HAD BEEN ADDED

Myeloma protein		Concentrations of myeloma protein (g/100 ml.)						
Donor	Sub-class	1	0·25	6×10^{-2}	$2·4 \times 10^{-4}$	6×10^{-5}	$1·5 \times 10^{-5}$	Saline control
Pu	G1	–	–	(+)	+ +	+ +	+ +	+ +
Sa	G2	(+)	+ +	+ +	+ +	+ +	+ +	+ +
Be	G3	–	–	+	+ +	+ +	+ +	+ +
Gu	G4	–	–	–	–	+ +	+ +	+ +
Re	G4	–	–	–	–	–	(+)	+ +
Du	G4	–	–	–	–	+	+ +	+ +
Me	G4	–	–	–	–	–	(+)	+ +
No	G4	–	–	–	–	–	+	+ +
Ro	G4	–	–	–	–	+	+ +	+ +

Table 2. COMPARISON OF TITRES OF THE FOUR "γG4 POSITIVE" ANTI D SERA WITH ANTI IgG (TOTAL) AND ANTI γG4

Donor	–IgG (total)	–γG4
Ric	1,024	128
Rus	256	128
Jud	4,096	32
Tri	1,024	16

Two of the "γG4 positive" sera were fractionated on carboxymethyl cellulose and it was found that the elution pattern of the γG4 molecules was different from that of the other anti D molecules in other anti Rh sera. Thus in a "typical" anti Rh serum the bulk of the IgG protein is eluted with the 0·06 M buffer, whereas most of the Rh antibodies are eluted with a buffer of higher molarity[8,9]. In serum "Ric", most of the γG4 antibody was eluted in the 0·06 M fraction with the bulk of the total IgG (Fig. 1). On the other hand, most of the antibody was eluted in the 0·1–0·15 M fractions, which sensitized red cells strongly to agglutination by anti-IgG but only weakly or not at all to agglutination by anti γG4, indicating that much of the antibody was of other subclasses—presumably γG1 or γG3, or both. The findings in serum "Jud" were similar.

Three additional anti D sera were tested after fractionation on CM–cellulose: "Avg", a potent serum which happened to contain both IgA and IgG anti D (ref. 10), "Jou", which had given doubtfully positive results with anti-γG4, and serum "Ripley" referred to previously. Not unexpectedly, all fractions from "Avg" were entirely negative, whereas the 0·06 and 0·08 M fractions from serum "Jou" gave a positive reaction with anti γG4 (which could be inhibited by diluted γG4 myeloma protein). An unexpected finding was that 0·06 M and 0·08 M fractions from serum "Rip" weakly sensitized red cells to anti γG4; these reactions were also inhibited by two γG4 myeloma proteins at a concentration of 10^{-3} g/100 ml.

Presumably it is easier to detect a low concentration of γG4 anti D molecules by sensitizing red cells with the 0·06 M or 0·08 M fractions, because in these fractions the ratio of γG4 to total γG is relatively high and competition for antigen sites with antibody molecules of other subclasses is reduced.

The demonstration of anti Rh antibodies of the γG4 subclass shows that with regard to antibody specificity there is less differentiation among the subclasses than was previously supposed. This type of antibody activity in γG4 molecules may have some biological significance because it has been reported that macrophages, derived from peripheral blood monocytes, have receptors for γG1 and γG3, but not γG2 or γG4 (ref. 11). Red cells sensitized with γG1 and γG3 molecules presumably can attach to monocytes and be ingested; those sensitized with γG2 and γG4 molecules cannot. The fate in the circulation of antibody-coated red cells that cannot be ingested by monocytes remains to be determined.

MARION FRAME
P. L. MOLLISON

MRC Experimental Haematology Unit,
St Mary's Hospital Medical School,
London W2.

WILLIAM D. TERRY

Immunology Branch,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014.

Received September 15, 1969.

[1] Terry, W. D., *J. Immunol.*, **95**, 1041 (1965).
[2] Ishizaka, T., Ishizaka, K., Salmon, S., and Fudenberg, H. H., *J. Immunol.*, **99**, 82 (1967).
[3] Spiegelberg, H. L., Fishkin, B. G., and Grey, H. M., *J. Clin. Invest.*, **47**, 2323 (1968).
[4] Yount, W. J., Dorner, M. M., Kunkel, H. G., and Kabat, E. A., *J. Exp. Med.*, **127**, 633 (1968).
[5] Anderson, B. R., and Terry, W. D., *Nature*, **217**, 174 (1968).
[6] Natvig, J. B., and Kunkel, H. G., *Series Haematol.*, **1**, 66 (1968).
[7] Waller, M., and Lawler, S. D., *Vox Sang.*, **7**, 591 (1962).
[8] Abelson, N. M., and Rawson, A. J., *J. Immunol.*, **83**, 49 (1959).
[9] Frame, M., and Mollison, P. L., *Immunology*, **16**, 277 (1969).
[10] Adinolfi, A., Mollison, P. L., Polley, M. J., and Rose, J. M., *J. Exp. Med.*, **123**, 951 (1966).
[11] Huber, H., Polley, M. J., Linscott, W. D., Fudenberg, H. H., and Müller-Eberhard, H. J., *Science*, **162**, 1281 (1968).

Identity of Chimpanzee with Human Fibrinopeptides

FIBRINOPEPTIDES, those portions of vertebrate fibrinogen molecules split off by thrombin during clotting, exhibit a very large degree of interspecific variability. The amino-acid sequences of these peptides have been determined for more than thirty different species of mammals, and the number of identified amino-acid replacements ranks the fibrinopeptides among the most variable polypeptide structures known. On the arbitrary scale developed by Dayhoff[1] these molecules have a variability index of 90, compared with values of 13 and 11 for the β and α chains of haemoglobin, and a score of 3 for cytochrome *c*. As a result of this propensity for rapid change, the fibrinopeptides are particularly useful for tracing phylogenetic relationships at the generic level.

Chimpanzees are the closest living evolutionary ancestors of man. It has previously been determined that the α and β chains of haemoglobin[2] and the cytochrome *c* molecules[1] of these creatures are identical with their respective human counterparts. We now report that the fibrinopeptides, in spite of their high degree of interspecific variability, are also identical in chimpanzees and humans.

Blood samples were collected from eight chimpanzees (four male and four female) into EDTA (1 mg/ml.). Approximately 100 ml. of the pooled plasma was used for the preparation of fibrinogen by previously described procedures[3]. After clotting (bovine thrombin), the fibrinopeptide mixture was isolated from the clot liquor by bulk adsorption on 'Dowex 50*X*2' (ref. 4). The peptide material was subsequently resolved into the constituent fibrinopeptides by gradient elution on a 1·2 × 40 cm 'Dowex 50*X*2' column (Fig. 1).

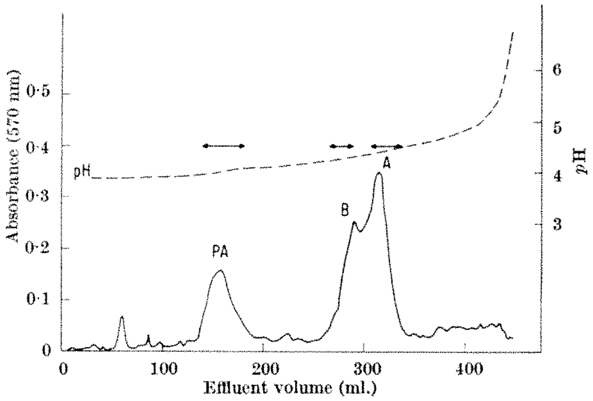


Fig. 1. Gradient chromatography of chimpanzee fibrinopeptides on 'Dowex 50*X*2'. Because the separation of A and B peptides was not complete, further purification had to be achieved by paper electrophoresis at *p*H 2.

Three different fibrinopeptides were isolated, eluting at identical *p*H positions as the corresponding human fibrinopeptide control sets, including a phosphorylated A peptide (eluting at *p*H 4·0), a B peptide (eluting at *p*H 4·3), and a non-phosphorylated A peptide (eluting at *p*H 4·4). The amino-acid compositions of these peptides were quantitatively determined after total acid hydrolysis and found to be indistinguishable from the corresponding human types, a large number of which had recently been analysed in this laboratory as part of a survey of individual human types (Table 1) (ref. 5). In addition, the chymotryptic peptides of the chimpanzee A and B fibrinopeptides were isolated and their amino-acid compositions found to be identical with human controls. In the case of the fibrinopeptide A, we isolated both the amino-terminal octapeptide produced by the chymotryptic attack on the phenylalanine residue at A9 (Fig. 2) and the overlapping nonapeptide produced by splitting at the leucine at A8.

Table 1. AMINO-ACID COMPOSITIONS OF CHIMPANZEE AND HUMAN FIBRINOPEPTIDES

Residues/mole

Amino-acid	PA			A			B		
	Chimp.	Human		Chimp.	Human		Chimp.	Human	
Aspartic acid	2·22	2·27	0·06	2·07	2·20	0·12	2·94	2·90	0·04
Serine	0·86	0·90	0·13	0·97	0·94	0·04	0·98	0·97	0·05
Glutamic acid	2·31	2·37	0·09	2·16	2·28	0·12	3·04	3·01	0·06
Glycine	4·80	4·60	0·22	5·16	4·64	0·40	2·32	2·48	0·22
Alanine	1·96	1·83	0·14	2·01	1·79	0·14	1·03	1·07	0·08
Valine	0·82	0·83	0·14	0·66	0·88	0·07	0·98	0·97	0·03
Leucine	0·90	0·86	0·09	1·00	0·89	0·15	0·14	0·18	0·06
Phenylalanine	1·13	1·16	0·08	0·95	1·18	0·14	1·87	1·88	0·05
Arginine	0·81	(1)	—	0·87	(1)	—	0	(1)	—
Total residues, *n*	16	16		16	16		14	14	

The values for the human controls are from ten sets of human fibrinopeptides isolated and resolved in the conditions used for isolation of the chimpanzee peptides. The human values are expressed as means and standard deviations, where $S.D. = \sqrt{\frac{\Sigma d^2}{n-1}}$. Arginine values were not measured in the human survey.

The corresponding carboxy-terminal fragments were also analysed. In the case of the B fibrinopeptide, the ninhydrin-negative ondecapeptide corresponding to the blocked amino-terminal peptide was isolated and analysed; the blocked amino-terminus leading to ninhydrin-negativity is attributable to terminal pyrrolidone carboxylic acid (PCA) residue. The carboxy-terminal tripeptide was also isolated and analysed. In all cases the electrophoretic mobilities (at *p*H 4·1) of the chymotryptic peptides

	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
Human A	Ala-	Asp-	Ser-	Gly-	Glu-	Gly-	Asp-	Phe-	Leu-	Ala-	Glu-	Gly-	Gly-	Gly-	Val-	Arg
Chimp. A	(Ala,	Asp,	Ser,	Gly,	Glu,	Gly,	Asp)	Phe-	Leu	(Ala,	Glu,	Gly,	Gly,	Gly,	Val)	Arg
Human PA	Ala-	Asp-	PSE-	Gly-	Glu-	Gly-	Asp-	Phe-	Leu-	Ala-	Glu-	Gly-	Gly-	Gly-	Val-	Arg
Chimp. PA	(Ala,	Asp,	PSE,	Gly,	Glu,	Gly,	Asp,	Phe,	Leu,	Ala,	Glu,	Gly,	Gly,	Gly,	Val)	Arg
Human B			PCA-	Gly-	Val-	Asn-	Asp-	Asn-	Glu-	Glu-	Gly-	Phe-	Phe-	Ser-	Ala-	Arg
Chimp. B			PCA	(Gly,	Val,	Asn,	Asp,	Asn,	Glu,	Glu,	Gly,	Phe)	Phe	(Ser,	Ala)	Arg

Fig. 2. Amino-acid sequences of human[5] and chimpanzee fibrinopeptides. PSE, Phosphoserine; PCA, pyrrolidone carboxylic acid.

corresponded exactly with those of the human controls run simultaneously.

Finally, the proof of phosphorylation of the chimpanzee PA peptide was accomplished by enzymatic removal of the phosphate group by alkaline phosphatase (*Escherichia coli*) digestion followed by paper electrophoresis at *p*H 2. The change in peptide mobility after phosphate removal was exactly the same as observed for the human PA control, both types being converted into peptides with mobilities corresponding to non-phosphorylated A peptides.

Previously the fibrinopeptides from four old world species of monkeys had been characterized (rhesus, baboon, green monkey and drill)[1]. These have been found to differ from the human fibrinopeptides in five to eight of the thirty amino-acid positions compared. The identity of the chimpanzee and human fibrinopeptides re-emphasizes the extraordinarily close relationship of these two species on the molecular level. Recently Wilson and Sarich[6], on the basis of immunochemical and other molecular criteria, have argued that the time of divergence for these two species may have been as recent as 5 million years ago, in contrast to the 20–30 million year figures postulated by palaeontologists and others. Our data would support their contention, the chimpanzee and human—as judged by their fibrinopeptide structures—being more closely related than such pairs as horse–donkey, water buffalo–Cape buffalo, cat–lion, or dog–fox, all of which have at least one amino-acid difference in their fibrinopeptides[1]. On the other hand, sheep and goat (and ibex) have identical fibrinopeptides A and B, as do llama and vicuna[1]. The relatedness of chimpanzees and humans and the time of their divergence should be judged in the same context as the relationships proposed for each of the pairs compared here, all of which are thought to have diverged in Pleistocene or Recent times[7].

The chimpanzee blood used in these experiments was provided by Major Ralph F. Ziegler and his staff at the Aeromedical Research Laboratory, Holloman Air Force Base, New Mexico. We thank Miss Carol Glasgow for technical assistance. We also thank Drs Wilson and Sarich for sending us their manuscript before publication.

R. F. DOOLITTLE
G. A. MROSS

Departments of Chemistry and Biology,
University of California,
San Diego,
La Jolla, California 92037.

Received November 3, 1969.

[1] Dayhoff, M. O., *Atlas of Protein Sequence and Structure*, **4** (National Biological Research Foundation, Silver Spring, 1969).

[2] Rifkin, D. B., and Konigsberg, W., *Biochim. Biophys. Acta*, **104**, 457 (1965).

[3] Doolittle, R. F., Schubert, D., and Schwartz, S. A., *Arch. Biochem. Biophys.*, **118**, 456 (1967).

[4] Blömback, B., and Vestermark, A., *Arkiv Kemi*, **12**, 173 (1958).

[5] Doolittle, R. F., Chen, R., Glasgow, C., Mross, G., and Weinstein, M., *Humangenetik* (in the press).

[6] Wilson, A., and Sarich, V., *Proc. US Nat. Acad. Sci.*, **63**, 30 (1969).

[7] Colbert, E. H., *Evolution of the Vertebrates* (Science Editions, New York, 1961).

[8] Blömback, B., Blömback, M., Edman, P., and Hessel, B., *Biochim. Biophys. Acta*,**115**, 371 (1966).

Separation of Isoenzymes by Zonal Centrifugation

THE commercial availability of zonal rotors and centrifuges within the past 3–4 years has permitted the exploitation of zonal centrifugation for large scale fractionation of cellular components. In particular, attention has been focused on the isolation of relatively large components such as cell organelles and sub-fractions, viruses and RNA, although the technique has also been applied to enzyme purification[1].

Our work has been concerned with the enzyme isocitrate dehydrogenase (EC 1.1.1.42) of *Acinetobacter lwoffi*. We have found[2] that in this organism, and certain other bacteria, there are two distinct NADP-linked isocitrate dehydrogenases which can be separated by DEAE-cellulose chromatography or gel filtration on 'Sephadex *G*-200'. We have termed these IDH-I and IDH-II. The two isoenzymes differ in kinetic, molecular and electrophoretic properties, and it is particularly interesting that IDH-II (the higher molecular weight enzyme) is strongly activated (about five-fold) by low concentrations (0·2 mM) of either glyoxylate or pyruvate. The considerable difference in molecular size of the two isoenzymes, as shown by their separation on 'Sephadex *G*-200', prompted an investigation of the usefulness of zonal centrifugation for their preparative separation.

A. lwoffi was cultured aerobically at 30° C on acetate as the sole carbon source. The cells were collected towards the end of logarithmic growth, washed, resuspended in buffer containing 20 mM Tris, *p*H 8·0, 10 mM $MgCl_2$, 1 mM EDTA, and disrupted by ultrasonication. After centrifugation, nucleic acid was removed by treatment of the extract with protamine sulphate (1·3 mg per 10 mg protein) and the mixture was again centrifuged. The clear supernatant solution was then fractionated with ammonium sulphate and the material precipitating between 45 per cent and 75 per cent saturation was redissolved in 50 mM phosphate, *p*H 8·0, containing 10 mM $MgCl_2$ and 1 mM EDTA and dialysed overnight at 4° C against this buffer. The dialysed solution, which contained 27 mg of protein/ml., was used in the zonal centrifugation experiments.

Isocitrate dehydrogenase activity was determined at 340 nm by following the formation of NADPH in a reaction mixture at 25° C containing 20 mM Tris, *p*H 8·0, 10 mM $MgCl_2$, 1 mM EDTA, 2 mM isocitrate and 0·2 mM NADP. Activation by glyoxylate was examined by incorporating 0·2 mM glyoxylate in the assay mixture.

A linear sucrose density gradient (5–20 per cent w/v) in buffer of composition 5 mM Tris, *p*H 8·0, 10 mM $MgCl_2$, 1 mM EDTA, was fed into an MSE *B* XIV titanium zonal rotor rotating at 2,500 r.p.m. in an MSE 'Superspeed 65' centrifuge. Enzyme solution (5 ml.) was introduced to form a layer at the core of the rotor, on the light end of the gradient, and then pushed further into the centrifugal field with 15 ml. of buffer. Centrifugation was carried out at 47,000 r.p.m. and 10° C for the times given in the figure legend, after which the rotor was slowed to 2,500 r.p.m. and the gradient gently displaced to a collector by pumping in 30 per cent sucrose solution. Fractions (20 ml.) were collected and assayed for enzyme activity.

Fig. 1*A* shows the separation of isoenzymes achieved after 12·5 h of centrifugation, and Fig. 1*B* shows the increased separation after 20 h. The activation of IDH-II by glyoxylate is also depicted. These results show that IDH-I and IDH-II may indeed be fractionated by this technique. Similar resolution has been achieved with as much as 25 ml. of applied enzyme solution. Moreover, centrifugation removes the enzymes isocitrate lyase and glutamate dehydrogenase, both of which sediment faster than either IDH-I or IDH-II. The removal of these enzymes is an added advantage of this zonal separation, for their presence interferes with kinetic studies of the IDHs; moreover, they are difficult to remove by other means.

For comparison, Fig. 1*C* shows the pattern obtained after 18 h of centrifugation of an extract of acetate-grown *Escherichia coli* prepared in the same way as before. We have not observed activation of *E. coli* isocitrate dehydrogenase by glyoxylate or pyruvate, and have found no evidence for the existence of isoenzymes. Consistent with this, the sedimentation pattern reveals a single peak of IDH activity.

This application of the technique of zonal centrifugation to the separation of IDH isoenzymes should, when sedi-

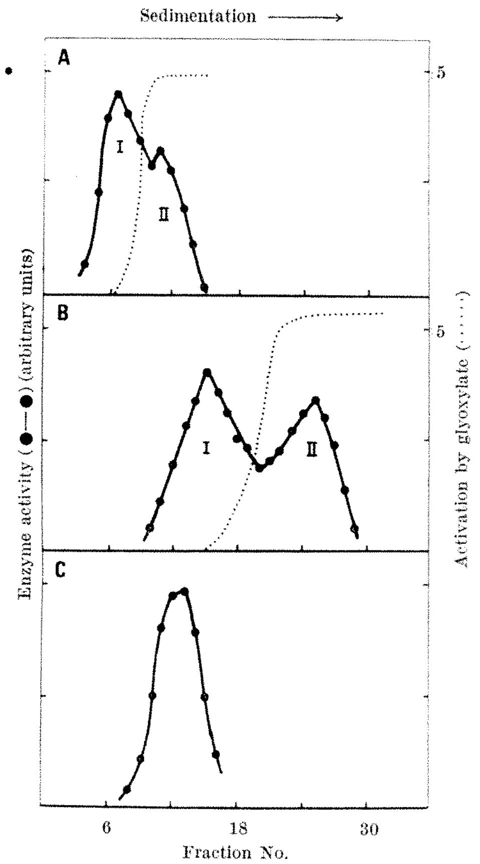


Fig. 1. Sedimentation patterns of isocitrate dehydrogenases of *A. lwoffi* and *E. coli*. Experimental details are given in the text. *A*, *A. lwoffi*, 12·5 h centrifugation at 47,000 r.p.m.; *B*, *A. lwoffi*, 20 h; *C*, *E. coli*, 18 h. The dotted lines show the activation by glyoxylate (0·2 mM) found only with the IDH-II of *A. lwoffi*. This activation is defined as the ratio of the additional enzyme activity in the presence of glyoxylate to that in its absence.

mentation characteristics are sufficiently distinct, prove useful in studies on other isoenzyme systems. Furthermore, our results with IDH-I demonstrate the applicability of the technique to relatively small molecules (molecular weight $< 100,000$).

This work was supported, in part, by the Science Research Council and was conducted during the tenure (by C. H. S.) of an MRC postgraduate training scholarship.

Colin H. Self
P. D. J. Weitzman

Department of Biochemistry,
University of Leicester.

Received November 10, 1969.

[1] Setlow, B., and Lowenstein, J. M., *J. Biol. Chem.*, **242**, 607 (1967).

[2] Self, C. H., and Weitzman, P. D. J., *Abst. of the Sixth FEBS Meeting*, Madrid, No. 249 (1969).

Antigenic Expression of a Murine Lymphoma during Growth *in vitro*

While studying a Moloney virus (MV) induced lymphoma in culture, we were puzzled by the disappearance of the antibody-mediated cytotoxic sensitivity of the cells several days after explantation. After a longer time in culture, however, these cells regained sensitivity to the same sera with complement. The experiments have been repeated in better controlled conditions, and some of the results obtained are reported here.

The mouse lymphoma YCAB was induced by intraperitoneal inoculation of MV in newborn mice of (A/Sn × A.CA)F_1 origin. The resulting solid tumour was converted to ascites form and later explanted in minimal essential medium (MEM) supplemented with 10 per cent foetal calf serum, L-serine and sodium pyruvate[1]; to each ml. medium 30 units of 'Mycostatin' (Squibb) were added. The cells were grown in suspension at 37° C and with 5 per cent CO_2. The antiserum with activities directed against *H*-2 alloantigenic specificities 4, 10, 13 and 31 was produced in (C57Bl × C3H)F_1 hybrids ($H2^b/H2^k$) by repeated weekly subcutaneous inoculation of spleen, liver and kidney tissues from DBA/2 mice ($H2^d$) (ref. 2). Sera from several mice were pooled and stored at $-20°$ C in aliquots of 0·06 ml. Anti-Moloney serum was produced in (DBA/2 × A.BY)F_1 hybrids by inoculation of irradiated (6,000 r) lymph node cells from syngeneic mice bearing the solid Moloney lymphoma YDYA.

Cytotoxicity tests were performed with 10^5 cells and 0·05 ml. serum of appropriate dilution. All dilutions were made in MEM without foetal calf serum. The cells were incubated with serum for 20 min at 37° C, washed once in MEM free of foetal calf serum, and incubated with complement (diluted 1 : 2) for 45 min at 37° C. The source of complement was fresh guinea-pig serum absorbed with mouse tumour cells 1 : 20, and stored at $-20°$ C. The viability of target cells was assessed by the trypan-blue exclusion test (0·025 per cent in balanced salts solution). During procedures other than incubations the cells were kept in cold. The absorptions of the (C57Bl × C3H)F_1 anti-DBA/2 and anti-Moloney sera were performed with 0·05 ml. serum on 10^6 YCAB cells at 6° C for 30 min with shaking. The (C57Bl × C3H)F_1 anti-DBA/2 serum was absorbed in 1 : 2 dilution; anti-Moloney serum was absorbed undiluted. Both absorbed sera were frozen and later tested against standard YCAB cells (see below).

To determine the modal cell volume during growth *in vitro*, cells were harvested every day and the cell volume was measured in a Coulter counter, model B. The population doubling time[3] was calculated according to the formula[4]

$$\log N = \log N_0 + kt \log 2$$

where N is the number of cells at time t, N_0 the number of cells at time 0, and k is the regression constant. The population doubling time is the reciprocal of k. The cytotoxicity index was calculated as described elsewhere[5].

Fig. 1 shows the growth curves and changes of the cytotoxin sensitivity, as well as the population doubling time of YCAB cells cultured in two different conditions: cells from a 3-day culture (in 400 ml. bottles with 100 ml. medium and 10^5 cells as initial inoculum) were put in 10 ml. of medium in 25 ml. screw-cap bottles at a concentration of either 2×10^5 cells/ml. (*A* and *B* in Fig. 1) or 6×10^5 cells/ml. (*C* and *D*). With the lower initial inoculum the cytotoxic sensitivity to the (C57Bl × C3H)F_1 anti-DBA/2 serum and complement decreased to very low values 48 h after seeding, remained low during the next 24 h, and rose within 48 h to 0·90. The sensitivity to the anti-Moloney serum followed the same pattern.

A striking correlation between the cytotoxic sensitivity and the population doubling time is observed in these (Fig. 1) and various other experiments (Table 1). With the initial cell inoculum of 6×10^5 cells/ml., in otherwise identical conditions, the changes in cytotoxic sensitivity occurred much more rapidly and again corresponded with the changes in population doubling time. An inverse relationship between the modal cell volume and cytotoxic sensitivity was observed in all experiments (see, for example, Fig. 2).

The absorption experiments (Fig. 3) indicate that the cytotoxic sensitivity of the cells is indeed reflecting the amount of detectable antigens present on the cell surface. The serum absorbed with the cells harvested two days after seeding (Fig. 1), when the cytotoxic sensitivity was low, and that absorbed on the cells harvested five days after

Table 1. CHANGES OF THE POPULATION DOUBLING TIME AND CYTOTOXIC SENSITIVITY OF THE YCAB CELLS DURING A PERIOD OF 4 DAYS IN CULTURE

	Dilution	Days after seeding 0	1	2	3	4
Cells/ml. $\times 1^5$		1·0	1·5	10·4	13·6	16·6
Population doubling time (h)		—	40	8·5	62	83·7
C.I. of (C57Bl × C3H)F_1 anti-DBA/2 serum	1 : 2	0·54	0·46	0·01	0·45	0·49
	1 : 4	0·44	0·15	0	0·19	0·27
C.I. of the anti-Moloney serum	1 : 1	0·41	0·35	0	0·19	0·26
	1 : 2	0·37	0·13	0	0·16	0·22

10^5 cells/ml. were seeded in 400 ml. bottles in 100 ml. medium. *C.I.* = cytotoxicity index. The population doubling time data refer to the period between two subsequent days (24 h).

seeding (in the same experiment), when it was high, are compared with the unabsorbed sera. All sera were tested simultaneously on standard YCAB cells grown in conditions favouring the expression of a relatively large amount of antigen on the cell surface; this can be achieved by manipulating the size of the bottles, the amount of medium, and the initial cell inoculum, as well as the growth phase of the seeded cells (to be published). The sera absorbed on the cells grown for two days in culture in the conditions of the experiment of Fig. 1*A* have only slightly decreased activity compared with the unabsorbed sera (Fig. 3). On the other hand, when the sera were absorbed in the same experiment on the cells grown for five days in culture, they almost completely lost the activity against the standard cells.

Large cells exhibited low cytotoxic sensitivity and low absorbing capacity, whereas small cells had high cytotoxic sensitivity and high absorbing capacity, so it follows that the number of expressed antigenic receptors per cell is directly proportional to the cytotoxic sensitivity. If the number of expressed antigenic receptors on a single cell were to remain constant during growth of a cell population in culture, the absorbing capacity of a constant number of cells should not vary with the cell volume.

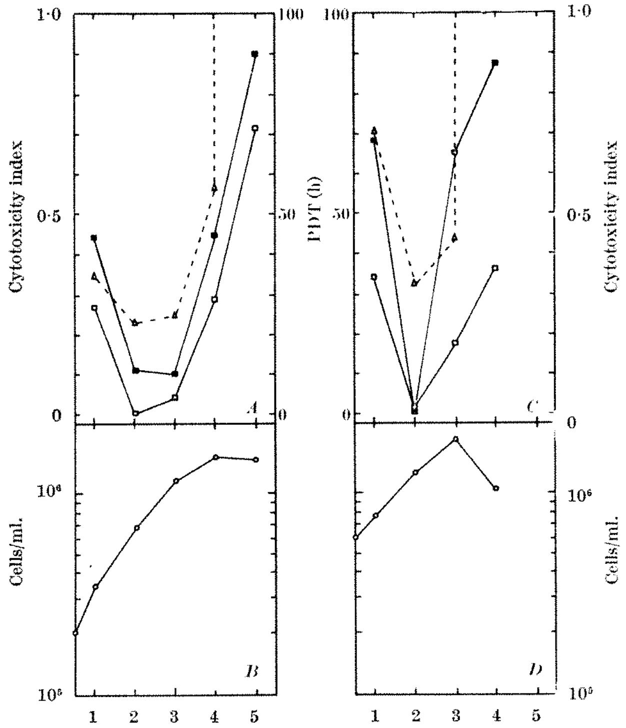


Fig. 1. Cytotoxic sensitivity, population doubling time (PDT), and growth curves of MV-induced lymphoma YCAB during 5 days in culture; two different experiments. *A* and *B* represent experiments with an initial inoculum of 2×10^5 cells/ml. in 25 ml. bottles and 10 ml. medium. *C* and *D* represent experiments with an initial inoculum of 6×10^5 cells/ml. but otherwise in the same conditions. *B* and *D* are the growth curves of the respective cell populations. *A* and *C* depict the cytotoxicity index for (C57Bl × C3H)F_1 anti-DBA/2 serum in 1 : 2 dilution (■—■), and for the undiluted anti-Moloney serum (□—□), as well as population doubling time (△ - - - △).

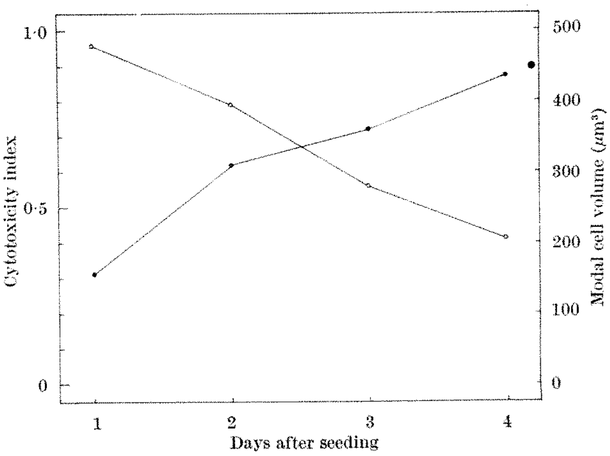


Fig. 2. Changes of the modal cell volume (○—○) and cytotoxic sensitivity (●—●) at various times.

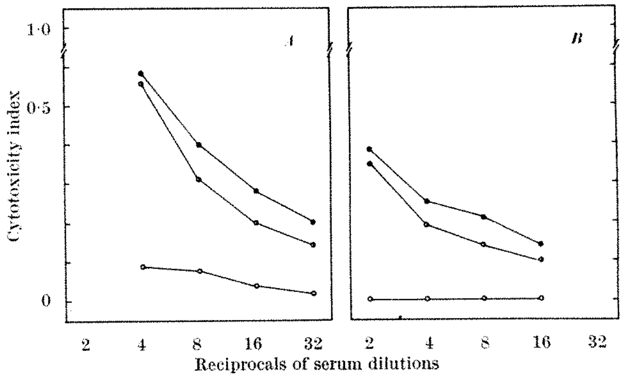


Fig. 3. Results of the absorption tests. *A*, Tests performed with (C57Bl × C3H)F_1 anti-DBA/2 serum; *B*, tests with anti-Moloney serum. ●—●, Unabsorbed sera; ◑—◑, sera absorbed on day 2 after seeding; ○—○, sera absorbed on day 5 after seeding. All sera were tested simultaneously against the same standard cells.

The experiments demonstrate that the expression of antigenic characteristics of YCAB cells during growth *in vitro* is directly proportional to the population doubling time and that an inverse relationship exists between cell volume and the concentration of antigenic receptors on the cell surface. The data do not determine, however, whether the changing pattern of expression of antigenic characteristics is the result of the coating of antigenic receptors[6], or of a restricted period of gene transcription during growth *in vitro*. Experiments are in progress to relate antigenic expression to different phases of the cell cycle. At this state no more than indirect conclusions can be drawn, namely, that the antigenic characteristics of the YCAB cells are fully expressed during *G*1 phase, and that at saturation density YCAB cells are arrested in this phase. These conclusions are based on the inverse relationship of cytotoxic sensitivity and population doubling time, on the one hand, and cell volume on the other[7,8]. It would be of interest to know whether similar events take place *in vivo*, and also whether other phenotypic characteristics of cells are dependent on growth phases.

I thank Professor George Klein for valuable suggestions. The work was supported by grants from the Swedish Cancer Society, the Medical Research Council, the Jane Coffin Childs Fund for Medical Research and Carl-Bertel Nathorst's Foundations; and was done during the tenure of a fellowship awarded by the International Agency for Research on Cancer.

MATKO CIKES

Department of Tumour Biology,
Karolinska Institutet,
Stockholm 60.

Received September 19, 1969.

[1] Herzenberg, L. A., and Roosa, R. A., *Exp. Cell Res.*, **21**, 430 (1960).

[2] Snell, G. D., and Stimpfling, J. H., in *Biology of Laboratory Mouse* (edit. by Green, E. L.) (McGraw-Hill, New York, 1966).

[3] Fedoroff, S., *In Vitro*, **2**, 155 (1967).

[4] Paul, J., *Cell and Tissue Culture* (Williams and Wilkins, Baltimore, 1965).

[5] Klein, E., and Klein, G., *J. Nat. Cancer Inst.*, **32**, 547 (1964).

[6] Bagshawe, K. D., and Currie, G. A., *Nature*, **218**, 1254 (1968).

[7] Killander, D., and Zetterberg, A., *Exp. Cell Res.*, **40**, 12 (1965).

[8] Nilausen, K., and Green, H., *Exp. Cell Res.*, **40**, 166 (1965).

Transplantation Antigens in the Central Nervous System

INVESTIGATIONS into the presence of histocompatibility antigens in mouse brain have given apparently conflicting results. Haemagglutinin[1,2] and cytotoxin[3] inhibition studies have revealed that the brain has an extremely small capacity for absorbing H-2 antibodies, compared with lymphoid tissues. Brain tissue, however, induces transplantation immunity[4] and histocompatibility antigens are detected in neurones of the cerebral cortex by immunofluorescence[5]. The selective localization of the specific fluorescence to the neurones can explain these conflicting results, for the number of stained cells represents only a small proportion of the total brain tissue. This will be reflected by a very low absorptive capacity in quantitative inhibition studies.

I report here the detection of transplantation antigens in the cerebellum of three inbred strains—C3H, CBA and C57Bl—and further confirmation of their selective distribution in the central nervous system.

Table 1. EXPERIMENTAL DESIGN

Tissue donor strain	Control antisera	Antisera to tissue donor
C3H (H-2^k)	C3H anti-E.L.4*	C57Bl-anti-B.P.8† Balb/C anti-B.P.8 129 anti-B.P.8
CBA (H-2^k)	CBA anti-E.L.4	C57Bl anti-B.P.8 Balb/C anti-B.P.8 129 anti-B.P.8
C57Bl (H-2^b)	C57Bl anti-B.P.8	C3H anti-E.L.4 CBA anti-E.L.4 Balb/C anti-E.L.4 A anti-E.L.4

* E.L.4 is an H-2^b leukaemia originating in C57Bl mice.
† B.P.8 is an H-2^k ascitic sarcoma originating in C3H mice.

H-2 antigens were detected with an indirect fluorescent antibody technique. Acetone-fixed or unfixed cryostat sections of mouse cerebellum were incubated with an alloantiserum known to contain H-2 antibodies, washed and treated with fluoresceinated rabbit antibodies to mouse gamma globulins. The experimental design, materials and methods were the same as those previously reported in detail[5]. Table 1 summarizes the experimental design. All antisera were used undiluted and had comparable haemagglutination and cytotoxic titres. The conjugate consisted of a preparation of IgG from a rabbit antiserum to mouse IgG coupled with fluorescein isothiocyanate. The purification of this conjugate and other technical factors were found to be of critical importance and have been fully described[5]. Five controls were performed in each test to assess the specificity of the fluorescent staining. These included incubation with only the conjugate, the "blocking test", absorption of the positive antiserum with tissue donor cells before use, and substitution of the positive antiserum with normal mouse serum. The most reliable control, however, was provided by the genetic system used in which the same antiserum was employed as a control with syngeneic tissues and as a positive antiserum with allogeneic cells (Table 1).

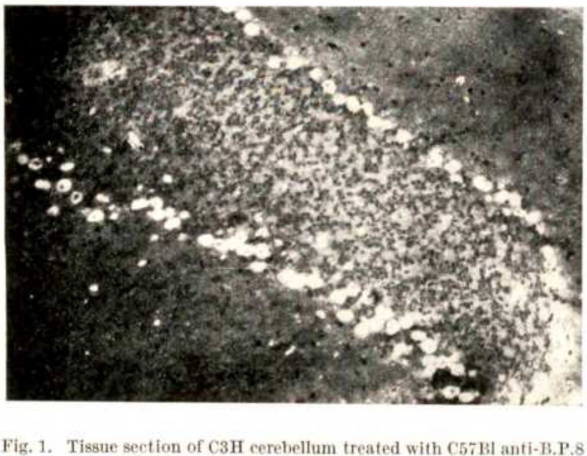


Fig. 1. Tissue section of C3H cerebellum treated with C57Bl anti-B.P.8 and the conjugate. Acetone-fixation. × 50.

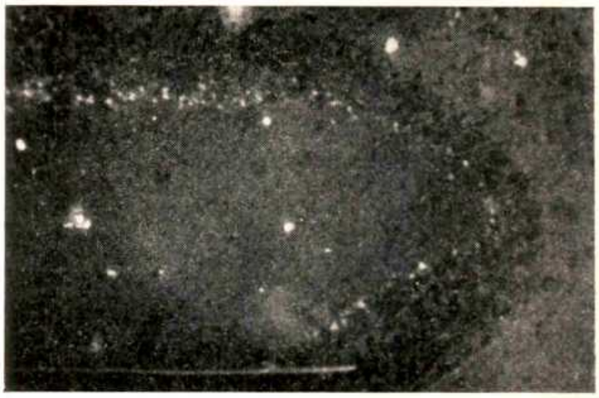


Fig. 2. Tissue section of C3H cerebellum treated with C3H anti-E.L.4 and the conjugate. Acetone-fixation. × 50.

The results showed that the localization of specific green fluorescence was better defined after acetone fixation. Tissue sections treated with positive antisera displayed bright cytoplasmic staining of the Purkinje cells, the nucleus remaining unstained. The cytoplasm of cells of the inner granular layer was also stained, but the fluorescence was not so intense (Fig. 1). In the "blocking test", there was moderate inhibition of the staining. In all other controls, with or without acetone fixation, specific fluorescence was absent (Fig. 2). Bright yellow autofluorescent granules were observed in all tissue sections. This has been attributed to autofluorescence of lysozymes[6].

My results show that the cerebellum, as well as the cerebral cortex, contains H-2 antigenic material and that these antigens are restricted to certain types of cell in the central nervous system. The cells of the granulosa have little cytoplasm and this may explain why their fluorescence appeared less intense than that of the Purkinje cells with abundant cytoplasm. These findings are consistent with the reported ability of cerebellar tissue to induce transplantation immunity[4].

A. G. GERVAIS*

Department of Pathology,
Guy's Hospital Medical School,
London SE1.

Received October 13, 1969.

* Present address: Department of Anatomy, University of Montreal, Canada.

[1] Pizarro, O., Hoecker, G., Rubinstein, P., and Ramos, A., *Proc. US Nat. Acad. Sci.*, **47**, 1900 (1961).

[2] Pizarro, O., Rubinstein, P., and Hoecker, G., *Guy's Hospital Report*, **112**, 392 (1963).

[3] Basch, R. S., and Stetson, C. A., *Ann. NY Acad. Sci.*, **97**, 83 (1962).

[4] Barnes, A. D., *Ann. NY Acad. Sci.*, **120**, 237 (1964).

[5] Gervais, A. G., *Transplantation*, **6**, 261 (1968).

[6] Sarnat, H. B., *Anat. Rec.*, **162**, 25 (1968).

Induction of Hepatic Tyrosine Aminotransferase mediated by a Cholinergic Agent

TYROSINE aminotransferase in the rat liver is subject to regulation by a number of hormones and amino-acids[1-3]. Recent work has indicated that the sympathetic neurotransmitter norepinephrine suppresses the synthesis of tyrosine aminotransferase by forming a complex with the pyridoxal 5′-phosphate cofactor (refs. 4–6 and I. B. B., manuscript submitted for publication). Evidence suggesting mediation of this effect by peripheral autonomic nerves has been presented[7]. Because many of the physiological consequences of sympathetic stimulation are antagonized by parasympathetic discharge with acetylcholine release, the effect of cholinergic agents on enzyme regulation was examined. I report here that the cholinergic agent carbachol (carbamylcholine) induces hepatic tyrosine aminotransferase and that this induction is antagonized by the cholinergic blocking agent atropine.

Adrenalectomized, female, Sprague–Dawley rats (160–180 g) were subjected to a diurnal lighting schedule previously described[4] and offered one per cent NaCl solution *ad libitum*. Animals were used 4 to 6 days after adrenalectomy and were fasted from the onset to the termination of each experiment. All studies were performed between 0800 h and 1200 h.

The effects of administered acetylcholine are evanescent, presumably because of the rapid hydrolysis by acetylcholinesterase and non-specific cholinesterases. The choline ester carbachol, which is resistant to these enzymes, was therefore used to mimic acetylcholine. After treatment, rats were killed by a blow to the head and livers were rapidly removed and frozen. Livers were homogenized in isotonic KCl solution and tyrosine aminotransferase activity was assayed by a modification of the method of Diamondstone[8].

Carbachol administration resulted in a two-fold rise in tyrosine aminotransferase activity within 2 h (Fig. 1). Bethanecol, another cholinergic agent, caused a similar increase in enzyme activity. To determine whether the increased enzyme activity was the result of cholinergic receptor stimulation, additional groups of rats were also treated with atropine. This compound, which competitively antagonizes the effects of cholinergic agents, blocked the rise in tyrosine aminotransferase activity after carbachol. Atropine alone, in the doses used, did not alter enzyme activity (Fig. 1).

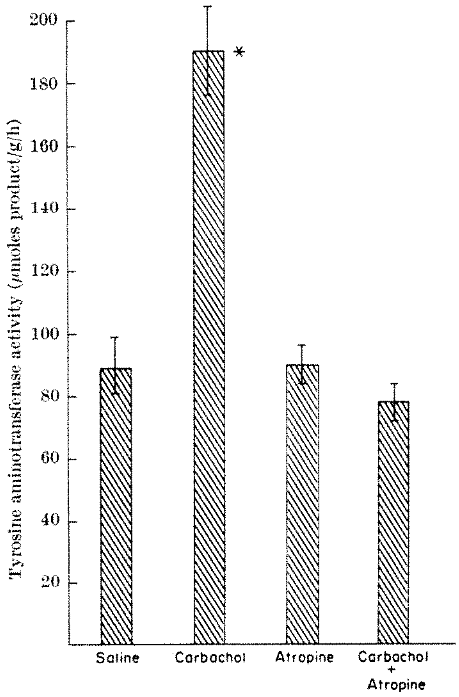


Fig. 1. Specificity of tyrosine aminotransferase activity elevation after carbachol. Rats were injected with carbachol 0·75 mg/kg at 0900 h and/or with atropine 10·0 mg/kg at 0845 h. Controls were treated with saline at appropriate times. All injections were intraperitoneal in a volume of 1 ml. All rats were killed at 1100 h. Results are expressed as mean ± *S.E.* (brackets). * Differs from all other groups at $P < 0\cdot001$.

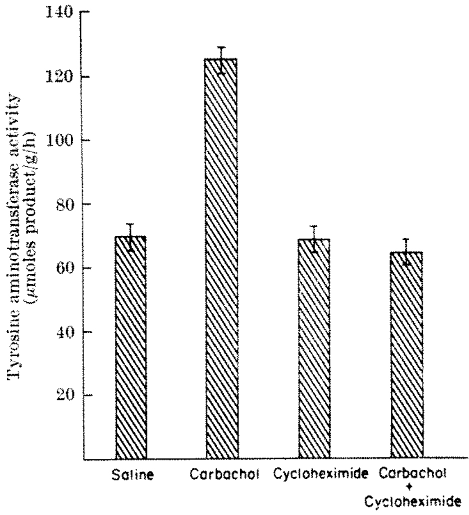


Fig. 2. Induction of tyrosine aminotransferase by carbachol. Rats were treated with carbachol as in Fig. 1, and/or with cycloheximide 20 mg/kg intraperitoneally 15 min earlier. Controls were treated with saline. Animals were killed 2 h after carbachol administration. Results are expressed as mean ± *S.E.* (brackets). The group treated with carbachol above differs from all other groups at $P < 0\cdot001$.

Enzyme activity may increase either through activation of existing molecules or as a result of an increase in the amount of enzyme protein. To distinguish between these alternatives, rats were treated with the inhibitor of protein synthesis, cycloheximide, before receiving carbachol. Cycloheximide prevented elevation of tyrosine aminotransferase by carbachol (Fig. 2), indicating that on-going protein synthesis is necessary for this rise in enzyme activity.

Induction of tyrosine aminotransferase by carbachol and blockage of this effect by atropine suggests that this induction is mediated by a cholinergic receptor. The effect of this cholinergic agent is opposite to that previously described for the sympathetic neuromediator, norepinephrine. Norepinephrine suppresses tyrosine aminotransferase synthesis by binding pyridoxal 5′-phosphate, whereas carbachol induces the enzyme. Thus, although these two neuromediators may be operating at different sites, this enzyme system provides a molecular model for the recognized antagonism of the sympathetic and parasympathetic nervous systems.

The locus of action of carbachol has not been determined. Because acetylcholine has been associated with the release of pancreatic insulin[9] (a known inducer of tyrosine aminotransferase[10]), the drug may be acting through hormonal mediation.

IRA B. BLACK

Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Maryland 20014.

Received October 27, 1969.

[1] Lin, E. C. C., and Knox, W. E., *Biochim. Biophys. Acta*, **26**, 85 (1957).
[2] Greengard, O., Smith, M. A., and Acs, G., *J. Biol. Chem.*, **238**, 1548 (1963).
[3] Rosen, F., and Milholland, R. J., *J. Biol. Chem.*, **238**, 3730 (1963).
[4] Black, I. B., and Axelrod, J., *Proc. US Nat. Acad. Sci.*, **59**, 1231 (1968).
[5] Axelrod, J., and Black, I. B., *Nature*, **220**, 161 (1968).
[6] Black, I. B., and Axelrod, J., *J. Biol. Chem.* (in the press).
[7] Black, I. B., and Axelrod, J., *Fed. Proc.*, **28**, 2632 (1969).
[8] Diamondstone, T. I., *Anal. Biochem.*, **16**, 395 (1966).
[9] Kaneto, A., Kajinuma, H., Kosaka, K., and Nakao, K., *Endocrinology*, **83**, 651 (1968).
[10] Holten, D., and Kenney, F. T., *J. Biol. Chem.*, **242**, 4372 (1967).

Actinomycin-*D*: Effects on Memory at Different Times after Training

INHIBITORS of macromolecular synthesis have been used extensively for studies of memory storage. The amnesic effects of two inhibitors of protein synthesis, acetoxycycloheximide and cycloheximide, have been attributed to specific interference with the synthesis of protein required for formation of long-term memory[1-4]. The effects of actinomycin-*D*, an inhibitor of DNA-dependent RNA synthesis, have been particularly difficult to interpret because doses sufficient to produce marked inhibition of cerebral RNA synthesis produce fairly rapid onset of irreversible systemic toxicity[5,6]. Even much smaller doses produce localized chromatolysis[7] and may lead to death. Studies with rodents injected before learning show that marked inhibition of RNA synthesis does not interfere with retention for a few hours after injection[5,6,8], but the marked toxicity of this drug has limited its usefulness in studies of the participation of cerebral RNA synthesis in long term memory.

In goldfish, intracerebral injection of actinomycin immediately after training has produced impairment of memory tested 3 days later, but injections 1 h after training had no effect[9]. These results were interpreted as supporting the view that participation of cerebral RNA synthesis is involved in the formation of long-term memory. Because of this report, we have investigated further the effects of actinomycin-*D* on memory in mice.

We trained mice in a shock-escape (0·4 mA) position discrimination to a criterion of only two correct responses. Mice learned this discrimination in an average of 3·4 trials (including the two correct trials) and showed good savings as long as 7 days later. Distributions of trials to criterion for learning and retention tests are shown in Fig. 1.

Male Swiss albino mice (25–50 per group) were injected intracerebrally in both temporal or frontal regions of the brain with a total of 30 µl. of mannitol (40 mg/ml.) containing either 30 µg (high dose) or 1 µg (low dose) of actinomycin-*D*. Injections were given 3 h before, 1 day after, or 7 days after training. Control animals were injected with mannitol. All mice were tested for retention 27 h after injection. In contrast to the substantial inhibition of cerebral RNA synthesis found previously[5,6] with high doses of actinomycin-*D*, we found that whole cerebral RNA synthesis was inhibited only 14 per cent or 18 per cent 3 h or 27 h after injection of the low dose.

Uninjected mice and mice injected with mannitol or actinomycin-*D* 3 h before training needed an identical number of trials to learn the position discrimination.

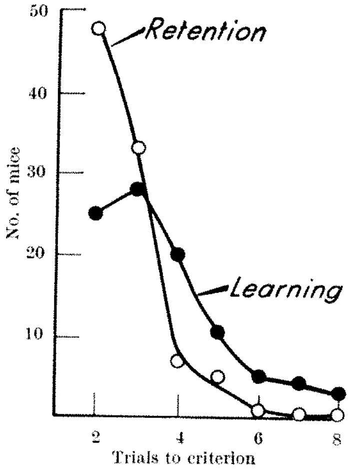


Fig. 1. Distributions of trials needed to reach a criterion of two correct during original learning and retention tests. These distributions represent the pooled results of all ninety-six control (mannitol-injected) mice in the study.

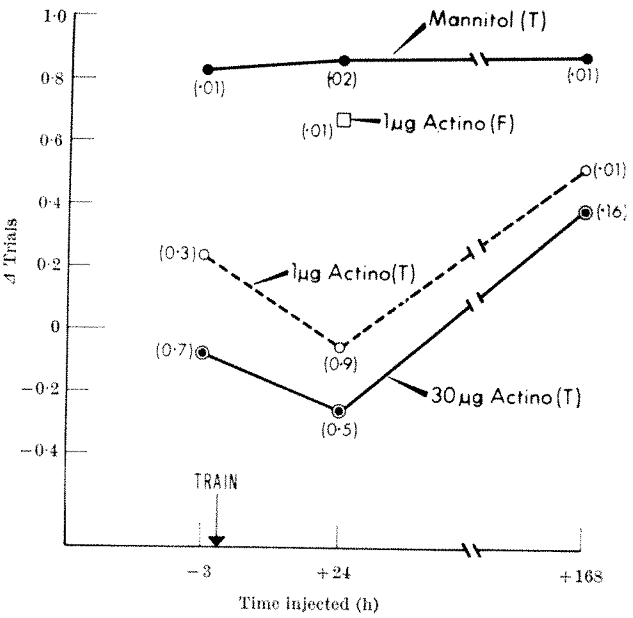


Fig. 2. Effect of injection of actinomycin-*D* or mannitol on retention. Mice were injected (25–50 per group) before or 1 or 7 days after training. Retention was always tested 27 h after injection. Five mice failed to reach criterion within eight trials and were eliminated. Mice ($N = 8$) requiring more than eight trials to relearn the task were assigned a relearning score of 8. T denotes bitemporal injection (1 mm rostral to the caudal sutures, 1 mm lateral to the sagittal suture, and 4 mm below the skull surface). F denotes bifrontal injections (6 mm rostral to the caudal sutures, 1 mm lateral to the sagittal suture and 2 mm below the skull surface). Δ Trials was computed by subtracting the mean number of trials needed to reach criterion on retest from the mean number needed for original learning. For each group, the probability that Δ trials was due to chance is shown in parentheses. The distributions of the difference scores (trials to learn—trials to relearn) for each group closely approximated a normal distribution. Accordingly, two-tailed *t* tests were used to evaluate differences between independent groups. Paired *t* tests, two-tailed, were used to evaluate the significance of savings within groups. Differences between the mannitol group and the group given a high dose of actinomycin-*D* at −3 h, $P < 0{\cdot}02$; at +1 day, $P < 0{\cdot}03$; at +7 days, $P > 0{\cdot}3$. Differences between the mannitol group and the group given the low dose of actinomycin-*D* at −3 h, $P < 0{\cdot}13$; at +1 day, $P < 0{\cdot}04$; at +7 days, $P > 0{\cdot}4$. Differences between the low dose (F) group and the low dose (T) group at +1 day, $P < 0{\cdot}06$. Differences between the 7 day actinomycin-*D* (high and low dose) group and the 1 day actinomycin-*D* (high and low dose) group, $P < 0{\cdot}03$.

Mannitol-treated mice showed significant retention when injected 3 h before, 1 day after or 7 days after training (Fig. 2). In contrast, mice injected bitemporally with actinomycin-*D* 3 h before or 1 day after training gave no evidence of retention. Mice given the high or the low dose of actinomycin-*D* at these times needed as many trials to relearn the task as they required to learn it originally. When injections were given 7 days after learning, there were no significant differences between mice injected with mannitol and mice injected with a high or a low dose of actinomycin-*D*. Mice given the high or low dose of actinomycin-*D* at this time showed, as a group, significantly better retention than mice given the high or low dose of actinomycin-*D* 1 day after learning ($P < 0{\cdot}03$). Further, frontal injections with the low dose of actinomycin-*D* 1 day after training had no effect on retention.

We also found that inhibition of protein synthesis by bitemporal injection of 20 µg acetoxycycloheximide, 1 day after learning this task, had no effect on retention 27 h later (savings significant, $P < 0{\cdot}01$), although substantial prolonged inhibition is known to result from such a dose[1-3]. Both the high and low doses of actinomycin-*D* did impair retention 1 day after training, so it seems that the drug is not acting by inhibiting RNA synthesis specifically needed to direct the synthesis of protein needed for long-term memory storage. Furthermore, the low dose (1 µg) of actinomycin-*D* has relatively little effect on cerebral RNA synthesis, although it is enough to produce hippocampal cellular damage and abnormalities in electrical activity[7]. We believe that these results have no direct bearing on the hypothesis that cerebral RNA is specifically required for long-term memory. Rather, these effects may result from non-specific (toxic) actions of the drug.

The report that actinomycin-*D* produces a brief temporal gradient of retrograde amnesia in goldfish[9] is in sharp contrast to the long gradient that we find. Differences in length of retrograde amnesia gradients are found with a variety of treatments[10], and may result from differences in motivation, task or species. It is possible that the necrosis and electrical abnormality known[7] to be produced by actinomycin-*D* may be responsible not only for the long gradient of retrograde amnesia which we found, but for shorter gradients as well. Results with goldfish and mice, however, have often been different[11-13]. Accordingly, in the absence of evidence that actinomycin-*D* produces neuronal abnormalities in the goldfish, care should be taken in generalizing this interpretation from one species to another.

Although they do not seem to be relevant to the possible participation of cerebral RNA synthesis in memory storage, our results are interesting in another respect. The fact that bitemporal actinomycin-*D* injections impaired retention if given 1 day but not 7 days after training suggests that some aspect of the memory formation process is changing during the interval. The effects of bitemporal injections of puromycin or of anticholinergic or anticholinesterase drugs suggest that the memory storage process changes for several days after training[13-17]. Our study thus neither supports nor falsifies the hypothesis that cerebral RNA synthesis participates in memory storage. Actinomycin-*D* seems to have limited usefulness for investigating the role of specific macromolecules in memory formation. The results do, however, support the suggestion that, at least in the case of undertrained habits, intact hippocampal function is needed for 1 or more days after training, but not 7 days after training. This suggestion may also apply to more overtrained tasks[13,14].

Our work was supported by grants from the National Institutes of Health, US Public Health Service.

LARRY R. SQUIRE
SAMUEL H. BARONDES

Albert Einstein College of Medicine,
Yeshiva University,
New York.

Received October 27; revised December 8, 1969.

[1] Barondes, S. H., and Cohen, H. D., *Proc. US Nat. Acad. Sci.*, **58**, 157 (1967).
[2] Cohen, H. D., and Barondes, S. H., *Nature*, **218**, 271 (1968).
[3] Cohen, H. D., and Barondes, S. H., *Comm. Behav. Biol.*, **1**, 337 (1968).
[4] Agranoff, B. W., Davis, R. E., and Brink, J. J., *Brain Res.*, **1**, 303 (1966).
[5] Barondes, S. H., and Jarvik, M. E., *J. Neurochem.*, **11**, 187 (1964).
[6] Cohen, H. D., and Barondes, S. H., *J. Neurochem.*, **13**, 207 (1966).
[7] Nakajima, S., *J. Comp. Physiol. Psychol.*, **67**, 457 (1969).
[8] Goldsmith, L., *J. Comp. Physiol. Psychol.*, **63**, 126 (1967).
[9] Agranoff, B. W., Davis, R. E., Casola, L., and Lim, R., *Science*, **158**, 1600 (1967).
[10] Booth, D. A., *Psychol. Bull.*, **68**, 149 (1967).
[11] Agranoff, B. W., Davis, R. E., and Brink, J. J., *Proc. US Nat. Acad. Sci.*, **54**, 788 (1965).
[12] Davis, R. E., Bright, P. J., and Agranoff, B. W., *J. Comp. Physiol. Psychol.*, **60**, 162 (1965).
[13] Flexner, J. B., Flexner, L. B., and Stellar, E., *Science*, **141**, 57 (1963).
[14] Rosenbaum, M., Cohen, H. D., and Barondes, S. H., *Comm. Behav. Biol.*, **2**, 47 (1968).
[15] Deutsch, J. A., Hamburg, M. D., and Dahl, H., *Science*, **151**, 221 (1966).
[16] Deutsch, J. A., and Rocklin, K. W., *Nature*, **216**, 89 (1967).
[17] Wiener, N., and Deutsch, J. A., *J. Comp. Physiol. Psychol.*, **66**, 613 (1968).

Effects of an Imprinting Procedure on Regional Incorporation of Tritiated Uracil into Chick Brain RNA

WE have demonstrated previously that an imprinting procedure influences the rate of incorporation of amino-acid into acid insoluble substances in the upper part of the chick's forebrain[1]. In so far as changes in incorporation rate reflect changes in the rate of protein synthesis, these should be reflected also in an altered rate of RNA production. In addition, the time sequence of events at the biochemical level within the brain should be one in which an increase in RNA production should precede the increase in protein. We describe here the effects of varying amounts of exposure to an imprinting stimulus on the incorporation of tritiated uracil into RNA of different regions of chick brain.

Eight batches of Chunky chicks were hatched and kept in a dark incubator until the start of the experiment. All the birds hatched 6–12 h before the peak period of hatching. Experiments were begun at a mean post-hatch age of 17·03 ± 0·25 h. Chicks from each of the batches were distributed into three groups (experimental, light control, dark control).

The experimental group was exposed to a flashing light, previously shown to be a highly effective imprinting stimulus[2], and the light control group was exposed to an overhead light as described earlier. A total of thirty-five chicks (dark control) were kept in darkness from hatching to death. The period of exposure of the experimentals and light controls was varied; twelve experimentals and twelve light controls received 38 min of exposure, nineteen experimentals and nineteen light controls received 76 min, and eighteen experimentals and eighteen light controls received 160 min. Each chick in the experimental group exposed for 160 min was placed in a testing alley for 10 min during which the speed of approach to a flashing light was measured.

Meanwhile the light control was kept in a lit pen at the same temperature (23·5°–26·5° C) as the testing alley. Immediately after the initial exposure each chick was weighed and given an intracardiac injection of 0·1 ml. Locke solution containing 20 μCi 5-^{3}H-uracil (1,000 mCi/mmole). For 150 min after injection the experimental group was exposed to the flashing light and each light control was isolated in a pen illuminated by a 60 W bulb. Both groups were kept at 28°–31° C. None of the chicks exposed to light for shorter periods of time was tested before injection; initially, after injection they were all placed singly in dark compartments at 28°–31° C. The 76 min groups were kept in the dark for 37 min before and after their period of exposure to light and the 38 min groups for 56 min before and after. All handling of chicks was done in the dark or under dim green light.

Each chick was placed in the testing alley 150 min after injection and its speed of approaching the flashing light was measured. It was then decapitated. The brain was removed and three regions dissected out (midbrain, forebrain roof and forebrain base). The brain samples and a piece of liver tissue were placed in individual pots and kept frozen on solid CO_2 until analysis. The samples were treated essentially as described previously[3]. They were homogenized in 5 ml. of ice-cold 0·9 per cent NaCl and portions of the homogenate taken for (*a*) protein assay, (*b*) determination of total (pool) radioactivity and (*c*) radioactivity associated with nucleic acid. Acid insoluble radioactivity was determined following precipitation and three washes with 10 per cent trichloroacetic acid containing 0·1 per cent uracil. Radioactivity in the samples was counted and specific activity calculated as described previously[1,3]. The figures were corrected to a standard body weight of 50 g. Preliminary experiments showed that incorporation of radioactivity from uracil was linear over 3 h from the injection of precursor, and amounted at the end of that period to some 4 per cent of pool radioactivity. Entry into the pool was thus not rate-limiting in these conditions.

To eliminate variability between batches, the specific activity of each sample of tissue in a batch was divided by the mean for all brain samples from the dark control group in that batch. The results are therefore standardized and expressed as percentages of the mean for the dark

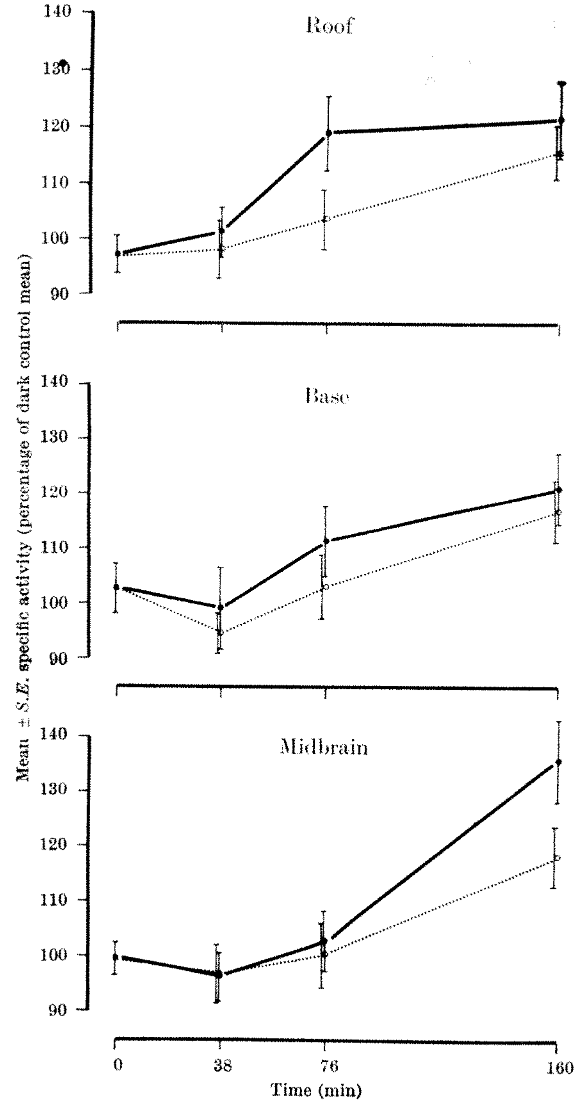


Fig. 1. Means and standard errors of standardized specific activities of RNA from the forebrain roofs, forebrain bases and midbrains of differently treated chicks. All chicks were injected with tritiated uracil and the time from injection to killing was kept constant. The experimental birds (———) were exposed to a flashing light and the light controls (. . .) to a continuous light.

controls. Fig. 1 gives the mean values and standard errors of the standardized specific activities (s.s.a.) for different regions of the brain after each treatment.

No change in the mean values of s.s.a. could be detected after 38 min of exposure in either the experimental or light control group. After 76 min exposure, a highly significant increase had occurred in the s.s.a. of the forebrain roofs of the experimental group (Table 1). No other differences between the dark controls (0 min exposure) and the experimental and light control groups exposed for 76 min are statistically significant. But after 160 min of exposure, the mean s.s.a. in all three brain regions in both experimental and light control groups are significantly greater than those in the dark controls (Table 1). The experimental group differs significantly from the light control in the forebrain roof after 76 min of exposure ($d = 1{\cdot}81$, $P < 0{\cdot}05$ 1-t) and in the midbrain after 160 min of exposure ($d = 1{\cdot}93$, $P < 0{\cdot}05$ 1-t). No significant differences were found in the liver at any time.

Table 1. VALUES OF d and PROBABILITY LEVELS FOR STATISTICAL COMPARISONS BETWEEN THE DARK CONTROL GROUP (0 MIN) AND (a) EXPERIMENTAL AND (b) LIGHT CONTROL GROUPS EXPOSED FOR 76 AND 160 MIN

Time of exposure (min)	Group	Roof	Base	Midbrain
76	Experimental	3·00†	1·13	0·48
	Light control	1·07	0·03	0·08
160	Experimental	3·23†	2·38*	4·70‡
	Light control	3·14†	2·05*	3·08†

* $P < 0{\cdot}05$. † $P < 0{\cdot}01$. ‡ $P < 0{\cdot}001$.

Clearly RNA synthesis in the brains of day-old chicks is very sensitive to visual stimulation. By varying the time of exposure to such stimulation while keeping the time from injection to killing constant, the effects of different treatments on different brain regions can be distinguished. Particularly striking is the rapid increase in RNA synthesis in the forebrain roof of experimental birds. This is the region where we previously detected an increase in protein synthesis after early hatching chicks had been exposed to an imprinting stimulus[1]. We should point out that because mere exposure to a continuously illuminated environment eventually affects RNA synthesis in all brain regions, the causes of the more rapidly occurring change in the forebrain roof of experimental birds are not clear. On the one hand, the difference between experimental and light control groups at 76 min might have arisen because the experimental birds merely received more visual stimulation. On the other hand, the difference might have been related in a more direct way to the imprinting process.

We thank Mr A. K. Sinha for technical assistance. This work was supported by grants from the Agricultural Research Council to P. P. G. B. and from the US Public Health Service to G. H.

S. P. R. Rose

Department of Biology,
Open University,
Walton, Bletchley, Buckinghamshire.

P. P. G. Bateson

Sub-Department of Animal Behaviour,
University of Cambridge.

Ann L. D. Horn
G. Horn

Department of Anatomy,
University of Cambridge.

[1] Bateson, P. P. G., Horn, G., and Rose, S. P. R., *Nature*, **223**, 534 (1969).
[2] Bateson, P. P. G., and Reese, E. P., *Anim. Behav.*, **17**, 692 (1969).
[3] Rose, S. P. R., *Nature*, **215**, 253 (1967).

Investigation of *p*-Methoxyamphetamine Excretion in Amphetamine Induced Psychosis

Many differences exist between the symptoms of schizophrenia and those of drug-induced states caused by agents like LSD or mescaline[1]. Amphetamine psychosis, by contrast, can in some cases replicate virtually all of the manifestations of natural schizophrenic illness[2–5]. Such a correspondence justifies a study of the biochemical basis of this condition.

A provocative hypothesis about the genesis of amphetamine psychosis has been proposed[6]. Smythies *et al.* suggest that *p*-methoxyamphetamine (PMA) may be formed from amphetamine in patients who develop psychotic symptoms. In an animal test system utilizing a conditioned avoidance response and analysis of reaction time which is designed to correlate with human psychotogenicity[6,7], Smythies has found PMA to be highly potent as a disruptor of behaviour—second only, in this respect, to LSD of all the compounds tested[6]. Shulgin *et al.* have established that PMA is psychotogenic in humans and also that it has an order of potency of approximately five times that of mescaline[8]. The doses of amphetamine required to induce a psychosis in humans are such that pharmacologically active amounts of this metabolite could be produced. We therefore examined the urine of subjects who were receiving amphetamine in psychotogenic doses to ascertain the presence or absence of PMA.

The subjects were four physically healthy non-schizophrenic volunteers to whom amphetamine was administered orally in doses of 5–50 mg/h for as long as was tolerated. A mean total cumulative dose of 629 mg was administered over an average period of 51 h. The method of administration and behavioural effects have been reported[5].

Urine was collected continuously in 6–12 h pooled specimens throughout amphetamine administration. The specimens representing hours before and immediately after amphetamine was last administered were examined. Urinary amphetamine was assayed by a method in which an internal standard, phenethylamine, is added to a 5 ml. aliquot of each urine sample, in duplicate. The amines, extracted with chloroform at *p*H 10, are acetylated directly with acetic anhydride. The residue on evaporation is dissolved in dioxane and a portion is injected into an F and M 'Biomedical 400' gas chromatograph fitted with a six foot U tube packed with 8 per cent ethylene glycol adipate on 'Chromosorb W'. For 8 inches at the two ends the packing was 3 per cent 'SE–30' on 'Diatoport S'. The column temperature was 215°.

The amount of amphetamine present can be calculated by comparing the height of its chromatographic peak with that of the internal standard[9]. PMA was assayed with the same method. Retention times for N-acetylamphetamine and N-acetyl-PMA were, respectively, 4 and 15 min. From peak height measurements of standard amounts (0·1–1·0 μg) of N-acetyl-PMA and, independently, from recovery studies of PMA added to urine, it is estimated that the limit of detectability of PMA in these urines is about 70–80 μg/l. of urine. In none of the nine studies was PMA detected even though large amounts of amphetamine were present in these urine samples (Table 1).

Table 1. SCHEDULE OF TESTING FOR PMA EXCRETION

Subject and trial	Cumulative dose (mg)	Duration of administration (h)	Urine volume (ml.)	Amphetamine excreted (mg)	Psychotic symptoms
1 A	325	28·75	95	10·0	Present
B	745	64·25	36·5	14·2	Present
C(+R)	950	54	735	123	Absent
2 A	390	45·75	485	18·3	Minimal
B	590	74·75	325	23·4	Minimal
3 A	955	71·5	245	21·4	Absent
B(+R)	465	26	405	9·20	Absent
4 A	595	46	79	9·05	Present
B(+R)	645	47	490	14·9	Absent

PMA was not detected in any of the trials. R indicates pretreatment with reserpine, which seemed to prevent the occurrence of psychotic symptoms[5].

Our study does not support the suggested formation of PMA from ingested amphetamine as a mechanism for the genesis of amphetamine psychosis, but the distinct possibility remains that amphetamine could undergo such transformation within the central nervous system and not peripherally, and then subsequently be further transformed before being excreted in the urine. Even the transient production of such a substance, should it occur at critical sites in the central nervous system, could cause significant behavioural effects. In partial support of this alternative, we have found that N-^{3}H-acetyl-PMA is almost entirely O-demethylated in rats *in vivo*.

We thank Dr Alexander Shulgin for the *p*-methoxyamphetamine used as a chromatographic standard for preliminary studies. The standards were synthesized in our laboratory.

B. M. ANGRIST
J. W. SCHWEITZER
A. J. FRIEDHOFF
S. GERSHON

Department of Psychiatry and Neurology,
New York University School of Medicine,
550 First Avenue, New York.

Received July 28; revised September 9, 1969.

[1] Hollister, L., *Chemical Psychosis, LSD and Related Drugs*, 117 (Thomas, Springfield, 1968).

[2] Connell, P. H., *Amphetamine Psychosis*, Maudsley Monographs, No. 5, 75 (Oxford University Press, London, 1958).

[3] Griffith, J. J., Oates, J., and Cauanaugh, J., *J. Amer. Med. Assoc.*, **205**, 39 (1968).

[4] Angrist, B. M., and Gershon, S., *Schizophrenia. Current Concepts and Research* (edit. by Siva Sankar, D. V.), 508 (P. J. D. Publications Ltd, 1969).

[5] Angrist, B. M., and Gershon, S., *Proc. Soc. Biol. Psychiat.*, 1970 (in the press).

[6] Smythies, J. R., Johnston, U. S., Bradley, R. J., Bennington, F., Morin, R. D., and Clark, jun., L. C., *Nature*, **216**, 128 (1967).

[7] Smythies, J. R., Copper, A., and Kreitman, N., *Biological Psychiatry, A Review of Recent Advances*, 70 (Springer-Verlag, New York, 1968).

[8] Shulgin, A. T., Sargent, T., and Naranjo, C., *Nature*, **221**, 537 (1969).

[9] Angrist, B. M., Schweitzer, J. W., Friedhoff, A. J., Gershon, S., Hekimian, L. J., and Floyd, A., *Internat. Pharmacopsychiat.*, **2**, 125 (1969).

Inhibition of ^{3}H-Thymidine Incorporation into Rat Liver Nuclei by *E. coli* L-Asparaginase

THERE have been numerous reports dealing with the antitumour activity of *Escherichia coli* L-asparaginase in the therapy of human leukaemia. It has been postulated that the agent is without toxic action on normal tissues and that it is specific for asparagine dependent tumour cells, but neither of these assumptions has been proved[1,2]. When L-asparaginase was used in the treatment of human cancer, a satisfactory effect could be seen only with acute lymphoblastic leukaemia[3–5], but many investigators emphasized the toxicity of L-asparaginase. An alteration of liver function[2,5–7] has been demonstrated by the finding of hepatic lipoidosis; a marked increase in bromsulphthalein retention, and in serum levels of cholesterol, triglycerides and phospholipid; a decrease in human serum proteins and the demonstration of various clotting disorders in the course of therapy. Other authors[8] observed the total inhibition of the early wave of mitotic activity in regenerating rat liver approximately 30 h after partial hepatectomy. We decided to study the effect of L-asparaginase preparations of high specific activity on DNA synthesis in rat liver nuclei, and to determine nuclear DNA and RNA polymerase activity in rat liver under the influence of L-asparaginase.

The methods used in this study—with a few modifications—have been described[9–11]. Female Wistar rats with an average initial weight of 90 g have been used throughout this study. *E. coli* L-asparaginase (220 IU/mg protein, crystal suspension in 3·5 M $(NH_4)_2SO_4$) was a gift from Boehringer, Mannheim. The enzyme was injected intraperitoneally in an adjusted dilution of the crystal suspension which contained the administered doses in a solution volume of 0·5 ml. In all experiments control animals were injected with the same volume of an equimolar solution of $(NH_4)_2SO_4$. Labelling of nuclear DNA was achieved by means of a 1 h pulse of ^{3}H-thymidine (20 μCi/animal; 5 Ci/mmole) given intraperitoneally in 0·5 ml. 0·9 per cent NaCl. The nuclei were isolated using the procedures described elsewhere[11–13]. DNA as well as RNA polymerase assay followed the description in previous studies[9–11]. Nuclear DNA was estimated essentially as described by Burton[14]. To determine the incorporation of ^{3}H-thymidine into nuclear DNA, aliquot suspensions of liver nuclei were precipitated and washed with 2 per cent perchloric acid and radioactivity was counted in a toluene scintillator[15]. The results are presented in Tables 1–3.

Table 1. ^{3}H-THYMIDINE INCORPORATION INTO NUCLEAR DNA OF RAT LIVER FOLLOWING INTRAPERITONEAL INJECTION OF L-ASPARAGINASE

Group No.	Time of L-asparaginase injection before death (h)	Dose L-asparaginase (IU/kg)	Radioactivity (c.p.m./mg DNA)	Per cent inhibition
1	—	—	2,194 ± 1,313	—
2	1	780	478 ± 191	78 $P < 0.02$
3	4	780	912 ± 468	58 $P < 0.02$

Four animals were used in each group.

Perkin-Elmer offers you the most complete range of U.V. Spectrophotometers in the world!

One of them is ideal for your application. For instance, the new Model 402 scans from 190 to 850 nm in only two minutes and gives you built-in ordinate scale expansion, selectable wavelength limit switches, time drive and repetitive scanning, Flowchart recording, plus automatic multiple sampling to follow enzyme reactions. The Model 124 is a double-beam instrument with manual or recording operation, whilst the Model 139 has an extensive range of accessories for unsurpassed versatility. The Model 6/20 is robust and reliable, ideal for routine clinical use. The Model 356 is a dual wavelength instrument which can record single-beam, double-beam or derivative spectra and give accurate measurements on turbid samples.

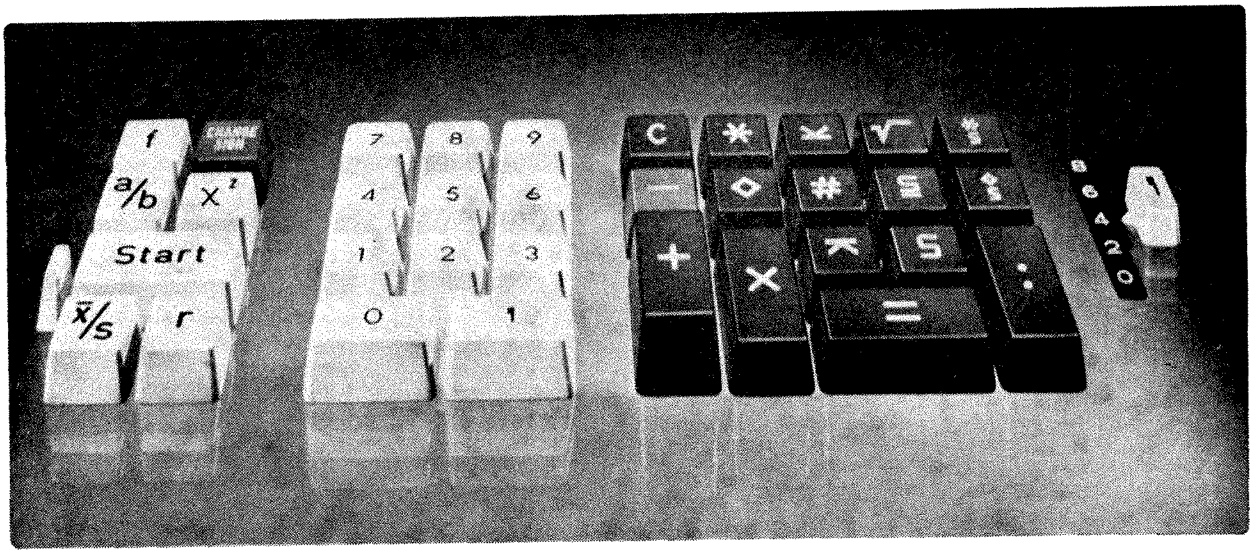
Get details from
Perkin-Elmer Ltd (UVGB),
Beaconsfield, Bucks,
Tel: Beaconsfield 2571
Telex 83257.

Perkin-Elmer Instruments: Infrared, Ultraviolet, Fluorescence and Atomic Absorption Spectrophotometers, Gas and Liquid Chromatographs, Spectropolarimeters, Elemental and Thermal Analysers, Mass and NMR Spectrometers.

PERKIN-ELMER

DIEHL sigmatron

Electronic Calculating System with Pre-programmed Statistical functions



DIEHL sigmatron is the first calculating system specifically designed for Statistical calculations. By pre-programmed functions, Statistical calculations are reduced to one key operations. For instance, to obtain the Arithmetic Mean and Standard Deviation enter data on the keyboard and touch the pre-programmed key. After entry of the data is completed you need only touch the same pre-programmed key to obtain the results immediately. Linear Regression, Chi Square, Coefficient of Correlation are all reduced to the simplicity of one key operation. DIEHL sigmatron also gives you a powerful and effective calculating performance with ten separate memories, automatic square root, automatic decimal selection up to 8 places and a complete check of each figure, each step and each result.

If you prefer to make your own programs, DIEHL combitron S has 10 Program/Memory registers providing a capacity of 120 steps with branching and looping.

For external input of data, DIEHL dilector – punched tape reader – is available.

Please cut out!

DIEHL Calculating Systems 8500 Nürnberg 2 W · Germany

Please send me details of DIEHL Calculating Systems

Name ______________ Title ______________

Address ______________________________

City ______________ Country ______________

Diehl

Table 2. DNA POLYMERASE ACTIVITY OF RAT LIVER NUCLEI

	c.p.m./mg DNA 0 min	10 min	20 min
Controls	461 ± 60	14,475 ± 1,990	22,400 ± 3,505
+ L-Asparaginase	640 ± 74	14,600 ± 1,410	22,800 ± 1,617

Four rats received 780 IU/kg L-asparaginase 2 h before being killed. Four control animals received 0·5 ml. 0·43 M $(NH_4)_2SO_4$. The incubation mixture contained 80 μM each of dATP, dGTP, dCTP; 1 μCi dTTP (5 Ci/mmole); 0·5 ml. nuclear suspension (corresponding to 0·8 mg nuclear DNA); 21·1 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (Hepes) buffer; 16·5 mM $MgCl_2$; 0·312 M sucrose. The *p*H was 8·1 and the total volume 0·8 ml.

Table 3. RNA POLYMERASE ACTIVITY OF ISOLATED RAT LIVER NUCLEI IN THE PRESENCE OF L-ASPARAGINASE

Group No. (3 tests/group)	Radioactivity (c.p.m./mg DNA) 0 min	10 min	20 min
1 (control)	400 ± 50	10,720 ± 400	16,030 ± 1,250
2 (+1·5 IU/sample)		12,500 ± 1,800	16,640 ± 2,100
3 (+7·5 IU/sample)		11,680 ± 390	16,280 ± 160
4 (+15 IU/sample)		11,380 ± 1,400	15,870 ± 110

L-Asparaginase was added directly to the incubation mixture which contained, in a final volume of 0·8 ml.: 50 mM Tris (*p*H 8·5); 5·6 mM spermidine · 3 HCl; 8 mM NaF; 2·5 mM phosphoenolpyruvate; 1·875 mM $MgCl_2$; 45 μM each of ATP, CTP and GTP; 0·45 μM ^{3}H-UTP (1 μCi); 1–2 IU pyruvate kinase; nuclear suspension corresponding to 0·6 mg nuclear DNA; 0·094 M sucrose. L-Asparaginase (Boehringer) was dissolved in 0·1 ml. H_2O; controls were dissolved in 0·1 ml. 0·43 M $(NH_4)_2SO_4$.

In another experiment, four rats were given 900 IU/kg L-asparaginase 90 min before being killed. In these conditions, the average labelling of nuclear DNA in the control group (four animals) was found to be 1,262 ± 220 c.p.m./mg DNA and that of the L-asparaginase treated group only 510 ± 158 c.p.m./mg DNA. This difference was highly significant ($P < 0.002$). There is no influence of the enzyme on nuclear DNA and RNA polymerase even when pre-injected *in vivo* (Tables 2 and 3). When L-asparaginase (15 IU/sample) was added to the incubation mixture directly, no effect was detectable either.

Although a satisfactory explanation for the rapid and remarkable inhibition of ^{3}H-thymidine uptake into rat liver cell nuclei caused by L-asparaginase cannot yet be given, these data constitute further evidence for the hepatotoxicity of the enzyme. A similar effect has been shown with leukaemic lymphocytes[2,16]. Using a highly purified preparation (specific activity 220 IU/mg protein), any toxic effects of contaminating *E. coli* endotoxins should have been excluded.

We thank Miss Margot Ickelsheimer for technical assistance. The study was aided by grants from the Deutsche Forschungsgemeinschaft.

SIEGFRIED SEEBER*
ULRICH WESER

Institute of Physiological Chemistry,
74 Tübingen,
Germany.

Received November 24, 1969.

---

* Present address: Department of Pharmacology, Baylor University, Texas Medical Center, Houston, Texas 77025.

[1] Schein, P. S., Rakieten, N., Gordon, B. M., Davis, R. D., and Rall, D. P., *Cancer Res.*, **29**, 426 (1969).

[2] Hirschmann, W. D., Seeber, S., and Warnecke, P., *Klin. Wschr.* (in the press).

[3] Dolowy, W. C., Henson, D., Cornet, J., and Sellin, H., *Cancer*, **19**, 1813 (1966).

[4] Hill, J. M., Roberts, J., Loeb, E., Khan, A., MacLellan, A., and Hill, R. W., *J. Amer. Med. Assoc.*, **202**, 882 (1967).

[5] Oettgen, H. F., Old, L. J., Boyse, E. A., Campbell, H. A., Philips, F. S., Clarkson, B. D., Tallal, L., Leeper, R. D., Schwartz, M. K., and Kim, J. H., *Cancer Res.*, **27**, 2619 (1967).

[6] Oettgen, H. F., and Schulten, H. K., *Klin. Wschr.*, **47**, 65 (1969).

[7] Stier, H. W., Gallmeier, W. M., and Schmidt, C. G., *Dtsch. Med. Wschr.*, **94**, 253 (1969).

[8] Becker, F. F., and Broome, J. D., *Science*, **156**, 1602 (1967).

[9] Weser, U., Seeber, S., and Warnecke, P., *Biochim. Biophys. Acta*, **179**, 422 (1969).

[10] Weser, U., Seeber, S., and Warnecke, P., *Z. Naturforsch.*, **246**, 866 (1969).

[11] Möller, H., thesis, Univ. Tübingen (1969).

[12] Higashi, K., and Busch, H., *Biochim Biophys. Acta*, **103**, 339 (1965).

[13] McGregor, R. R., and Mahler, H. R., *Arch. Biochem. Biophys.*, **120**, 136 (1967).

[14] Burton, K., *Biochem. J.*, **62**, 315 (1958).

[15] Weser, U., *Z. Physiol. Chem.*, **349**, 989 (1968).

[16] Seeber, S., Warnecke, P., and Straub, O. C., *Z. Arzneimittelforsch.*, **19**, 1745 (1969).

Changes underlying Halothane-induced Malignant Hyperpyrexia in Landrace Pigs

MALIGNANT hyperpyrexia is a rare but often fatal complication of inhalation anaesthesia in man[1]. The fluorinated hydrocarbon halothane, $CF_3CHBrCl$, is among the many anaesthetic agents which have been implicated[2]. A similar syndrome has been described in a pure Landrace strain of pig[3]. The onset of this phenomenon is rapid (10–15 min after administration of halothane) and there is extreme rigidity of skeletal muscles, a rapid rise in body temperature to 42°–45° C, severe acidosis and a fatal outcome in spite of cooling, muscle relaxants and intravenous alkalis. Once initiated, the process seems independent of the tissue concentration of halothane, which appears to act as a trigger. A few animals have recovered after brief exposure to halothane.

Wilson *et al.* have suggested uncoupling of oxidative phosphorylation as a possible mechanism[2]. The process is augmented by dinitrophenol and halothane is known to uncouple oxidative phosphorylation in normal rat liver mitochondria *in vitro*[4].

All animals studied were of pure Landrace or Landrace–Large White cross strains. No untoward effects of thiopentone were observed in susceptible pigs over periods of up to 150 min. Monitoring was accordingly established under controlled steady state conditions of thiopentone anaesthesia with pigs breathing oxygen. Observations made during thiopentone anaesthesia were compared with those following halothane. Arterial blood samples and pressure readings were taken from a femoral catheter. Venous blood was withdrawn from an indwelling right atrial catheter. Temperatures of the core (oesophagus and rectum), liver, muscle and skin were recorded by thermistor probes. Oxygen uptake was measured with a Benedict–Roth spirometer and respiration was assisted during apnoeic periods by manual chest compression. Carbon dioxide output was determined gravimetrically after absorption in $CaCl_2$/soda-lime. The acid-base status of the blood was measured by the Astrup technique[5]. Total CO_2 content, serum concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, total protein and inorganic phosphate (P_i) were determined by standard clinical laboratory techniques. Blood lactate[6] and pyruvate[7] were determined enzymatically. Blood oxygen tensions were measured with a Clarke electrode. Biopsies of muscle were assayed for glycogen[8] and ATP[9].

Anaesthesia with thiopentone for up to 150 min had no effect on organ temperatures. Within 10 min (usually 3–4 min) of introducing halothane, the temperature of all organs rose, except skin (Fig. 1). Cooling of the latter and cyanotic blotchiness indicated decreased circulation. After discontinuing halothane, the temperature continued to rise and maximum core temperature (oesophagus) was reached at 30–45 min.

O_2 consumption and CO_2 output after giving halothane were compared with control periods of isothermia on thiopentone anaesthesia. The results (Fig. 2) reveal augmented uptake of oxygen, but the disproportionate rise in CO_2 output resulted in high respiratory quotients (1·69 in one experiment). Oxygen consumption rose 20–40 per cent per °C rise in core temperature. Between 38° and 39° C, passive heating raises O_2 consumption 18 per cent in rats[10] and 12–15 per cent in normal human adults[11]. Increased oxygen consumption appeared therefore to be slightly greater than that expected from elevated temperature alone.

Table 1 presents some metabolic changes observed during the development of malignant hyperpyrexia. These changes, initiated by halothane, were: (*a*) Shift of water into the intracellular space; serum Na^+ and total protein concentrations both increased. (*b*) Shift of Ca^{2+} and Mg^{2+} into the extracellular space.

Concentrations of these two cations rose disproportionately with respect to serum protein concentration assuming 50 and 30 per cent, respectively, to be protein bound. (*c*) Release of P_i into plasma. (*d*) Increase in blood glucose. In control animals blood glucose tended to fall from values of 60 to 80 to below 50 mg/100 ml. after 2 to 3 h of anaesthesia. (*e*) Uncompensated metabolic acidosis resulting from a rise in plasma lactate. The base deficit was equivalent to the increase in plasma lactate and reflected a pure lactic acid acidosis. A respiratory defect, denoted by lowered arterial pO_2 in spite of administering 100 per cent oxygen, could be partly responsible for elevated pCO_2. In spite of diminished respiratory gaseous exchange, however, raised CO_2 output, measured gravimetrically (Fig. 2), doubtless arose from the reaction: $H^+ + HCO_3^- \rightarrow H_2O + CO_2$. Although both plasma lactate and pyruvate concentrations increased, "excess lactate", calculated according to Huckabee's formula[12], indicated a net shift in the equilibrium $NADH + H^+ + pyruvate \rightleftharpoons lactate + NAD^+$, in favour of lactate; presumably the result of accumulation of NADH.

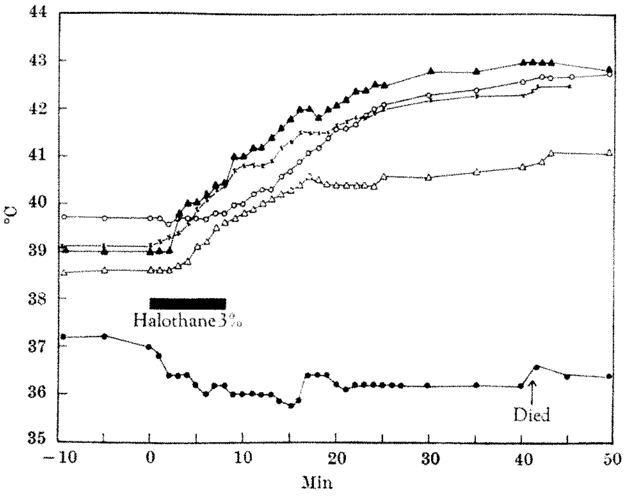


Fig. 1. Temperature changes in organs of pig 82A induced by halothane. While anaesthetized with thiopentone alone, no significant changes took place in those portions of the curves from −150 to −10 min. Halothane, 3 per cent (v/v) in O_2, was commenced at 0 min. and discontinued at 8 min. ▲, Liver; ○, rectum; ×, oesophagus; △, muscle (gluteus); ●, abdominal skin.

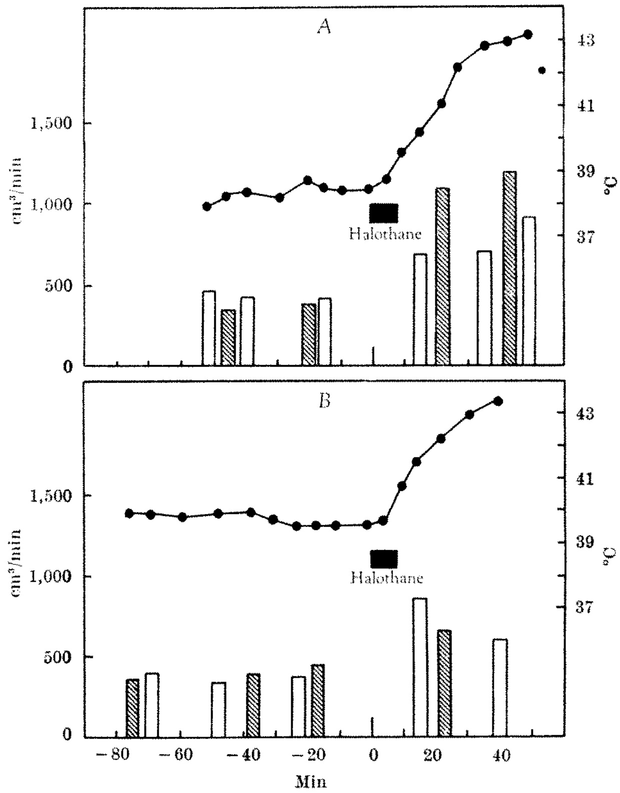


Fig. 2. *A*, Pig 39A, weight 80 kg; *B*, pig 82A, weight 56·1 kg. Effect of halothane on O_2 consumption and CO_2 output. O_2/CO_2 exchange was measured over timed 4 min periods. Halothane, 3 per cent (v/v, in O_2), was given from 0 to 8 min. White columns, O_2; hatched columns, CO_2. ●, Oesophageal temperature.

To establish more precisely the interrelation between the observed biochemical and temperature changes, these were carefully timed in a separate experiment on a susceptible pig (82A) in the first 10 min after halothane induction. During this period, oesophageal temperature rose from 39·5° to 40·8° C. All the expected changes had occurred within 4 min and Ca^{2+} and Mg^{2+} shifts and P_i and lactate production were apparent at 2 min. It was therefore clear that biochemical changes preceded the onset of pyrexia and were not secondary to heat injury to tissues. The sequence of metabolic changes that we have encountered in pigs sensitive to halothane seems to be completely different from that associated with heat injury[13], in which rise in blood lactate is a late manifestation.

Halothane, in susceptible pigs, initiates massive and uncontrolled glycogenolysis with production of "excess lactate" and a rise in blood glucose. The mechanism responsible for this is not immediately clear, because the usual anaerobic causes of lactate accumulation can be excluded. The animals breathed pure oxygen throughout the experiment and arterial oxygen tensions were maintained above 100 mm Hg in all cases. Blood pressure was over 100 mm systolic, but did fall terminally after lactacidosis had become well established. Cyanotic blotchiness and decline in temperature of the skin, however, provided evidence of a local perfusion defect, but its contribution to the stimulation of glycogenolysis in this and other tissues, for example, muscle, cannot be evaluated. Skin anoxia is considered unlikely to be responsible for the lactacidosis, for it appeared after 8–10 min on halothane (after serum lactate had risen) and was transient. The magnitude of the lactacidosis, moreover, continued to increase after the skin had returned to a healthy pink

Table 1. PIG 39A. EFFECT OF HALOTHANE ON BLOOD COMPOSITION

Time	(min)	−60	−3	+3	+8	+21	+31	+80
Oesophageal temp.	(°C)	38·3	38·4	38·6	39·0	42·0	43·0	44·1
Na^+	(mequiv./l.)	136	142	142	154	151	154	155
K^+	(mequiv/l.)	4·5	4·6	4·5	5·4	8·2	8·4	11·4
Glucose	(mg/100 ml.)	68	68	68	78	112	125	150
Total protein	(g/100 ml.)	6·5	6·5	6·3	6·9	7·7	7·9	6·3
Ca^{2+}	(mg/100 ml.)	10·8	11·1	10·3	11·7	13·5	12·9	10·5
Mg^{2+}	(mequiv./l.)	1·12	1·44	2·16	2·40	2·00	2·24	2·56
P_i	(mg/100 ml.)	9·6	9·4	9·1	14·5	25·8	29·3	31·7
Lactate	(mequiv./l.)	1·63	1·43	1·70	7·00	19·9	20·9	18·0
Pyruvate	(mequiv./l.)	0·15	0·16	0·17	0·27	0·18	0·20	0·21
"Excess lactate"	(mequiv./l.)	0·62	0·35	0·56	5·18	18·7	19·5	16·6
pH		7·36	7·36	7·07	6·92	6·60	6·60	6·82
pO_2 arterial	(mm Hg)	120	350	340	265	200	215	78
pO_2 venous	(mm Hg)	46	55	52	51	28	58	47
pCO_2 arterial	(mm Hg)	49	49	68	119	268*	168*	153*
Base excess	(mequiv./l.)	+3·0	−1·5	−3·2	−7·2	−21·5	< −22·0	< −22·0

Experimental details are as in Fig. 2*A*.
* Calculated from total blood CO_2 and pH.

colour. The possibility remains that muscle rigor resulted in occlusion of blood vessels causing an anaerobic state in muscle. The concentrations of muscle glycogen after halothane fell in two animals from 0·56 and 0·35 to 0·28 and 0·20 g/100 ml., respectively. In one instance muscle ATP fell from 2·7 to 2·2 mg/100 ml., concentrations above those at which fully developed rigor mortis is to be expected[14]. In the pigs we studied, the mechanism of muscle rigor seemed to be intrinsic, for the relaxant, tubocurarine, was ineffective in dispelling the rigidity. Impairment of blood supply to a limb consistently delayed onset of rigidity, suggesting that the effect of halothane on muscle is humoral rather than neural.

For the purpose of discussion of the origin of heat, we may consider pig 82A (weight 56·1 kg). Assuming a water content in the body of 67 per cent by weight and disregarding body solids (whose specific heat is not easily measured), the rise in temperature observed (3·9° C) represents extra heat of 147 kcalories, which—averaged over 40 min—is equivalent to 3·68 kcalories/min. Initially, however (5–10 min), the rate was 10·5 kcalories/min. If this extra heat were strictly related to increased O_2 utilization, 2·18 l./min additional O_2 consumption should have resulted (assuming that 1 l. O_2 consumed generates 4·825 kcalories[15]) whereas the measured increment at 15 min ($T = 41{\cdot}5°$ C) was 0·38 l./min and by 40 min ($T = 43{\cdot}4°$ C) the increment had fallen to 0·22 l./min. Non-oxidative mechanisms seem to be at least partly responsible for the excess heat production. Possibly anaerobic sources of heat include: (*a*) Glycolysis:

$$(\text{Glucose})_n + H_2O \longrightarrow (\text{glucose})_{n-1} + 2\ \text{lactate} + 2H^+$$

for which $\Delta F^{\circ\prime} = -52{\cdot}4$ kcalories/mole[16]. In pig 82A, heat from this source = 21·7 kcalories. (*b*) Neutralization of lactic acid:

$$H^+ + OH^- \longrightarrow H_2O;\ \Delta H = -13{\cdot}6\ \text{kcalories/g equivalent}$$

In the experimental animal this process provided 11·3 kcalories. These calculations are based on the rise in serum lactate concentration and are minimal because the quantity of lactate oxidized was unknown. (*c*) Hydrolysis of high energy phosphate esters.

During anaerobiasis, fall of creatine phosphate in frog sartorius muscle corresponds to rise in its P_i content[17]. Assuming that the released extracellular P_i was derived from compounds possessing high phosphate bond energy, for example creatine phosphate, ATP or phosphoenal pyruvate, capable of yielding 10 kcalories/mole, the rise in serum phosphate from 10 to 30 mg/100 ml. represents 2·4 kcalories in this animal. The sum of accountable anaerobic heat sources amounted to 35·4 kcalories or about 25 per cent of that actually evolved. We are investigating other potential heat sources which could be activated by halothane.

The mechanism by which halothane stimulates excess heat production in susceptible pigs is still obscure. Certain conclusions can, however, be reached which will influence further investigations and possibly management of the similar condition occurring in man.

(1) Excessive heat production is not accountable in terms of oxidative mechanisms and uncoupling of oxidative phosphorylation. Halothane induced an anaerobic state favouring breakdown of glycogen and organic phosphate esters. Anaerobic mechanisms have not previously been considered as possible sources of physiological or pathological heat.

(2) Alterations in the distribution of water and electrolytes and lactic acid production preceded significant rise in temperature and must be regarded as primary and not secondary to non-specific heat damage. They were apparently closely related to the development of muscle rigor. The lactate acidosis was uncompensated and blood pCO_2 rose to levels which could cause narcosis. Immediate treatment with intravenous HCO_3^- is contraindicated because death could ensue from CO_2 poisoning rather than H^+ accumulation.

(3) Known causes of defective tissue oxygenation could not account for the extent of lactate production. Future studies will aim at localizing a possible defect in mitochondrial electron transport.

We thank Misses P. du Toit and A. Munro for technical assistance. Dr R. Hickman selected the susceptible animals. We acknowledge financial support from Anglo-American and De Beers Anaesthetic Research Fund.

M. C. BERMAN
G. G. HARRISON
A. B. BULL
J. E. KENCH

CSIR Protein Research Unit,
Department of Chemical Pathology,
and the Department of Anaesthetics,
University of Cape Town Medical School,
Cape Town.

Received March 25; revised September 15, 1969.

[1] Editorial, *Brit. Med. J.*, **3**, 69 (1968).

[2] Wilson, R. D., Nichols, R. J., Dent, T. E., and Allen, C. R., *Anaesthesiology*, **27**, 231 (1966).

[3] Harrison, G. G., Biebuyck, J. F., Terblanche, J., Dent, D. M., Hickman, R., and Saunders, S. J., *Brit. Med. J.*, **3**, 594 (1968).

[4] Snodgrass, P. J., and Piras, M. M., *Biochemistry*, **5**, 1141 (1966).

[5] Siggaard-Andersen, O., Engel, K., Jørgensen, K., and Astrup, P., *Scand. J. Clin. Lab. Invest.*, **12**, 172 (1960).

[6] Hohorse, H., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H.), 266 (Academic Press, New York, 1963).

[7] Bucher, T., Czok, R., Lamprecht, W., and Latzko, E., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H.), 253 (Academic Press, New York, 1963).

[8] Pfleiderer, G., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H.), 59 (Academic Press, New York, 1963).

[9] Adam, H., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H.), 539 (Academic Press, New York, 1963).

[10] Einhorn, J., Dimitriadou, A., and Gadhok, R. S., *J. Endocrinol.*, **41**, 373 (1968).

[11] Brown, A. C., and Brengelmann, G., in *Physiology and Biophysics* (edit. by Ruch, T. C., and Patton, H. D.), 1046 (Saunders, Philadelphia, 1965).

[12] Huckabee, W. E., *Amer. J. Med.*, **30**, 833 (1961).

[13] Frankel, H. M., Ellis, J. P., and Cain, S. M., *Amer. J. Physiol.*, **205**, 733 (1963).

[14] Erdos, T., in *Studies, Muscular Contraction, Blood Coagulation* (edit. by Szent-Györgyi, A.), **3**, 52 (Karger, Basel, New York, 1943).

[15] Guyton, A. C., *Textbook of Medical Physiology*, 979 (Saunders, Philadelphia, 1966).

[16] Mahler, H. R., and Cordes, E. H., *Biological Chemistry*, 411 (Harper and Row, New York, 1966).

[17] Helmreich, E., Karpatkin, S., and Cori, C. F., in *Control of Glycogen Metabolism* (edit. by Whelan, W. J.), 224 (Churchill, London, 1964).

Transformation of Hamster Macrophages into Giant Cells with Antimacrophage Serum

THE sequence of cellular changes leading from monocyte to macrophage, epithelioid cell and finally multinucleated giant cell is now well documented both *in vitro* and *in vivo*. These changes, however, occur only in prolonged culture and in normal conditions only a small number of cultured macrophages have a tendency to undergo such transformation[1,2]. It is known that antimacrophage serum (AMS) has a marked influence on the physiology of the macrophage, the target structure probably being the cell membrane[3]. Agglutination of macrophages and decrease in the rate of phagocytosis have been described by several authors[3,4]. Moreover, Unanue[4] found macrophages showing aberrant nuclear changes in the peritoneal cavity of AMS-treated mice. This report concerns the influence of the protracted action of AMS on the development of giant cells in the cultures of mouse and hamster peritoneal macrophages.

AMS was produced in rabbits by four intravenous injections of 10^7 cultured mouse (Porton strain) or Syrian hamster macrophages at weekly intervals. Thioglycollate stimulated macrophages, for immunization, were cultured for 3 days in Eagle's basal medium supplemented with 40 per cent of inactivated horse serum. Ten days after the last injection the animals were exsanguinated and the sera, after heat inactivation, were absorbed twice with an equal volume of packed mouse or hamster red blood cells. For our experiments, sera with cytotoxic titres of at least 1/160 were used. Mouse or hamster antilymphocyte serum (ALS) was prepared in rabbits by the schedule used for AMS and absorbed twice with mouse erythrocytes. For immunization, peripheral lymph node cells were used. The unstimulated mouse or hamster macrophages were obtained by washing the peritoneal cavity with Eagle's basal medium. The fluid containing peritoneal cells was withdrawn and transferred to Leighton tubes with flying coverslips. The medium was changed every second day. Between 2 and 5 days later (sometimes even longer) AMS or ALS in different concentrations was added to the fresh medium. To the control tubes inactivated normal rabbit serum was added in similar dilutions. Each culture was observed daily in the light microscope. At different times the coverslips were removed and the cells examined, unfixed, by phase contrast and interference microscopy or by staining with haematoxylin-eosin. In 5 day cultures, macrophages form about 97 per cent of the total cell count, the remaining cells being classified as fibroblasts. In these conditions the number of hamster macrophages containing two or three nuclei does not exceed 5·5 per cent; the number of multinucleated giant cells is less than 0·5 per cent. These numbers do not alter significantly in older cultures.

Hamster macrophages after 2–5 days of culture (Fig. 1*A* and *B*), and after the addition of AMS—even at the final dilution of 1/50—exhibit the following sequence of changes in the course of several hours. Initially, because of the disappearance of cell extensions, the macrophages become round and then stick together to form aggregates containing two to four cells (Fig. 1*E*). After 3–4 h the aggregation of macrophages is more pronounced and many cells are arranged in coral-bead structures (Fig. 1*C* and *D*). The degree of agglomeration of cells exceeds 96 per cent, whereas in untreated controls it is 3–5 per cent. At this stage only a slight increase in the number of cells containing two or three nuclei is visible. At 24 h nearly all the macrophages fuse (96 per cent) and form multinucleated cells containing from a few up to several scores of nuclei (Fig. 1*F*). The fusion is preceded by a stage of strong cyto-adherence with pronounced duplication of the cell membrane (Fig. 1*C* and *E*). The possibility that some of the giant cells arise as a result of cell division cannot be entirely excluded, although the mitotic index of AMS-

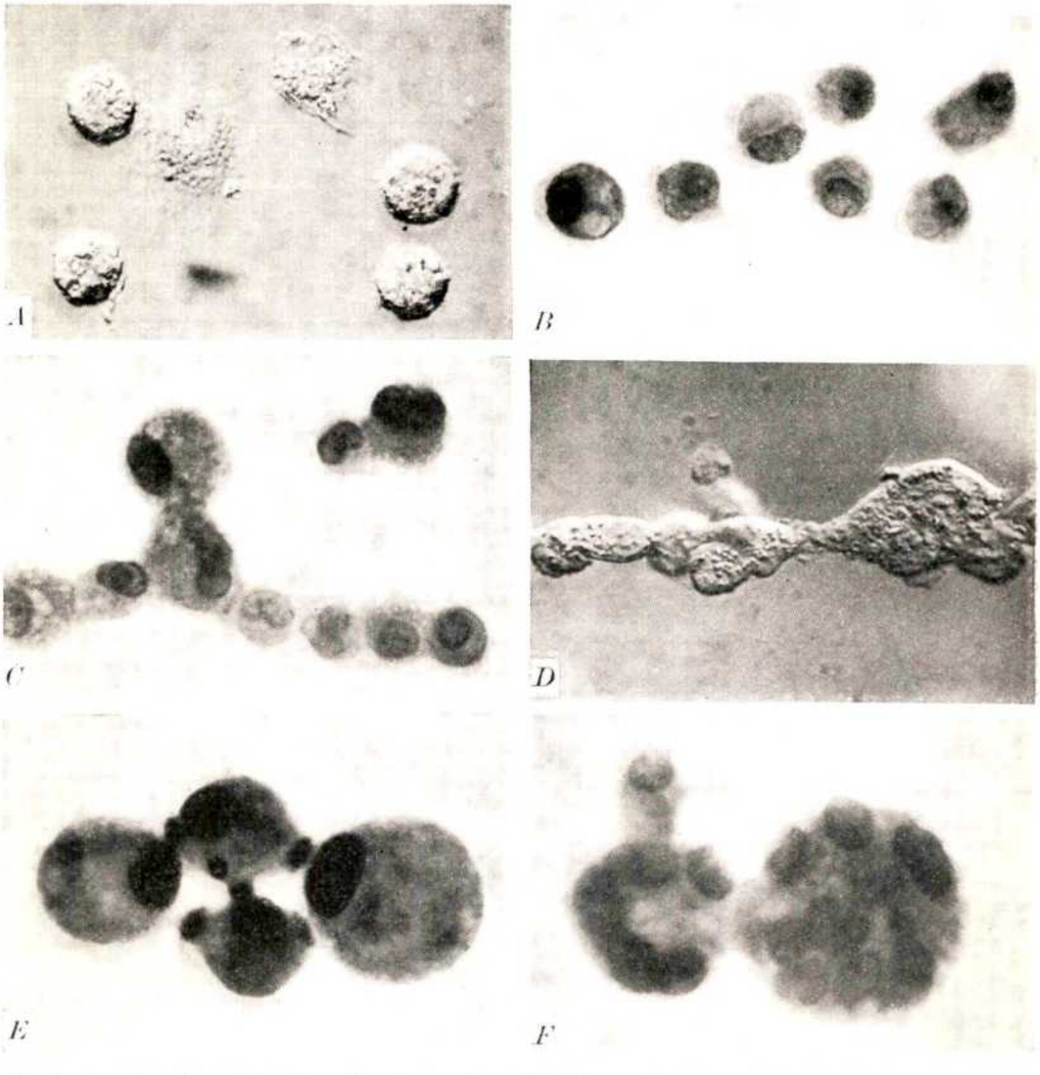


Fig. 1. *A*, 5 day culture of hamster peritoneal macrophages; interference microscope, × 300; *B*, the same, stained with haematoxylin-eosin, × 300; *C*, bead-like arrangement of macrophages exposed to antimacrophage serum, stained with haematoxylin-eosin, × 300; *D*, bead-like arrangement of macrophages and giant cell formation; 24 h exposure to AMS; interference microscope, × 150. *E*, Early stage of macrophage fusion and giant cell formation; note the duplication of cell membranes; stained with haematoxylin-eosin, × 900; *F*, giant multinuclear cells, 36 h exposure to AMS, stained with haematoxylin-eosin, × 300.

treated macrophages is lower (0·2 per cent) than that of untreated cells (0·4 per cent). Measurements in the interference microscope revealed a distinct increase in the dry mass of giant cells as compared with macrophages. The macrophage cultures treated with ALS—even at the final dilution of 1/5—showed no such changes, nor were they visible in control cultures. Higher concentrations of ALS or normal rabbit serum exert a cytotoxic effect on macrophages. The effect of AMS can therefore be attributed to its specific action on hamster macrophages. In none of the many experiments with macrophages from various strains of mice were similar changes observed, following the action of specific AMS, except for cell aggregation.

It has been shown that antibody against the surface antigens of Krebs ascites tumour cells can have a stimulating effect on the surface activity of these cells appearing in the interdigitations of the cell membranes of adjacent cells[5]. The differing reactions of hamster and mouse macrophages after treatment of AMS may be related to the different degree of maturity of these cells. The mononuclear phagocytes in the unstimulated peritoneal cavity of the hamster, by contrast with those in the mouse, more closely resemble blood monocytes than macrophages. During their cultivation, however, typical macrophages develop. We have observed that monocyte-like macrophages from 2–5 day cultures react more readily to AMS than mature macrophages from later phases of cultivation. It can therefore be assumed that during the differentiation of macrophages substantial changes in the structure of the cell membrane take place. The factors influencing the development of giant cells from macrophages are not fully understood. The origin of these cells has frequently been attributed to the increased rate of phagocytosis by macrophages[6,7]. It seems, however, that AMS, acting specifically on the surface of the macrophage, promotes cell development in the same direction. This assumption is substantiated further by the fact that the cultured macrophages can be fused by means of Sendai virus acting on the cell membrane[8].

This work was supported partly by a grant from the Polish Academy of Sciences.

W. PTAK
Z. PORWIT-BÒBR
Z. CHLAP

Departments of Medical Microbiology
and Pathological Anatomy,
Medical Academy,
Cracow, Poland.

Received July 28; revised September 11, 1969.

[1] Cohn, Z. A., Hirsch, J. G., and Fedorko, M. E., *J. Exp. Med.*, **123**, 747 (1966).
[2] Sutton, J. S., *Nat. Cancer Inst., Monographs*, **26**, 71 (1967).
[3] Jennings, J. F., and Hughes, L. A., *Nature*, **221**, 79 (1969).
[4] Unanue, E. R., *Nature*, **218**, 36 (1968).
[5] Easton, J. M., Goldberg, B., and Green, H., *J. Exp. Med.*, **115**, 275 (1962).
[6] Sutton, J. S., and Weiss, L., *J. Cell Biol.*, **28**, 303 (1966).
[7] Patch, P. R., and Bernick, S., *Anat. Rec.*, **138**, 27 (1960).
[8] Harris, H., *Nature*, **206**, 583 (1965).

Stimulation of Mitosis in Amphibian Kidney by Organ Specific Antiserum

IT has been shown that the rate of mitosis in the pronephric kidney of larval *Xenopus* is inhibited by factors present in soluble fractions of homogenized adult *Xenopus* kidney (mesonephros) or rat kidney (metanephros)[3,10] and it was suggested that the rate of cell division in the kidney may be controlled by a tissue-specific mitotic inhibitor or chalone such as has been described in other tissues[1,9,11,13]. Although the rate of mitosis in the larval pronephros is sensitive to the "kidney chalone" we do not know whether it is controlled by the chalone in natural conditions, because this would imply that the pronephros already produces the chalone at this early stage in development. Extraction of the chalone from such a small organ would be difficult and we have therefore sought a different way of demonstrating its presence.

An antiserum can be produced to the epidermal chalone[6] and if this is also true for the kidney we should expect that the immediate effect of a kidney chalone antiserum on the pronephros would be to neutralize any endogenous chalone and hence to stimulate mitosis. This hypothesis has been examined.

The effect of test substances on the mitotic rate in the pronephros was measured in the way previously described[3,10]. Trunk regions containing the pronephros and epidermis and variable amounts of skeletal muscle, spinal cord and in some cases heart, were dissected from stages thirty-two to thirty-three (post-tailbud) *Xenopus laevis* larvae. These pronephric explants were cultured in groups of five for two days in Niu and Twitty balanced saline solution (BSS) with 250 µg/ml. of streptomycin at 25° C. Four hours before fixation, test substances were added together with colcemid (0·025 mg/ml.) to accumulate mitoses at metaphase. The number of arrested metaphases was counted in a sample of 1,000 pronephric or epidermal cells from each explant (5,000 cells per group of five) and the mean mitotic incidence (*MI*) expressed as the proportion of arrested metaphases per 10^5 cells.

An extract of rat kidney was prepared in the manner described earlier for *Xenopus* kidney[3,10]. The perfused kidneys were homogenized in 3·5 times their volume of BSS using a teflon pestle. The homogenate was centrifuged at approximately 100,000*g* and the clear supernatant freeze-dried and stored at –70° C. Extracts of rat liver and lung were made in the same way.

The specific inhibitory activity of this extract preparation was assessed by adding it to pronephric explants at a concentration of 25 mg dry weight equivalent/ml.[3,10]. The *MI* of pronephros in control explants was $1,771/10^5$, compared with $262/10^5$ for kidney extract. The kidney extract thus depressed mitosis by 85 per cent ($P < 0\cdot001$). No significant alteration in *MI* was achieved by the addition of rat lung ($1,721/10^5$) or liver ($1836/10^5$) extracts to the medium at the same concentration. These results are very similar to those obtained with *Xenopus* organ extracts and it thus seems that the inhibitory factor present in the kidney is tissue specific but not species specific.

An adult rabbit was immunized with the inhibitory kidney extract following a schedule described in detail elsewhere[14]. A total of four series of injections was given at intervals of fifteen days. For the first series a quantity of extract equivalent to 0·3 mg of protein was made up to 3 ml., emulsified with an equal volume of Freund's complete adjuvant, and injected intramuscularly at four different sites. For the second and third series of injections the dose was increased to 3 mg protein emulsified with Freund's adjuvant, and for the fourth series of injections 3 mg protein suspended in normal saline was given. Seven days after the final injection a sample of 20 ml. of blood was taken from the marginal vein of the ear. This was stirred to remove fibrinogen, incubated at 37° C for 3 h and centrifuged at 4,500 r.p.m. for 30 min. The clear supernatant serum ("kidney antiserum") was stored at –70° C. Normal rabbit serum was prepared in the same way using a sample of blood taken from a non-immunized animal.

Kidney antiserum or normal serum was added to the culture medium to give a final concentration of 5 per cent and the effects of these two treatments on the *MI* of pronephros and epidermis are compared in Figs. 1 and 2. In explants treated with kidney antiserum the *MI* of the pronephros was increased by 42 per cent ($P < 0\cdot02$) but that of the epidermis was decreased by 42 per cent

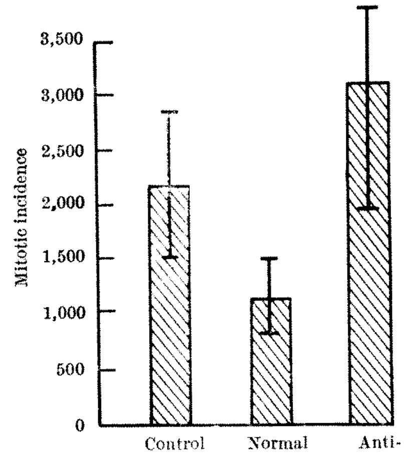


Fig. 1. Effect of normal serum (5 per cent) and kidney antiserum (5 per cent) on mitotic incidence in the pronephros of larval explants of *Xenopus laevis*.

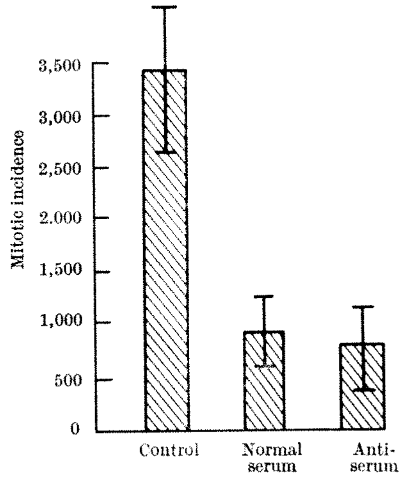


Fig. 2. Effect of normal serum (5 per cent) and kidney antiserum (5 per cent) on mitotic incidence in the epidermis of larval explants of *Xenopus laevis*.

($P < 0{\cdot}001$) compared with untreated control explants. In explants treated with normal serum the *MI* in both pronephros and epidermis was decreased ($P < 0{\cdot}001$) by approximately the same amount as in the epidermis of antiserum-treated explants.

Preparations of serum from adult non-immunized animals are frequently found to be toxic to cells in culture[12] probably because they contain antibodies to common tissue antigens[4]. This is the probable explanation for the mitotic depression in pronephros treated with normal serum and that in epidermis treated with either normal serum or kidney antiserum. The probable explanation for the increased rate of mitosis in pronephros treated with kidney antiserum is that the pronephros contains a growth inhibitor identical to that contained in adult kidney extracts and that this inhibitor is neutralized by the antiserum. Our results therefore support the view[2] that synthesis of the "kidney chalone" begins during the development of the larval pronephros.

The observation that kidney antiserum stimulates cell division in the pronephros of cultured explants may have a bearing on the aetiology of autoimmune glomerulonephritis. In some forms of this disease proliferative changes occur in a number of cell types[5,7] including the glomerular epithelium[8], and we propose that these localized increases in the rate of cell production may in some cases be caused by circulatory antibodies against tissue specific mitotic inhibitors present in the kidney.

This work was supported by a grant from the North of England Council of the British Empire Cancer Campaign for Research.

D. P. Chopra
J. D. Simnett

University of Newcastle upon Tyne,
Department of Pathology,
Royal Victoria Infirmary,
Newcastle upon Tyne, NE1 4LP.

Received October 9, 1969.

[1] Bullough, W. S., and Laurence, E. B., *Exp. Cell Res.*, **33**, 176 (1964).
[2] Chopra, D. P., and Simnett, J. D., *J. Embryol. Exp. Morphol.*, **21**, 539 (1969).
[3] Chopra, D. P., and Simnett, J. D., *Exp. Cell Res.*, **58**, 319 (1969).
[4] Fedoroff, S., and Doerr, J., *J. Nat. Cancer Inst.*, **29**, 331 (1962).
[5] Heptinstall, R. H., and Joekes, A. M., in *Diseases of the Kidney* (edit. by Strauss, M. B., and Welt, L. G.), 306 (Churchill, London, 1964).
[6] Hondius-Boldingh, W., and Laurence, E. B., *Europ. J. Biochem.*, **5**, 191 (1968).
[7] Howie, J. B., and Helyer, B. J., *Advan. Immunol.*, **9**, 215 (1968).
[8] McManus, J. F. A., in *Diseases of the Kidney* (edit. by Strauss, M. B., and Welt, L. G.), 255 (Churchill, London, 1964).
[9] Rytömaa, T., and Kiviniemi, K., *Cell Tissue Kinet.*, **1**, 329 (1968).
[10] Simnett, J. D., and Chopra, D. P., *Nature*, **222**, 1189 (1969).
[11] Simnett, J. D., Fisher, J., and Heppleston, A. G., *Nature*, **223**, 944 (1969).
[12] Terasaki, P. I., Esail, M. L., Cannon, J. A., and Longmire, W. P., *J. Immunol.*, **87**, 383 (1961).
[13] Voaden, M. J., and Froomberg, D., *Exp. Eye Res.*, **6**, 213 (1967).
[14] Weir, D. M., in *Handbook of Experimental Immunology* (edit. by Weir, D. M.), 844 (Blackwell, Oxford, 1967).

Absolute, Unambiguous Ultramicroanalysis of Metabolites present in Complex Biological Extracts

The analysis of substances of biogenic interest is usually difficult and often ambiguous because the metabolite of interest is usually present at low concentration in a complex mixture. Quantitation usually proceeds via prefractionation (organic solvent extraction, ion exchange or absorption), chromatographic separation and then a relatively specific chromogenic or fluorogenic reaction either directly on the chromatogram or, after elution, in solution. The certainty of identification using these, or variants of these, procedures is limited. This is particularly so in the field of biochemical psychiatry where over the past few years there have been numerous examples of chromatographically separated chromogenic factors which have meant different things to different people (further details in ref. 1). We present here preliminary data on the use of a new technique—the so-called "integrated ion current (IIC) technique"—involving the high resolution double focusing mass spectrometer (in our case, the MS9 manufactured by Associated Electrical Industries).

When the mass spectrometer is used conventionally in the quantitative analysis of sample mixtures, a large reservoir of sample vapour is usually kept at a constant low pressure while allowing a very small fraction to flow into the instrument through a constriction such as a sinter. The ion currents recorded on ionization of the sample in the instrument are proportional to the partial pressures of the respective components of the sample mixtures in the reservoir. The ratio of the ion current at any appropriate m/e value to the partial pressure of the component responsible is substantially constant during times which are long compared with the duration of the recording. This ratio, which is called the sensitivity of the instrument for that particular component, may be determined by calibration with a standard sample of the component.

This technique is inappropriate when only small samples in the nanogram range of mixtures of materials of biological origin having a comparatively low vapour pressure are available. It is possible, however, to use an alternative technique in which the ion current at a significant m/e value for each component is recorded separately while small samples of the solid mixture are evaporated from a probe. This probe is inserted into the vacuum system and sample evaporation takes place close to the ionizing beam. It is then possible to relate the integrated ion current at the selected m/e value to the weight of pure component evaporated and so calibrate the instrument. This technique enables samples in the subnanogram range to be estimated[2]. In order to compensate for any instrumental instability, it is convenient to make a series of measurements of ion current at intervals of 1 s using the peak matching facilities.

This technique has been applied to the analysis of *p*-tyramine in human urine and in various anatomical regions of the rat brain. *p*-Tyramine was selected in this first instance because it has been positively identified in human urine[3], but its concentration is different from normal in some patients suffering from Parkinson's disease, migraine and schizophrenia[3,4]. It has not hitherto been positively identified in brain material. Furthermore, two relatively specific chromatographic analytical procedures, one macro[5] the other semi-micro (A. A. B. and L. Quan, paper presented at the second International Neurochemistry meeting, Milan, September 1969), were available for comparative purposes.

A record of the evaporation of a 1 ng sample of *p*-tyramine at a temperature of 200° C is shown in Fig. 1. Each vertical line represents a single determination of the ion current at m/e 108. It is the area under the envelope which is proportional to the integrated ion current and hence to the weight of *p*-tyramine evaporated. A calibration curve for *p*-tyramine in the 10–100 ng region is shown in Fig. 2.

Co-evaporation of any compounds which do not have peaks in their mass spectra at m/e 108 has no effect on the sensitivity of the instrument for *p*-tyramine provided that the source pressure does not reach so high a value that ionization is suppressed. If, however, a compound is co-evaporated which can produce an ion with an m/e value of 108, then the integrated ion current is the sum of the individual contributions and interference takes place. The possibility that two compounds will evaporate at the same temperature and provide an identical ion is a slight one and it has been demonstrated that even in the case of geometrical isomers such as, for example, cis- and trans-stilbene the integrated ion current curve shows fine structure caused by the differential evaporation of the isomers (J. R. M., unpublished work).

There remains the possibility that two such substances may co-evaporate, each providing an ion at the integral m/e value of 108 but with ions having a different atomic constitution. As a result, the precise mass of these ions may differ by a few hundred parts per million. A high resolution mass spectrometer can record ion currents due to ions differing by this slight mass interval as two separate peaks. Such an effect is observed when the phenolic amine extract[6], containing *p*-tyramine, isolated from rat brain is examined by the integrated ion current technique. Each determination of the ion current at m/e 108 is represented by two lines each corresponding to a different ion. The two ions responsible are $C_7H_8O^+$ (from *p*-tyramine) with a precise mass of 108·0575 and $C_8H_{12}^+$ with a precise mass of 108·0939. The envelopes of these two separate ion peaks can readily be identified and so the amount of *p*-tyramine in the mixture may still be estimated using the calibration graph. In cases where double envelopes appear at one m/e value, it may sometimes be possible to find an alternative m/e value where only one ion current is registered or alternatively modify the substance of interest by deuterium exchange labelling or by the formation of a simple derivative.

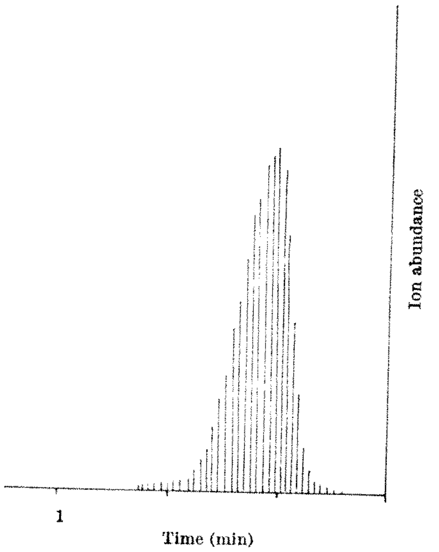


Fig. 1. Record of the ion current at m/e value 108 during evaporation at 200° C of 1 ng of *p*-tyramine.

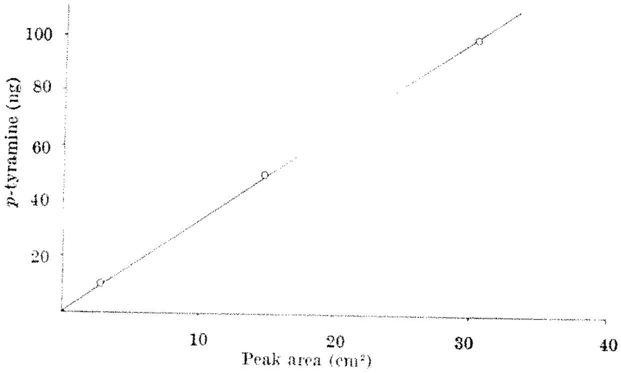


Fig. 2. Calibration curve for *p*-tyramine using the integrated ion current technique.

Table 1. REGIONAL DISTRIBUTION OF *p*-TYRAMINE IN RAT BRAIN

Sample No.	Brain area	IIC technique		Chromatographic technique
		ng *p*-tyramine	ng *p*-tyramine per g tissue	ng *p*-tyramine per g tissue
1	Whole brain	43	200	192 ± 24 (8 estimations)
2	Whole brain	43	224	
3	Whole brain + 500 ng	91	478	—
4	Whole brain + 500 ng	97	483	—
5	Caudate nucleus	11·3	587	—
6	Hypothalamus	14	1,690	—
7	Cerebellum	14	229	—
8	Stem	10	201	—
9	Rest	15·5	54·7	—
10	Blank	Nil	—	—

Rats were stunned, decapitated and their brains removed. The various areas were then dissected out. Whole brains or the dissected areas from two brains were washed in ice cold saline solution and then homogenized at 2° in 0·4 N $HClO_4$. After centrifugation, the supernatant was neutralized with 1 N NaOH and the phenolic amine fraction isolated by ion exchange chromatography as described by Kakimoto and Armstrong[6]. For IIC (see text) estimations the extract was triturated in 50 µl. ethanol : ammonia, 70 : 30 (v/v) and a 5 µl. sample placed directly into the mass spectrometer. Recovery of added *p*-tyramine is effectively 100 per cent. Quantitative chromatographic analysis was achieved following separation of the total phenolic amine extract on Kodak (silica gel) TLC plates in the solvent system butanol : acetic acid : water, 4 : 1 : 1 (v/v). The *p*-tyramine zone was eluted, danyslated and separated twice more on Kodak (silica gel) TLC plates in the solvent systems chloroform : butyl acetate, 5 : 1 (v/v) and ethyl acetate : cyclohexane, 3 : 2 (v/v)[7]. The fluorophore was then finally eluted in benzene : acetic acid, 99 : 1 (v/v)[7], and measured in the spectrophotofluorometer. The overall recovery of added *p*-tyramine by this procedure is 54 ± 10 per cent Further details were given in a paper presented by A. A. B. and L. Quan at the second International Neurochemistry Meeting, Milan, September 1969.

In this present case, the alternative m/e value of 137 also showed a double envelope caused by the ions $C_8H_{11}ON^+$ (from *p*-tyramine) with a precise mass of 137·0841 and $C_{10}H_{17}^+$ with a precise mass of 137·1330. The precise origin of the hydrocarbon ions is uncertain, but they may be fragments of lipid originating in the homogenization procedure (A. A. B. and L. Quan, paper presented at the second International Neurochemistry Meeting, Milan, September 1969) and carrying through in the subsequent aqueous operations. They were not present in urinary phenolic amine extracts. Using this new technique, however, their presence does not interfere with the estimation of *p*-tyramine in the samples and, further, it is possible that if they could be identified their concentration could be determined at the same time as that of *p*-tyramine in a single evaporation, a result which has not previously been possible.

The results of *p*-tyramine determination on extracts of various areas of rat brain using this technique are compared with those obtained by a fluorimetric procedure based on the chromatographic separation of the 1-dimethlyaminonaphthalene-5-sulphonyl (Dansyl) derivative.

The further development of this technique towards analysis at the picrogram level and its use in the analysis of other amines and their respective metabolites in various neurochemical specimens is currently in progress.

We thank Miss L. Quan for making available her chromatographic data and for help in obtaining the various brain areas, and Miss M. J. A. Reade for carrying out the mass spectrometric determinations. Parts of this work were supported by the Psychiatric Services Branch of the Province of Saskatchewan and the Canadian Medical Research Council.

J. R. MAJER

Department of Chemistry,
University of Birmingham.

ALAN A. BOULTON

Psychiatric Research Unit,
University Hospital,
Saskatoon,
Saskatchewan, Canada.

Received August 11, 1969.

[1] Boulton, A. A., *Nature* (in the press).
[2] Jenkins, A. E., and Majer, J. R., *Talanta*, **14**, 777 (1967).
[3] Boulton, A. A., Pollitt, R. J., and Majer, J. R., *Nature*, **215**, 132 (1967).
[4] Smith, I., and Kellow, A. H., *Nature*, **221**, 1261 (1969).
[5] Boulton, A. A., in *Methods of Biochemical Anal.* (edit. by Glick, D.), **16**, 327 (Interscience, New York, 1968).
[6] Kakimoto, Y., and Armstrong, M. D., *J. Biol. Chem.*, **237**, 208 (1962).
[7] Seiler, N., in *Methods of Biochemical Anal.* (edit. by Glick, D.), **17** (Interscience, New York, in the press).

Flying Speed of the Largest Aerial Vertebrate

THE Cretaceous pterosaur *Pteranodon* was the largest flying animal known to exist in vertebrate history (see Fig. 1). Its wing-span reached 25 feet, far exceeding in size the largest modern bird, the albatross, which has a wing span of only 12 feet[1,2]. Although *Pteranodon* was so large, its weight was surprisingly low. This has been estimated as a maximum of 55 pounds or a minimum of 25 pounds[1,3]. About 40 pounds may be taken as a reasonable weight. In any case, *Pteranodon* was an animal of great size and fragility. Its weight had become reduced during the course of its evolution in many different ways; for example, the heavy hind legs and pelvic region of the earlier pterosaurs had been much reduced, the teeth had been lost, and the bone itself was no thicker than blotting-paper, the wing bones being strengthened internally by crosswise struts.

Hankin and Watson[4] have studied the flight of *Pteranodon* in detail, and they have suggested that *Pteranodon* was primarily a glider and that it was not constructed for continuous flapping flight. This is shown by the long, narrow wing shape and the relative smallness of the pectoral musculature.

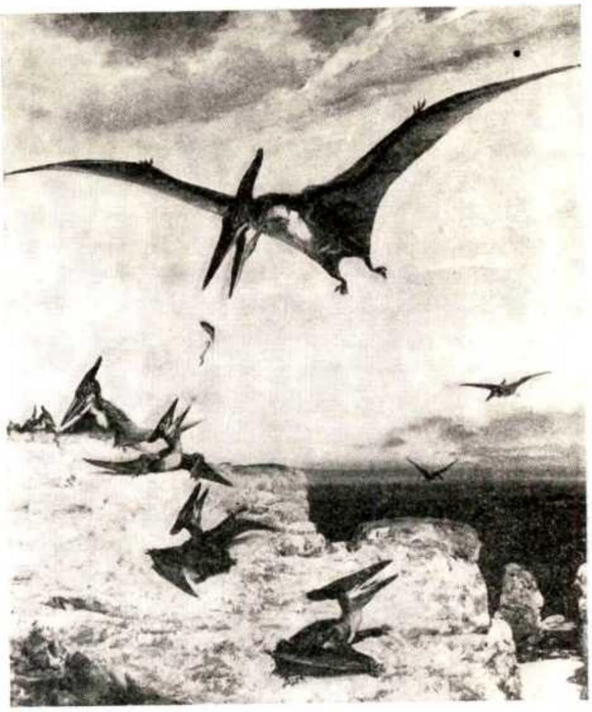


Fig. 1. *Pteranodon* feeding its young (after J. Burian).

Take-off in pterosaurs has always been considered a problem, because it has been assumed that the animals had to obtain a considerable velocity before becoming airborne. Most authors suggest that *Pteranodon* must have obtained sufficient speed for taking off by jumping from cliffs rather than trying to get up speed from ground level: Bellairs[5] states that "*Pteranodon* had great difficulty in taking off from flat surfaces", while Romer[6] comments "how this animal could get itself into the air from level ground is difficult to understand". Several authors have also observed that *Pteranodon* must have landed very gently, because its delicate body could not have stood the force of a sudden impact[9].

These difficulties are resolved when the flying speed is calculated. Using the standard formula[7], weight = $\frac{1}{2}C_L \rho v^2 S$, and assuming a maximum lift coefficient, C_L, of 1, and a body weight of 40 pounds, the minimum flying speed of *Pteranodon* is 22 feet/s (or 15 m.p.h.). Even at the upper limit of weight, 55 pounds, the minimum flying speed is only 17 m.p.h.

These speeds are so low that take-off and landing (into wind, of course, like modern birds and aeroplanes) would have been easy. In fact, in winds above 15 m.p.h. *Pteranodon* would only have needed to spread its wings to become airborne, with negligible effort. It is still probable that *Pteranodon* took off more frequently from cliffs than from level ground because it was an oceanic animal and was likely to have nested on cliff sites; but if it landed on the water it could have easily taken off from the crest of a wave.

After take-off, *Pteranodon* would have had to rely largely on soaring. Its low wing loading would have ensured a low sinking speed, facilitating slope soaring even in light winds, the best slopes being cliffs, but at sea large waves would have sufficed, as they do for the albatross[8].

Thus many of the problems envisaged by palaeontologists for the pterosaurs did not exist for the pterosaurs themselves.

CHERRIE D. BRAMWELL

Department of Geology,

G. R. WHITFIELD

Department of Applied Physical Sciences,
University of Reading,
Berkshire.

Received November 19, 1969.

[1] Kripp, d. von, *Nova Acta Leopoldina*, **83**, 217 (1943).
[2] Landsborough-Thomson, Sir A., *A New Dictionary of Birds*, 43 (Nelson, London, 1964).
[3] Brown, B., *Bull. Amer. Mus. Nat. Hist.*, **52**, 104 (1943).
[4] Hankin, E. H., and Watson, D. M. S., *Aeronaut. J.*, **18**, 324 (1914).
[5] Bellairs, d'A., *Reptiles*, 118 (Hutchinson, London, 1957).
[6] Romer, A. S., *Vertebrate Palaeontology*, third ed., 146 (Univ. of Chicago Press, 1966).
[7] Welch, A., Welch, L., and Irving, F., *New Soaring Pilot*, 34 (Murray, London, 1968).
[8] Simkiss, K., *Bird Flight*, 79 (Hutchinson, London, 1963).
[9] Halstead, L. B., *The Pattern of Vertebrate Evolution*, 140 (Oliver and Boyd, Edinburgh, 1969).

Absence of Antimicrobial Substances in the Egg Capsules of Millipedes

MILLIPEDES lay their eggs in hollow capsules of their own construction, within which the emerged young spend the early part of their existence. The capsules are moulded primarily of soil and vegetable debris, and are built with the apparent addition of maternal enteric constituents, both oral and aboral in origin[1,2], which suggests that they might be impregnated chemically for defence against microorganisms. Here we present evidence indicating that for at least two species of millipede this hypothesis does not apply, because neither antibacterial nor antifungal substances could be detected in the walls of their capsules.

The millipedes studied were *Glomeris marginata* (order Glomerida) and *Narceus annularis* (order Spirobolida), from Europe and Ithaca, New York, respectively. They produced their capsules in laboratory cages, which were set up with soil and leaf litter, simulating their natural habitat. In the case of *Narceus*, the contents in the cage came from the actual collection sites. Several hundred *Narceus* capsules were obtained and several dozen of *Glomeris*. Extracts were prepared by separating the eggs from the capsules, homogenizing the capsules in water (ratio of capsule to water = 1 : 2 for antibacterial tests; 3 : 10 for antifungal tests), and sterilizing the homogenates by membrane filtration.

The bacteria tested included *Pseudomonas fluorescens*, *Escherichia coli*, *Aerobacter aerogenes*, *Sarcina lutea*, *Bacillus megaterium* and *B. subtilis*, as well as several Gram-negative cocci and bacilli and Gram-positive spore-forming rods isolated from the soil in which the capsules had been deposited. The tests were carried out by addition of capsule extract (droplets or soaked filter paper disks) to tryptose blood agar base (Difco) plates on the surface of which were spread standardized bacterial suspensions. The plates were incubated at 30° C for 18 h and examined for antibiotic effects at a magnification of 12. Even at maximal concentrations, the extracts had no inhibitory effect.

The fungi tested were *Candida albicans*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis* and—with the *Glomeris* extract only—*Paecilomyces varioti*. Growing cultures of the fungi, in suitable defined media, were uninhibited by the addition of extract to medium in the ratio of 1 : 5. Moreover, capsules of *Narceus* placed directly on peptone glucose agar were quickly overgrown with saprophytic moulds.

It seems probable that the principal function of the capsules is to offer concealment and mechanical protection from predators. A single negative finding of this sort should not be taken to indicate that antimicrobial agents are likely to be generally absent from soil-inhabiting arthropods and other animals. Clues to the occurrence of such agents are not numerous (probably because biologists are not generally alert to their possible significance), but they do occur. In some centipedes, for example, the eggs are tended by the mother, and when unattended become overgrown with mould[3,4]. Comparable brooding behaviour has been reported for crickets[5], earwigs[6] and ants[7]. In our opinion, a systematic pursuit of such leads is likely to pay off, particularly with regard to the eventual discovery of new antifungal agents.

This work was supported in part by grants from the US Public Health Service. The *Glomeris* were supplied by Dr W. Dohle (Berlin) and Dr R. H. Cobben (Wageningen).

THOMAS EISNER
STANLEY A. ZAHLER
JAMES E. CARREL
DOROTHY J. BROWN

Division of Biological Sciences,
Cornell University,
Ithaca, New York.

GEORGE W. LONES

Laboratory of Microbiology,
National Institute of Allergy and Infectious Diseases,
National Institutes of Health,
Bethesda, Maryland.

Received November 12, 1969.

[1] Loomis, H. F., *J. Wash. Acad. Sci.*, **23**, 100 (1933).
[2] Shaw, G. G., *Ecology*, **47**, 322 (1966).
[3] Auerbach, S. I., *Ecol. Monog.*, **21**, 97 (1951).
[4] Palmén, E., and Rantala, M., *Ann. Zool. Soc. Vanamo*, **16**, 1 (1954).
[5] West, M. J., and Alexander, R. D., *Ohio J. Sci.*, **63**, 19 (1963).
[6] Weyrauch, W. K., *Biol. Zentralbl.*, **49**, 543 (1929).
[7] Wheeler, W. M., *Ants, their Structure, Development, and Behavior* (Columbia University Press, New York, 1910).

Sterol Metabolism as a Basis for a Mutualistic Symbiosis

MUTUALISM makes it possible for certain organisms with unusual nutritional requirements to survive and reproduce[1]. Species of this kind may be invaluable in studies of metabolism if the aposymbiotic (symbiote-free) organisms can ultimately be raised easily on defined substrates in sterile conditions[2]. During efforts to develop such a laboratory culture of the beetle *Xyleborus ferruguineus*, fungus-free females feeding on media containing cholesterol as the sterol source produced second-generation larvae that did not pupate[3]. Here we report evidence that the mutualistic fungus *Fusarium solani* associated with the beetle produced ergosterol as its only sterol in pure culture on a chemically defined substrate in sterile conditions. Ergosterol proved to be adequate as the sole sterol source for continued growth, development and reproduction of the fungus-free beetle. Subsequent tests of other sterols showed that 7-dehydrocholesterol was similarly adequate.

The fungal sterol fraction was isolated from *F. solani* grown on Neutral-Dox-Yeast medium[4]. Batches of fungus harvested after five, ten or fifteen days' growth were washed, freeze-dried, weighed and analysed for sterols. For each analysis, 15 g of freeze-dried fungus of a given age was homogenized in and extracted with a 2 : 1 mixture of chloroform–methanol. The total lipid extract was chromatographed on a silicic acid column and the sterol-containing fraction was eluted with chloroform. Thin-layer chromatography of this fraction using several solvent systems showed that significant amounts of sterol esters were not present. Free sterols were precipitated by digitonin treatment[5].

Preliminary identification of the sole sterol, ergosterol, in the fungus was based on its retention time as the free form and as the trimethylsilyl derivative on 1 per cent 'QF-1' on 'Gas-Chrom Q' at two temperature programmes using dual-hydrogen gas–liquid chromatography. Retention times of the isolated ergosterol were compared with recrystallized commercial ergosterol. This identification was confirmed by mass spectrometry. Gas–liquid chromatography quantitation of the ergosterol per 15 g dry fungus was based on a standard curve derived from gas–liquid chromatography of known concentrations of authentic ergosterol.

Ergosterol content increased with the age of the fungus, and ranged from 0·17 to 0·24 per cent on a dry weight basis. Because insects generally require 0·01 to 0·1 per cent sterol in their diets[6], the fungus, irrespective of age, should be an adequate source of this nutrient.

For studying the nutritional adequacy of various sterols or related compounds for the growth, development and reproduction of the fungus-free beetle, the basic diet was: sucrose (10 g), vitamin-free casein (10 g), Wesson salt mixture (2·5 g), linolenic acid as methyl ester (0·1 g), sorbic acid (0·4 g), streptomycin (0·1 g), powdered cellulose (15 g), fibrous cellulose (4 g), Bacto-agar (10 g), 95 per cent ethanol (2·5 ml.), 20 ml. of a vitamin solution[3], and de-ionized water (230 ml.). One of the following sterols was added at 0·4 g (0·13 per cent) per batch of diet: cholesterol, 7-dehydrocholesterol, ergosterol, lanosterol or lumisterol. Vitamin D_2, D_3 or squalene was also tested at 0·4 g in place of a sterol. The purity of all treatment chemicals was affirmed by gas–liquid chromatography and/or thin layer chromatography. Twenty fungus-free female beetles were originally placed on each test diet.

In the first fungus-free generation, viable progenies, including at least pupae, were present at 6 weeks in diets with each treatment except sterol-free (control), lumisterol, vitamin D_2 or D_3. Even though one pupa was found in the diet with squalene, it is considered equivalent to the no-sterol control. In the second generation (or brood), pupation had only occurred at 6 weeks in diets containing ergosterol or 7-dehydrocholesterol. Diets with cholesterol or lanosterol did support egg production and larval growth in the second generation.

The only structural characteristic present in both ergosterol and 7-dehydrocholesterol but absent in both cholesterol and lanosterol is the Δ^7 bond. Pupation of *X. ferrugineus* therefore required this double bond. Structural differences at C_{10} between variously useful sterols and the deleterious lumisterol and two vitamins apparently caused the latter compounds to inhibit larval growth even in the first generation.

In summary, *X. ferrugineus* is apparently unique among studied insects in its use of cholesterol for egg production and larval growth, but not for pupation. Cholesterol as a sole sterol was reportedly[7] inadequate for both larval growth and pupation of *Drosophila pachea*. With the development[8] of a completely chemically defined diet for the sterile culture of fungus-free *X. ferrugineus*, investigations of steroid and hormone metabolism with the beetle should bring about significant developments in insect endocrinology.

This study was supported in part by the Institute of Allergy and Infectious Diseases, US National Institutes of Health.

LOKE TUCK KOK
DALE M. NORRIS
HSIEN MING CHU

Department of Entomology,
University of Wisconsin,
Madison.

Received November 3, 1969.

---

[1] Büchner, P., *Endosymbiosis of Animals with Plant Microorganisms* (Interscience, New York, 1965).

[2] Baker, J. M., and Norris, D. M., *J. Invert. Path.*, **11**, 246 (1968).

[3] Norris, D. M., Baker, J. M., and Chu, H. M., *Ann. Entomol. Soc. Amer.*, **62**, 413 (1969).

[4] Norris, D. M., and Baker, J. M., *Ann. Entomol. Soc. Amer.*, **62**, 592 (1969).

[5] Bergmann, W., *J. Biol. Chem.*, **132**, 471 (1940).

[6] Clayton, R. B., *J. Lipid Res.*, **5**, 3 (1964).

[7] Heed, W. B., and Kircher, H. W., *Science*, **149**, 758 (1965).

[8] Norris, D. M., and Chu, H. M., *Ann. Entomol. Soc. Amer.*, **63** (in the press).

Inheritance of Sex Ratio in the Butterfly *Acraea encedon*

IN equatorial Africa, the butterfly *Acraea encedon* occurs chiefly in disturbed habitats in populations that are ecologically and genetically isolated from each other. Many of these populations are composed mainly of females, with males making up as little as 2 per cent[1,2]. In other parts of Africa, mainly in relatively dry or undisturbed habitats, the sex ratio is normal. There is no evidence of parthenogenesis[2]. In at least one population (Lubya, Uganda) the very low frequency of males seems to regulate the size of the population; many females remain unmated and contribute nothing to succeeding generations[1,2].

At Newton, Sierra Leone, a population of *Acraea encedon* is established in a small area of irrigated rice fields. The larvae feed on a weed, *Commelina* sp., associated with the cultivation of rice and other crops. In the period September 1968–August 1969 the sex ratio fluctuated from 1–2 per cent male to just over 30 per cent male, with an overall frequency of 15·6 per cent ($n = 1307$, obtained by marking and releasing the butterflies once a week over 44 consecutive weeks). In October 1968–March 1969, twenty-eight clusters of eggs, each from a different female and each comprising 50–100 eggs, were brought into the laboratory and the butterflies reared. Twenty-six clusters produced only female butterflies and two clusters males and females in ratios that do not depart significantly from the expected 1 : 1. There was no conspicuous mortality of larvae or pupae during the rearing. From this stock four successive generations of butterflies were obtained in the laboratory. Three kinds of mating were made: brother × sister, male × female from all-female broods, and male × female from male/female broods that were not sisters of the males. The results are summarized in Table 1. Males mated to females from all-female broods produced only female offspring. Brother × sister matings and males × females from male/female broods that were not sisters of the male produced males and females. In the twenty-nine broods that produced males and females there was no overall significant departure from the expected 1 : 1 sex ratio. One male was mated successively with three different females: two were its sisters and one a female from an all-female brood. The matings with its sisters produced male and female offspring, but the mating with the female from the all-female brood produced only females. There are thus two kinds of female, one producing males and females and one producing females only.

Table 1. SEXES APPEARING FROM DIFFERENT KINDS OF MATINGS IN *Acraea encedon*

Mating	Broods obtained	Sexes present in broods obtained
Brother × sister	24	Males and females
Male × female from male/female brood	5	Males and females
Male × female from all-female brood	17	Females only

The larvae of *Acraea encedon* are gregarious until the last instar and pupation takes place on or near the foodplant. Adult butterflies from a brood emerge from pupae over a period of several days, the males tending to appear slightly earlier than the females. Females are able to mate on the day they emerge. Because of this and because of the overall scarcity of males in a predominantly female population most matings that eventually produce males and females must be brother × sister.

It is possible that the predominantly female populations of *Acraea encedon* are of relatively recent origin[3], and that the sex ratio phenomenon is the result of hybridization of formerly isolated populations caused by human disturbance of the environment. Hamilton[4] has suggested that the phenomenon could be caused by a driving *Y* chromosome. If he is correct it is likely that there are populations in which the effects of a driving *Y* chromosome are covered up by the selection of epistatic suppressors on other chromosomes. But when a female from such a population enters a new population (perhaps as the result of human alteration of the environment) the trait could spread very rapidly as the new population may not have evolved a suppressing system. In these conditions a population could become predominantly female in quite a short time, but the sex ratio should return to normal again once suppressors have been evolved. If suppressors are not evolved the population could become extinct, although there are other possible ways of avoiding extinction, such as increased fitness of normal females, multiple matings by males (which are known to occur[1]) and non-random mating. In Uganda, one predominantly female population did become extinct, while another population seemed to become extinct but was apparently revived by butterflies arriving from elsewhere[1].

Acraea encedon is not a pest of crops—indeed, it is capable of defoliating an important agricultural weed—but the sex ratio phenomenon described here could have implications in the control of insect pests. Females that produce only females could be introduced into pest populations and numbers would then be regulated. Provisional sampling suggests that similar distortions of the sex ratio, always in favour of the females, occur in other African butterflies. All the species so far examined are characteristic of disturbed agricultural areas.

My results will be published in full elsewhere as part of a wider investigation.

D. F. OWEN

Department of Zoology,
Fourah Bay College,
University of Sierra Leone,
Freetown.

Received September 23, 1969.

[1] Owen, D. F., and Chanter, D. O., *J. Zool.*, **157**, 345 (1969).
[2] Owen, D. F., *Heredity*, **21**, 443 (1966).
[3] Owen, D. F., *Nature*, **206**, 744 (1965).
[4] Hamilton, W. D., *Science*, **156**, 477 (1967).

Alkaloids of an *Ipomoea* Seed known as *Kaladana* in Pakistan

Ipomoea muricata Jacq. (*Calonyction muricatum* G. Don) has now been confirmed as the biological origin of the *Ipomoea* seed mentioned in a previous communication[1]. Seeds of Indian origin (obtained by courtesy of Dr O. Fervidi, Simes Servizi Scientifici, Milan), identical to those from Pakistan, showed on germination in Beirut the typical shape and size of the cotyledonary and first leaves of *Ipomoea muricata* Jacq. described by Kassner[2]. The identity was further confirmed by the characteristically tubercled stem[3] and the aristate sepals[4]. *Kaladana* seeds, obtained from Pakistan (Gohar and Co., Karachi) in 1965 and 1967, differed from the original seeds (and those from India) in being hairy and greyish-black but otherwise identical to the original seeds in size and shape. All the seeds, however, had identical alkaloid chromatograms. A few of the 1967 seeds were grown in Lafayette in 1968 (by courtesy of Dr J. E. Robbers, Purdue University, Lafayette). Seeds obtained from these plants and grown in Beirut produced plants identical to those obtained from the Indian seeds. Strangely enough, the seeds obtained from Lafayette were devoid of alkaloids. In this connexion it is interesting to note that Der Marderossian[5] reports no indole alkaloids from "*I. muricata*". Besides lysergol and chanoclavine-I, reported[1] as present in *Kaladana* seed, other alkaloids were also found but in much smaller amounts[6].

CHARLES I. ABOU-CHAAR

School of Pharmacy,
American University of Beirut,
Beirut, Lebanon.

Received November 27, 1969.

[1] Abou-Chaar, C. I., and Digenis, G. A., *Nature*, **212**, 618 (1966).
[2] Kassner, H. C., *Pharm. J.*, **112**, 232 (1924).
[3] Hutchinson, J., Dalziel, J. M., and Hepper, F. N., *Flora of West Tropical Africa*, **2**, 347 (London, 1963).
[4] Chopra, R. N., Badhwar, R. L., and Ghosh, S., *Poisonous Plants of India*, second ed., **1**, 601 (ICAR, New Delhi, 1965).
[5] Der Marderossian, A. H., *Econ. Bot.*, **18**, 67 (1964).
[6] Abou-Chaar, C. I., *Leban. Pharm. J.*, **9**, 93 (1966–67).

Achene Spacing of Strawberries as an Aid to calculating Potential Yield

THERE have been attempts to determine potential biological yield on the basis of the size and efficiency of the photosynthetic apparatus. Economic yield, however, depends on the successful partition of assimilates to useful parts of the plant and, in the case of fruit crops, to the reproductive organs[1,2]. In the strawberry, one component of yield is fruit size, which we have shown to be determined by the number of fertilized ovules (achenes) on the surface and the degree of receptacle expansion associated with each achene. Berry weight is highly correlated with achene spacing and achene number, so that for each value of achene spacing there is a linear relation between berry weight and achene number (Table 1).

Table 1. LINEAR REGRESSION RELATING BERRY WEIGHT, NUMBER OF ACHENES AND ACHENE SPACING FOR REDGAUNTLET BERRIES

No. achenes/cm²	6	7	8	9	10
Slope of line	0·109	0·093	0·081	0·073	0·072
Constant	82	78	48	67	87
Correlation coeff. *r*	0·98	0·92	0·95	0·91	0·96
Achene spacing × slope	0·654	0·655	0·645	0·660	0·657
Percentage shortfall in berry weight	0	14·3	25·0	33·3	40·0

A general formula for berry weight has been developed:

$$\text{Berry fresh weight (g)} = \frac{(\text{Total No. achenes} - C)F}{\text{No. achenes/cm}^2}$$

where C is the mean constant of the regression lines and F is the mean value for achene spacing × slope. It follows that for an individual berry where the total number of achenes is unalterable and constant, fresh weight × achenes/cm² is constant[3]. For the variety Redgauntlet, C and F have the values 67 and 0·653 respectively.

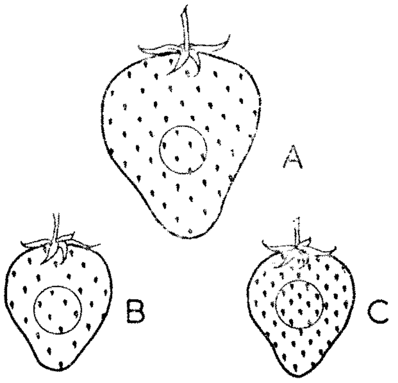


Fig. 1. Diagram showing relation of achene spacing to strawberry fruit size. Circles represent 1 cm².

All the data so far available to us show that a limit to surface expansion is reached at about 6 achenes/cm², so that it is now possible to calculate the potential maximum weight of a berry. In Fig. 1, berries A and B have both reached their potential size with six achenes/cm², and their weights are proportional to the total number of achenes on each. If berry C, with twelve achenes/cm², had expanded to the same extent as *A* it would have been of equal size because it has the same total number of achenes.

It is thus possible, using achene spacing measurements, to calculate the shortfall in yield resulting from submaximal receptacle development. The percentage shortfall in berry weight has been determined for achene spacings in the range 6–10 achenes/cm² (Table 1). Other experimental plants, yielding well by accepted standards, appear to have been capable of producing a 50–100 per cent greater yield of berries; and yields from a local commercial strawberry holding suggest that the crop could have been doubled if conditions had been more favourable to berry expansion. A large gap thus clearly exists between present yields and what could be achieved by manipulation of the environment (for example, irrigation, or better nutrition) during the final phase of development alone. A similar approach to the calculation of potential yield and the theoretical shortfall it reveals may be possible for other fruits, such as the raspberry, pineapple, blackcurrant and gooseberry.

A. J. ABBOTT
R. A. WEBB

Long Ashton Research Station,
University of Bristol.

Received November 24, 1969.

[1] Nichiporovich, A. A., and Strogonova, L. E., *Agrochimica*, **2**, 26 (1957).
[2] Bleasdale, J. K. A., *Ann. Appl. Biol.*, **57**, 173 (1966).
[3] Abbott, A. J., Best, G. R., and Webb, R. A., *J. Hort. Sci.* (in the press).

Non-biological Reduction of Nitrite in Soil

CHEMICAL decomposition of nitrite in soil[1-7] resembles microbial denitrification by producing gaseous end-products, N_2 and N_2O (refs. 8–11). NO and N_2 are formed in sterile soil over a wide *p*H range[12], and N_2 is also formed in sterile aqueous solutions containing nitrite and iron[13,14]. We show here that NO, N_2 and N_2O are products of non-biological nitrite decomposition in soil.

Soil was sterilized by autoclaving, brought to 60 per cent water-holding capacity with sterilized distilled water, and equal amounts of membrane-filtered $Na^{15}NO_2$ (60·019 atom per cent ^{15}N) and $Na^{14}NO_2$ were added. Final concentration was 250 μg N/g dry soil. The soil was incubated in respirometer flasks[15] at 25° C in an oxygen-free helium atmosphere for 168 h. Flask atmospheres were analysed by mass spectrometry. Atom per cent of ^{15}N in excess of normal was determined from isotope beam ratios. Quantitative analysis of respirometer gases was done by gas chromatography, using commercially available columns. Soil *p*H was measured at the sticky point with a glass electrode.

Table 1. ^{15}N ENRICHED GASES EVOLVED FROM SOILS AMENDED WITH NITRITE-^{15}N*

Soil	Per cent ^{15}N excess† NO	N_2	N_2O
Amity silty clay loam	29·40	4·10	5·22
Cove clay	14·70	3·13	11·70
Chehalis silty clay loam	19·40	2·94	14·20
Cloquato silt loam	14·60	2·48	9·70
Willamette silty clay loam	14·80	2·09	11·80

* Equal amounts of $Na^{15}NO_2$ (60·019 atom per cent ^{15}N) and $Na^{14}NO_2$ added to soil respirometer flasks to give a final concentration of 250 μg N/g dry soil.

† Excess ^{15}N in normal abundance determined from isotope beam ratios obtained by mass spectrometric analysis of flask atmospheres.

Soil containing added nitrite–^{15}N produced NO and N_2O heavily enriched with ^{15}N (Table 1). No microbial growth occurred in soil extracts incubated for 3 weeks in a variety of media and environmental conditions. The relative proportion of gases evolved from Amity, Cove and Cloquato soils was $NO > N_2 > N_2O$; whereas, for Chehalis and Willamette soils the order was $N_2 > NO > N_2O$. Gas analyses of many other soil systems in similar conditions revealed NO, N_2 and occasional small quantities of N_2O. Formation of N_2 and N_2O in sterile soil together with NO, a gas which is not usually associated with microbial denitrification, provides strong evidence for non-biological mechanism(s).

Table 2. PATTERN OF GAS PRODUCTION IN SEVERAL SOIL TYPES AMENDED WITH NITRITE*

Soil	*p*H	Per cent nitrite–nitrogen converted to gas† Autoclaved N_2	N_2O	NO	Non-autoclaved N_2	N_2O	NO
Astoria silt loam	4·8	7	0	71	9	0	68
Cove clay	5·8	14	0	37	17	0	41
Chalis silty clay loam	6·1	23	0	33	25	0	34
Willamette silty clay loam	6·2	26	0	63	28	0	60
Walla Walla silt loam	7·3	14	0	7	17	0	7
Corvallis sandy loam	8·9	1	0	11	1	0	10

* Nitrite-nitrogen added as $Na^{14}NO_2$ to give a final concentration of 200 μg N/g dry soil.

† Quantitative analysis of gases in respirometer atmospheres by gas chromatography.

The pattern of gas production in several soil types with added $Na^{14}NO_2$ is presented in Table 2. Evolution of N_2 and NO from both alkaline and acid soil indicates that *p*H is not the only factor involved in converting nitrite–N to gases[16]. Additional evidence, not presented here, suggests that organic matter and clay also affect the stability of nitrite in sterile soil systems. Autoclaving did not alter those soil properties which affect nitrite decomposition.

Copper, iron, manganese and certain aluminium salts may promote non-enzymatic decomposition of nitrite[13,14]. Metal ions can be reduced by metabolic intermediates and end-products of anaerobiosis[17] and then combine with nitrite, organic matter and colloidal clay. Such a complex could become reactive and liberate N_2 and other gases which are significant in soil denitrifying systems[11].

We thank Dr F. E. Broadbent, University of California, for assistance and interpretation of the ^{15}N analyses.

L. A. BULLA, JUN.*
C. M. GILMOUR†
W. B. BOLLEN

Oregon Agricultural Experiment Station,
Department of Microbiology,
Oregon State University,
Corvallis, Oregon 97331.

Received September 22, 1969; revised January 1, 1970.

* Present address: Northern Regional Research Laboratory, USDA, Peoria, Illinois 61604.

† Present address: Center for Environmental Biology, University of Utah, Salt Lake City, Utah 84112.

[1] Dhar, N. R., and Pant, N. N., *Nature*, **153**, 115 (1944).
[2] Allison, F. E., in *Soil Nitrogen* (edit. by Bartholomew, W. V., and Clark, F. E.), 573 (American Society of Agronomy, Madison, 1965).
[3] Broadbent, F. E., and Clark, F. E., in *Soil Nitrogen* (edit. by Bartholomew, W. V., and Clark, F. E.), 344 (American Society of Agronomy, Madison, 1965).
[4] Broadbent, F. E., and Stevenson, F. J., *Agricultural Anhydrous Ammonia Technology and Use*, 169 (Agricultural Ammonia Institute, Memphis, 1966).
[5] Allison, F. E., *Soil Sci.*, **96**, 404 (1963).
[6] Ewing, G. J., and Bauer, N., *Soil Sci.*, **102**, 64 (1966).
[7] Bremner, J. M., and Führ, F., *The Use of Isotopes in Soil Organic Matter Studies*, 169 (Pergamon Press, New York, 1966).
[8] Smith, D. H., and Clark, F. E., *Soil Sci.*, **90**, 86 (1960).
[9] Reuss, J. O., and Smith, R. L., *Proc. Soil Sci. Soc. Amer.*, **29**, 267 (1965).
[10] Nelson, D. W., thesis, Iowa State Univ. (1967).
[11] Wullstein, L. H., *Agric. Sci. Rev.*, **5**, 8 (1967).
[12] Bulla, L. A., thesis, Oregon State Univ. (1968).
[13] Wullstein, L. H., and Gilmour, C. M., *Soil Sci.*, **97**, 428 (1964).
[14] Wullstein, L. H., and Gilmour, C. M., *Nature*, **210**, 1150 (1966).
[15] McGarity, J. W., Gilmour, C. M., and Bollen, W. B., *Canad. J. Microbiol.*, **4**, 303 (1958).
[16] Wullstein, L. H., *Soil Sci.*, **108**, 222 (1969).
[17] Motomura, S., *Soil Sci. Plant Nutrit.*, **8**, 20 (1962).

Book Reviews

EVOLUTION OF AN INDUSTRY

A History of Coal Mining in Great Britain

By Robert L. Galloway. Introduction, Bibliography and Index by Baron F. Duckham. Pp. xi + 273. (David and Charles: Newton Abbot, April 1969. First published in 1882.) 63*s*.

THIS book is a reprint of Galloway's original text, first published in 1882, to which has been added an introduction, bibliography and index by Baron F. Duckham. It is difficult to imagine Galloway's historical synopsis of the British coal mining industry being superseded, for he and his family were contemporary to so much of what is now one of the most fascinating periods in the evolution of the industry. Written by a mining engineer as well as scholar and coalfield tourist, Galloway's book invites, and indeed enjoys among those who know it, approval as an accurate and well-balanced account of technical and scientific development of coal mining art and practice. He locates, as accurately as we are ever likely to define, an industrial embryo and traces its emergence from obscure beginnings, through periods of quiescence and vigorous growth, to a gigantic industrial activity gathering in economic momentum as it approaches the twentieth century. The story is simply told and is easy to read. It makes compelling reading not only for those associated with the industry but for anybody remotely interested in Britain as an industrial power either ancient or modern.

Baron F. Duckham has provided a historical supplement to the main text extending from 1880 to 1914. His treatment of the period is necessarily brief and claims to be no more. Fortunately, he resists what must have been a compelling temptation for an economic historian to comment on the social and political activity associated with the industry at that time. In this respect, Duckham keeps faith with his "co-author", thereby presenting a readable, stylish and accurate contiguity albeit chronologically misplaced. Rather than précis the events of 1880 to 1914 in his introduction, it might have been more meaningful to the reader to find the updating as an epilogue to Galloway's text.

Although undeniably accurate in his unfolding of events, Duckham fails to impress the importance of certain inventions and ideas to the development of the industry. For example, the invention of the face conveyor and the concept of conveyor transport has had a greater impact on technical progress in coal mining than any other single factor. Its introduction at the turn of the century completely changed the development of working methods. Indeed, the highly sophisticated mining systems of today are direct descendants of this invention. It is a pity that he was unable to find the time to tell more of the weird and wonderful inventions of the day in the field of mining machinery. These were mostly abortive but none the less fascinating. "Burnett's Patent Roller Wedge and Nicking Machine", for example, would excite the imagination of any engineer.

The publication of this book is opportune, coming at a time when today's coal mining industry is suffering the pains of rejuvenation. The reader is made aware that such tribulations are not new to this industry and our perspective of modern coal mining may be improved on learning that a mere 90 years ago, 4,231 collieries were needed to produce a gross output similar to that of today. Logically, one is at liberty to conclude that we have been averaging a net annual closure rate of forty mines for almost a century.

The book will provide interesting and informative weekend reading for anybody. But more particularly, the many college students who choose, as a study project, the history of coal mining in their region will find it an invaluable aid. There is an excellent bibliography which provides selected references appropriate to all the major coalfields. Engineers, be they mining or mechanical, civil or electrical, would do well to give the book more than a glance. For there is no industry in Britain that has not been either spawned or significantly influenced by the coal mining industry.

Duckham concludes his introduction with the comment ". . . The history of modernization since 1918, and particularly since nationalization, is a stirring one. It deserves another Galloway to do it justice". Indeed it is and indeed it does. Perhaps Duckham should look no further than the nib of his own pen.

F. F. ROXBOROUGH

SUNDIAL CONSTRUCTION

Sundials

A Simplified Approach by Means of the Equatorial Dial. By Frank W. Cousins. Illustrations by Malcolm Chandler. Pp. 247. (Baker: London, December 1969.) 126*s*.

MR COUSINS's book is the first major work on sundials in the English language to be published in the past 20 years (although one must not entirely overlook another recent work in a slightly lighter vein). It therefore deserves serious consideration, and it must be said at once that it fills a gap in the scientific literature and is to be welcomed.

The introduction is a little discursive, but the chief part of the work is mathematical and technical in content. Cousins sets out to describe the astronomical and mathematical foundations of the theory and practice of the construction and use of sundials, and shows in considerable detail how dials of many types, with their hour-lines inscribed on surfaces of a great variety of shapes and aspects, may be constructed by an intelligent layman. For each chief type of dial he illustrates three constructional methods for laying out the dial lines: one involving three dimensions and using the equatorial dial as described in the title to the work; a second involving two-dimensional drawing; and a third using simple trigonometry with the aid of tables. About one fifth of the book consists of a series of illustrations showing a large variety of existing sundials, both fixed and portable, with very brief descriptions; then comes the principal part of the book, consisting of about half of the total, in which the author gives full details for the construction of most of the chief types as described above. The work is rounded off with sections devoted to the layout of zodiacal lines, the use of dialling scales, a useful bibliography, and appendices including comprehensive tables of atmospheric refraction, the Sun's declination and the equation of time.

The author has been well served by his collaborators: Dr J. G. Porter, to whom is due much of the mathematical and astronomical detail; and Mr Malcolm Chandler, who has struggled manfully with the difficult task of representing in two dimensions diagrams involving three-dimensional geometry, and has been in most instances highly successful, although his upper illustration of a cone dial on page 31 could be misleading.

Cousins's own technical descriptions are well conceived but in some instances a little too brief for maximum clarity. There are a few errors here and there and some rather surprising omissions. The author touches only lightly on the large subject of portable and pocket sundials. He makes no mention of the important fact that there are

Mechanical equinoctial dial by Wm. Deane of London, c. 1690 (from *Sundials*, by F. W. Cousins, p. 65). (Photo by courtesy of the National Maritime Museum.)

two chief classes of such sundials—compass and altitude dials—nor does he draw attention to the defects of altitude dials, and he omits any description of ring, disk and quadrant dials. His historical series starts with the dial of Ahaz of about 700 BC and he omits the important Egyptian dials of about 1500 and 900 BC in the Berlin Museum. A minor point of criticism is that in dealing with the Equation of Time on page 74 he makes no attempt to dissect this into its two principal harmonic components. The only specific errors I wish to quote are the name of Hepslow for Henslow at the top of page 74, and in the photograph of a proposed Noon Mark on page 203, where the lower XI should be IX, and the month-end dots in the same region have a strange spacing.

Summing up, the book (apart from the blemishes I have mentioned) is an excellent compendium, attractively presented, of essential facts and figures relating to the theory and practice of the construction of sundials; and the fact that three alternative methods of layout are given for most of the principal types of dial should make it useful to a wide range of potential users. F. A. B. WARD

LANDMARK IN GEODESY

Mathematical Geodesy

By Martin Hotine. (ESSA Monograph No. 2.) Pp. xvi+416. (US Department of Commerce, Environmental Science Services Administration: Washington, DC, October 1969.) $5.50.

THIS book is indeed a new book on geodesy in that it treats the whole subject from a completely new standpoint and with the powerful technique of tensor calculus which is also completely new to most geodesists. The basic premise is that all geodetic measurements and concepts can be expressed as geometrical properties of a three-dimensional manifold, and a Euclidean metric suffices. Tensor calculus then enables geodetic and physical properties to be expressed in general form, particularly those which are invariant with the coordinate system, and they may be transformed very simply from one system to another.

The following illustrates the power of Hotine's use of tensor calculus. It is shown that the geometry of the Newtonian gravitational field can be treated as a special case of a (ω, φ, N) coordinate system, in which ω is longitude, φ latitude and N potential. Using Fermat's principle of least action, a light ray becomes a geodesic of the curved space obtained by conformally transforming the space in question to a curved space with scale factor n, the index of refraction. The whole of the theory of dynamic satellite geodesy is derived from Newton's second law of motion, which is first expressed in a general coordinate system. Then, although the whole concept of least action is Newtonian and the equations of motion referred to accelerating (rotating) axes are not Newtonian, the geodesic principle, the principle of least action and Newton's second law of motion are all shown to be equivalent.

Parts one and two of the book explain tensor notation and discuss the geometric properties of three-space expressed in this notation. Part one derives, or indicates the derivation of, formulae which will be used later. Part two derives coordinate systems of special interest in geodesy, and transformations between them, from a general class of three-dimensional systems. Part three applies the results of the first two to geodetic problems —to atmospheric refraction, computation and adjustment of control frameworks, potential and gravity anomalies, and to geometric and dynamic satellite geodesy. Finally, there is an index of symbols and a complete summary of formulae.

I disagree with some of the strictures made on current "classical" geodetic theory, in particular those on the classical use of the Laplace azimuth equation (paragraphs 14 and 15 of chapter 19). The tenor of the book could be considered to be given by the opening sentence of the preface: "This book is an attempt to free geodesy from its centuries-long bondage in two dimensions". When one comes to chapter 26, entitled "Internal Adjustment of Networks", it is, however, stated that, in adjustment by the widely used method of "Variation of Coordinates", the β (geodetic zenith distance) equations should properly be given less weight than the α (geodetic azimuth) equations because of uncertainties in angular refraction and, as their interaction is so limited, "the two sets of equations might be solved separately". This is indeed what is done in "classical" theory and practice, for the very reasons stated. Recent research, however, gives hope that it may soon be possible to measure angular refraction in the field with relatively simple field equipment to an accuracy commensurate with other measurements. When this day dawns, geodesy will indeed be freed from "its centuries-long bondage in two dimensions".

The real tenor of the book, however, is the unification of the whole of geodetic theory with complete mathematical rigour and elegance. In concept and in execution it is a beautiful piece of work and a fitting finale to the lifetime's work of the author. It is indeed a book which appears once in a generation and it is tragic that the author did not live to see the fruits of his labours. A. R. ROBBINS

STRUCTURES REVEALED

Methods for the Study of Sedimentary Structures

By Arnold H. Bouma. Pp. xvi+458. (Wiley (Interscience): New York and London, October 1969.) 190*s*.

THE interest shown by geologists in sedimentary structure has fluctuated over the years. Sir Charles Lyell and James Hall, working early in the past century, gave for the first time detailed descriptions of many structures, and Henry Clifton Sorby, in the 1850s, showed how such structures as ripple marks and cross-bedding could be understood physically and used for environmental interpretation.

In the 80 years following Sorby's work, there was little interest in sedimentary structures on account of the overwhelming need to solve purely stratigraphical problems, in which features indicative of sedimentary environment were widely thought to be irrelevant.

The wheel has turned once more, and geologists now realize the importance of a knowledge of sedimentary structures to an appreciation of both stratigraphy and sedimentary environments. But first, one must find the structures and then develop them to reveal as much as possible about themselves. Dr Bouma's important book is concerned with the techniques available to reveal sedimentary structures in the greatest possible detail according to circumstances. In studies of modern sediments, the field worker is faced with two problems which must be solved together: how to consolidate the sedimentary material and how to pick out with clarity the structures present in the deposit. The worker on consolidated sediments, on the other hand, is often faced with rocks showing structures only obscurely or not at all, and techniques are therefore needed to reveal any structures which, by reason of diagenetic processes, have become obscured or hidden in the rock.

Solutions to these and many related problems are to be found in Bouma's thorough and detailed account. The making of sedimentary peels is described together with the impregnation of sediments, either dried out or in a wet or moist state. Radiography, with application to unconsolidated as well as to lithified sediments, is discussed in detail and many useful practical hints on this relatively troublesome and expensive technique are given. Bouma also describes a variety of minor techniques such as staining, sand-blasting (also useful in fossil preparation), and moulding. The book has an exhaustive list of references, helpful appendices containing conversion tables and receipts, and a particularly useful list of manufacturers and suppliers of materials and apparatus.

This book will be an essential reference for all whose work involves sedimentary structures. It is unfortunate that its length and therefore price could not have been kept smaller by the rigorous exclusion of unnecessary detail and illustration for its own sake. For example, there are twenty-three full pages of costly half-tone illustrations that do little more than show that radiography will reveal the (not always unobvious) fabrics and structures present in rocks of different kinds. It is a pity that readers should be burdened in this way.

J. R. L. ALLEN

ENGINEER'S VIEW OF GEOLOGY

Engineering Geology and Rock Mechanics

By Neil Duncan. Vol. 1: Pp. xxii + 252 with 37 plates. Vol. 2: Pp. xxiv + 270 with 68 plates. (Leonard Hill: London, 1969.) Vol. 1, 55*s*; Vol. 2, 60*s*.

THE recent appearance of several new books on engineering geology suggests that new ideas and techniques have made it an opportune time for a re-statement of the subject for students. This two-volume work is related to the author's own experience of the ground covered in the first and second years of a civil engineering degree course. The approach is a creditable one because it is a new attempt to look at geology for engineers from the viewpoint of the engineer. Yet it does seem to fall short in the amount of real geology that it contains.

Volume one deals with the properties of "rock materials" and begins with the properties of minerals. (A short introductory chapter giving the nature and scope of engineering geology in relation to pure geology and engineering would perhaps have been appropriate here.) The structure of the Earth is briefly described and then there is a discussion of igneous, metamorphic and sedimentary rocks. The content of this discussion, however, is perfunctory, and the use of the now obsolete silica-percentage classification for igneous rocks is unfortunate. The nature and occurrence, structure and texture of each rock group are described together with a brief classification. The author then goes on to erect an engineering classification of rocks based on texture, structure, composition, colour and grain size, in which rocks fall into six groups. Such an approach may be of value to an engineer but might also tend to absolve him from making any geological identification at all, besides involving him in descriptions of texture, and the like, not catered for in earlier chapters. Presumably this is where a thorough course of practical work is essential—as indeed the author implies in his preface.

There is then a very detailed and lengthy consideration of the physical, mechanical and dynamic properties of rocks and the ways in which these properties may be measured. The volume ends with a description of soils (in the engineering and not the geological sense)—in what is a particularly comprehensive introduction to soil mechanics.

The second volume is concerned with rock masses and the application of geology to engineering problems. Because the reading lists in this and the first volume are quite adequate, it might have been better to omit the structural and stratigraphical chapter of this volume altogether rather than give it so brusque a treatment. The greater part of the section, however, is concerned with the methods by which joints may be studied in rock masses, the effects of groundwater, and the overall description of forces acting on the masses and their significance. The second part of this volume describes all types of engineering problems and practice, and provides a good practical demonstration of what has gone before. It also stresses by implication that field-work is an integral part of the course.

The text is very well illustrated with diagrams and graphs, but the choice and reproduction of some of the photographic plates make their interpretation almost meaningless (for example, plate 6, vol. 1, and plate 62, vol. 2). Any understanding of the graphs is also marred by the absence of any mention of the number of samples, and frequency and geographical location of the samples taken.

The approach of this work is very interesting and it can be recommended to teachers in the subject because it is different. Those that find sympathy with the approach will no doubt recommend the book to their students. It can also be recommended to geologists, particuarly those who are reluctant to quantify, for whom the chapters relating to rock–soil mechanics might prove particularly useful. But because it was written with students in mind, it would doubtless be better received if the volumes were combined in a paperback—perhaps without so many plates—at a reduced price.

JOHN R. V. BROOKS

SOLID STATE TUNNELLING

Tunneling in Solids

By C. B. Duke. (Solid State Physics: Advances in Research and Applications, Supplement 10.) Pp. x + 353. (Academic Press: London and New York, September 1969.) 149*s*.

TUNNELLING has enjoyed a golden age for the past decade, when it developed from a "good example in wave mechanics" to a major study and tool in solid state research. The publication of the first book on the subject probably indicates that this era is over and that both theoretical and experimental techniques will have to be refined to discover small deviations from the behaviour that can now be understood.

Duke has undertaken to tell the whole story of tunnelling. He considers electrons tunnelling through barriers with and without excitation states, between electrodes which are combinations of semiconductors, metals and superconductors. The achievements of both theory and experiment are critically examined, but the author's own interest has led to a greater emphasis on theory and rather less understanding is shown towards experiments. For example, he says that all systems claimed to be tunnelling should be proved to be so by the observation of the gap characteristic if one of the electrodes becomes superconducting, whereas this, of course, only indicates tunnelling over a small fraction of the energy range that is of interest.

The book is divided into two. The first half is concerned with independent electron effects which are usually large and can be understood quite well with relatively simple theory. The theory is presented first and then the measurements are critically compared with it. The second half deals with many body effects, and the author adopts the more cautious approach of describing the experiments and their interpretation before the detailed theory.

An admirable attempt has been made throughout the book to unify the diverse studies in tunnelling. The treatment and emphasis throughout are clearly personal, but this is the price of coherence. An honest effort has been made to present the arguments clearly and failures are emphasized as much as successes. I am sure people will find parts controversial, but on the whole it is, nevertheless, a very useful review. A. F. G. Wyatt

PHYSICS OF SCATTERING

Scattering Theory

New Methods and Problems in Atomic, Nuclear and Particle Physics. Edited by A. O. Barut. Pp. viii + 431. (Gordon and Breach: New York and London, 1969.) n.p.

This book consists of a collection of articles presented by theoretical physicists well known in their respective fields on aspects of quantum scattering theory and its applications to atomic, nuclear and elementary particle physics. Because quantum scattering theory is the chief underlying theme in the description of the various processes in atomic, nuclear and particle physics, an exposition of certain of its basic ideas and their relevance and use in these branches of physics could hopefully serve to bring out the unifying features of these different physical processes. This, together with a hope that such an effort may lead to a cross-fertilization of ideas between atomic, nuclear and particle physicists, forms, as the editor points out in the introduction, the chief objectives of this book. Given the enormous growth in these branches of physics in the past decade or so and their ever continuing expansion at an even faster pace, any significant accomplishment of these objectives is, in my opinion, quite impossible within the confines of a modest sized volume. Even a small contribution towards these objectives is, however, a praiseworthy venture and I think that this book does indeed make such a contribution.

The book consists of five parts. Most of the first part consists of a lucid article by F. Calogero on the variable phase approach to potential scattering. There is also a useful section by A. Degasperis on the inverse problem—that of obtaining the potential from the experimental scattering data.

The second part, which has the slightly misleading title of "General Scattering Theory", consists of three topics in quantum scattering theory. The first, written by G. C. Ghirardi, deals with the evaluation of upper and lower bounds for the number of bound states of a physical system. An elementary knowledge of the spectral theory of linear operators in a Hilbert space should prove useful in acquiring a deeper understanding of this material. The second topic, presented by J. Werle, is on relativistic polarization and angular correlation which is essentially a small portion of our up to date knowledge of the non-dynamical theory of relativistic particle reactions. Finally, the third topic, by L. Fonda, is on many-channel collision processes. Its relevance to the theory of nuclear reactions is obvious.

Parts three, four and five deal with the applications of scattering theory to atomic, nuclear and particle physics respectively. M. H. Choudhury

CRITICAL PATH CALCULATIONS

The Critical Path Method

Application of the PERT Method and its Variants to Production and Study Programs. By A. Kaufman and G. Desbazeille. Pp. x + 167. (Gordon and Breach: New York and London, August 1969.) 195*s*.

This book should be entitled "Theory of the PERT Method and its Variants". It is an account of the mathematical theory of critical path calculations, starting from an assumed knowledge of set theory, and extending to methods of parametric linear programming in the optimization of cost functions. It is well printed and laid out, with a consistent scheme of chapter, paragraph, table and figure numbers. As a result, it is possible to treat with amusement the occasionally quaint wording resulting from its translation from the French. But care is needed in following the terminology, as, for example, "float" becomes "margin", and "precedence diagrams" are variously called "method of potentials" or "tasks and constraints". Although the authors do take care to define all their terms as they come to them, there is no glossary or index to facilitate subsequent reference to definitions.

According to the foreword, this book is aimed at engineers, organizers, entrepreneurs and all who are responsible for the elaboration and control of programs. The first half of the first sentence reads "Without stretching the theoretical aspects relative to the search for ordinal relations in a graph, this concept, as will be seen, being nothing else but a set of elements among which oriented relations exist . . . ". If the reader survives the first chapter, which is a revision of the theory of graphs, he will find an impressive and well illustrated mathematical text, but one into which any really practical examples seem to have been introduced very grudgingly. The two mapping examples in section two are both borrowed from other authors, and Fig. 11.1, which is intended to explain the first of these, seems to be both irrelevant and inconsistent. The example in section thirty used to illustrate the Fulkerson algorithm is commended to the reader in the preface, where it is admitted that the algorithm itself is somewhat forbidding. But to work through the example requires the reader to have studied most of the rest of the book in any case.

For a dedicated operational research specialist who cares about the theory of critical path calculations, this book might well prove valuable. P. L. Cloot

POISONS INTRODUCED

Essays in Toxicology

Vol. 1. Edited by Frank R. Blood. Pp. xi + 169. (Academic Press: New York and London, September 1969.) 70*s*.

The increased use of chemical substances such as drugs, food additives, pesticides, fertilizers, and the like, and the fear of environmental pollution have stimulated the development of toxicology in its widest sense. Toxicology, the study of poisons, is today a multidisciplinary system of knowledge which acquires and interrelates information from many sciences including chemistry, physics, biochemistry, physiology, pharmacology, pathology, entomology, industrial and forensic medicine and food technology. The aim of *Essays in Toxicology*, of which this is the first volume, is to provide a publication which is scientifically accurate and at the same time sufficiently general to be read with profit by the "non-toxicologist". This is an ambitious aim in a world of increasing specialization, but the four essays in this volume illustrate the wide range of what may be regarded as toxicology. The titles of the four essays in this volume illustrate this point. They are "Poisons as tools in studying the nervous system", "Teratology", "The significance of methemoglobinemia in toxicology" and "Lead poisoning. An old problem in a new dimension".

The first essay, by R. D. O'Brien, occupies a third of the book and is an account of how poisons are used to study various forms of nervous transmission, namely axonic, excitatory synaptic and inhibitory synaptic transmission. The essay is an excellent introduction to nerve physiology with a brief account of the history and chemistry of each of the poisons used. It describes how natural and synthetic poisons have been used to study and differentiate the various forms of nerve transmission, the natural tetrodotoxin and synthetic DDT for axonic transmission, the plant poisons atropine, physostigmine, and the like, and the synthetic carbamate and organophosphate insecticides for excitatory synaptic transmission, and strychnine and picrotoxin for inhibitory synaptic transmission. The author suggests that whilst these poisons have served and will serve as tools for the nerve physiologist, they also represent one of the very few available starting points for biochemical work in this field in which our knowledge of biochemical mechanisms is as yet poor apart from the work on GABA (γ-aminobutyric acid), a transmitter substance in invertebrates. This could be regarded as an essay on nerve physiology rather than toxicology, but it illustrates admirably how the information from other sciences can be used to explain certain toxic actions.

The second essay, on teratology, by R. S. McCutcheon, is relatively short but with an extensive and useful bibliography. Although abnormal development and foetal malformations have been studied for more than a century, the author regards teratology as a new science stimulated by the thalidomide episode. The widespread occurrence of thalidomide malformations has led to the need for careful assessment of new and old drugs from the point of view of their action upon the foetus. The terms such as embryo and foetus and conceptus are defined, then the essay covers briefly placental physiology and the part of nutrition, genetic factors, drugs and infectious diseases in teratogenesis. The chief emphasis is on the need for increased basic and analytical research in teratology to replace much of the empirical animal work being done.

The third essay, on aspects of methaemoglobinaemia, by Roger P. Smith, discusses the concept of using a toxic response as a therapeutic measure, namely the generation of methaemoglobin by sodium nitrite in cyanide and sulphide poisoning. The other points which are interestingly discussed are toxic methaemoglobinaemia and its treatment and the contradictory reports concerning the effect of methylene blue in cyanide and carbon monoxide poisoning.

In the fourth essay, on lead poisoning, by P. B. Hammond, attention is drawn to the general problem of environmental pollution, lead being taken as an example. The author surveys lead poisoning in infants, industry and domesticated and wild animals, the metabolism of lead and the mechanism of its toxicity especially through the inhibition of haem synthesis. The new dimension mentioned in the title is that of foresight regarding general environmental pollution. In the case of lead, the author shows that, in the United States in 1966, 1,277,000 tons of lead were consumed, 573,000 tons were recovered, leaving 704,000 tons in the environment. About 20 per cent of the US lead consumption is in the form of the petrol additive, tetraethyl lead (TEL), and 70–80 per cent of the TEL used is released into the atmosphere to be inhaled or deposited on vegetation, soil and water.

These essays show the wide scope of toxicology, which is really a combination of many sciences. The "non-toxicologist" should therefore have no great difficulty in assimilating their content.

R. T. WILLIAMS

Obituaries

Professor Max Born

THE account that follows is a translation of the main part of a memorial address by Professor Werner Heisenberg. The address was given on January 12 at a meeting in Göttingen, where Max Born had died a week before at the age of 87.

Göttingen was Born's true scientific home. By the time he was 22, he was reading mathematics under Felix Klein and David Hilbert; he was introduced by Minkowski to problems in the electrodynamics of moving bodies, and it was here that he heard of the very beginnings of Einstein's theory of relativity and Einstein's light-quantum hypothesis, both of which made a very deep impression on him. Born's own thesis brought him into contact with questions of the elastic oscillations of rigid bodies, and in 1912, together with Theodore von Karman, he published his first scientific work of great significance; this dealt with the oscillations of crystal lattices. At that time the concept of a crystal as a regular ordering of atoms in a three dimensional lattice was generally accepted in principle; but it became a true physical statement only when it was possible to obtain concrete measurable results from the concept of a lattice. Born and von Karman applied the lattice model to the specific heat of the crystals at low temperatures for which Einstein had given a preliminary, although not entirely satisfactory, explanation in terms of Planck's quantum theory. Born and von Karman were able to demonstrate that the specific heat could be expressed even better if instead of considering the individual atoms as vibrating oscillators (as Einstein did), one considers the fundamental oscillations of the crystal lattice as the carriers of the oscillation-quanta. Henceforth much attention was to be paid to the lattice-structure of the crystal. In the same year, X-ray diffraction in crystals was discovered, providing a tool to throw light on the interior of crystals and to test the speculations of theoreticians. In the years that followed, Born continued to publish research on the lattice theory of crystals and assigned to his students doctoral theses connected with this sphere of problems. As late as 1954, when he was 72, he wrote in collaboration with a Chinese colleague a definitive report on this subject.

In 1915, Born was invited to Berlin as professor-extraordinary, where he entered into close association with Planck and Einstein. The four years in Berlin founded, first and foremost, the close friendship of Max Born and his wife Hedwig with Albert Einstein, which endured with undiminished cordiality until Einstein's death in 1955. A great part of Born's scientific deliberations was set down in his extensive correspondence with Einstein. After a brief period as professor in the University of Frankfurt, Born succeeded in 1921 to the professorship of theoretical physics at the University of Göttingen, and he was successful in obtaining at the same time the position of experimental physicist at Göttingen for his friend James Franck. Thus began the decisive new chapter in the history of the Mathematical Scientific Faculty at Göttingen. Up to this time, Göttingen had been a stronghold of mathematics; the tradition of Gauss, Riemann and Weber was consolidated by Felix Klein and Hilbert. And now, through Born and Franck, Göttingen also became a focus of atomic physics. Through his close cooperation with Franck, Born's interest was turned towards quantum theory, which he already knew very well from his contacts with Planck but which had originally repelled him somewhat by its obscurity, since he was so steeped in mathematical precision. With Niels Bohr's invitation to Göttingen in the summer of 1922 there began for Born a period of great activity on Bohr's theory of atomic structure, which found its expression in numerous lectures, seminars and colloquia.

Max Born proved to be an excellent teacher. Very soon he had attracted to his institute a number of gifted young theoreticians from various countries whom he inspired to take part in the study of the difficult problems of quantum theory. Together with Copenhagen, where Niels Bohr founded the new atomic theory, and Munich, where Sommerfeld with his work on atomic structure and spectral lines had founded a school for the new atomic science, Göttingen became a centre of the emerging discipline.

When I look back and compare the two schools of Munich and Göttingen where I was educated, and the teachers Sommerfeld and Born who headed them, I would say that Born bound his young colleagues to him first and foremost by making them still more sceptical of the contemporary state of the Bohr–Sommerfeld theory of atomic structure, and by awakening in his students the conviction that the most important work still remained to be done. In Munich there was still a widely held opinion that the atom could be approached by the old Newtonian mechanics provided that it was supplemented by the quantum conditions as formulated by Planck, Bohr and Sommerfeld. We at Göttingen had already more or less lost faith in this approach as a result of Bohr's lectures at Göttingen in 1922, and in his seminars Born expounded systematically his conviction that ultimately the problem was not one of working out complicated models of atoms and molecules, but rather of creating an entirely new mechanics. In spite of that fact—or rather, because of it—he required his students and colleagues to make a thorough study of the mathematical methods of the older mechanics and to consider them in seminars and discussions. For he rightly expected that only from a mastery of the older mechanics in all its details could there be awakened a consciousness of the scope which could perhaps be found for a new formulation of mechanics.

The seminars often took place in the evenings in a very small circle in the living room of the Borns' villa in an atmosphere of personal contact to which Frau Born, too, made a not inconsiderable contribution. I recall that at the time when I took part in these seminars, the participants very often included Pauli, Fermi, Hund, Jordan, the later Frau Goppert-Mayer and Delbrück. At that time most of them were still young students and only a few of them were over 25 years old. It was only later that they became known as co-founders of a new science, but it was here that they received their training for their future roles.

While speaking of personal contact, it should be mentioned that Born's home provided a good deal of social life for the young people, in which music played a great part. Born often played sonatas with Einstein, and as there were two grand pianos in Born's living room, I was occasionally able to play Mozart or Bach concertos with him. In the discussions at Born's seminars the ground was certainly prepared for the new atomic physics. As early as 1924 the word quantum mechanics was among those concepts which were obvious subjects of study. Born succeeded in producing among his young collaborators a sense of enthusiasm which meant that in the refectory or in the ski-lifts of the Harz mountains, quantum mechanics and the theory of astronomical perturbation were discussed far more than the notable events of the day.

If Born's school is to be compared with Bohr's school in Copenhagen, it should perhaps be said in favour of the Göttingen school that Born had a stronger conviction than Bohr that ultimately there must be a quantum mechanics that is closed and strictly formulated mathematically, and that quantum theory could not long remain in a state of contradiction between Newtonian mechanics, quantum conditions and the light quantum hypothesis. For Bohr, who had picked up the pragmatic tradition from his studies in England, this idea was not of such compelling importance as it was for Born with his mathematical training.

In 1925 and 1926, as a result of the joint work of Born's school, the final form of the new atomic theory was evolved. From his exceptional mathematical knowledge, Born was able to provide the methods of modern linear algebra, matrix calculus and theory of mappings for the representation of the new-found physical relationships. In the spring of 1926, when Schrödinger published his first researches in wave mechanics and proved the mathematical equivalence of quantum mechanics and wave mechanics, Born showed that the Schrödinger waves should be interpreted statistically so that their intensity is taken as a measure of the probability of finding a particle at a given point. The statistical character of natural law was for a long time the great stumbling-block to acceptance of the new theory; even Born and Einstein themselves could not agree on this, although their long-standing friendship was not, of course, affected. Later developments, however, proved that Born's views were the right ones.

In the years that followed, Born, together with his younger colleagues and students, applied the new quantum mechanics to problems of atomic structure, molecular physics and solid state physics. During this period a number of people destined to become the leading minds in physics, including Heitler, Fock, Oppenheimer, Teller, Weisskopf, Wigner and von Weizsäcker, belonged to the Göttingen circle. The intensive work they achieved is too far-reaching in its implications to be discussed here. The University of Göttingen at that time contributed far more to the prestige of German science abroad than most other German universities.

In the spring of 1933, the work came to a sudden halt with the devastation inflicted by the Nazis on Göttingen and all the other German universities. Born and Franck had to leave Göttingen. First of all, Born obtained a lectureship in Cambridge—the Stokes lectureship in applied mathematics, a position far lower than he had held in Göttingen. In 1936 he was offered the Tait professorship in natural philosophy at Edinburgh, and here he remained until his retirement in 1953. In Edinburgh too, Born gathered colleagues and students around him for a combined assault on physics. A series of works appeared on the statistical mechanics of condensed systems, the theory of fluids and associated problems. But the great activity of the Göttingen years could not be repeated in the Scottish university city, if only because in the meantime the interest of many physicists had turned to other problems. Nuclear physics was now the chief point of discussion. But Born did not take part in these developments. The possibility of the technical application of nuclear physics and the use of atomic energy became, at the end of the thirties, with Otto Hahn's discovery of uranium fission, the most exciting topic for atomic physicists. Born observed this development with great concern; he was dismayed at the possible consequences of these significant scientific advances and strove to minimize the danger.

Correspondence

X-Ray Stars and Infrared Excess

SIR,—In the course of his article on January 3, your Astronomy Correspondent says that Cowsik and Pal[1] are credited with the observation that " . . . the infrared flux might also be the cause of the X-ray background in the galactic plane which the Leicester group reported in October. . . . "

The fact is that the first article suggesting this mechanism for X-rays is the *Phys. Rev. Lett.*[2] article of the undersigned. Furthermore, when the Leicester results were reported in *Nature*, we published a further article (in *Nature*[3])—which also pre-dated the Cowsik–Pal article—discussing the anisotropy of the X-rays.

Yours faithfully,

ROBERT F. O'CONNELL
SATYA DEV VERMA

Louisiana State University
and Agricultural and Mechanical College,
Baton Rouge,
Louisiana 70803.

[1] Cowsik, R., and Pal, Y., *Phys. Rev. Lett.*, **23**, 1467 (1969).
[2] O'Connell, R. F., and Verma, S. D., *Phys. Rev. Lett.*, **22**, 1443 (1969).
[3] O'Connell, R. F., and Verma, S. D., *Nature*, **224**, 505 (1969).

Diffusion in Embryogenesis

SIR,—As an embryologist who started work during the heyday of "fields" and "gradients", I suppose I ought to be grateful to Dr Francis Crick for allowing me a nostalgic look back at these long-discredited concepts which he has now resurrected—or should I say, canonized—with the double halo of his own reputation and some elegant mathematics (*Nature*, **225**, 420; 1970). There is, however, one point that he appears to overlook: the extreme rarity with which sheer diffusion processes occur in living systems. Twenty years ago my better-informed colleagues told me about active transport and permeases. Ever since then, if materials have diffused in and out of my experimental embryos, I have regarded it as a sign that they are dying or dead. A sheet of frozen-dried tissue, extended between source and sink, might fit Dr Crick's formulae, but—alas—it would not differentiate !

Yours faithfully,

ELIZABETH M. DEUCHAR

The Medical School,
University of Bristol.

British Diary

Monday, February 16

Contention or Polling Techniques for Data–Computer Systems (5.30 p.m. discussion) Institution of Electronic and Radio Engineers. Joint IEE/IERE Computer Group, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Insect Hormones and Insect Control (5 p.m.) Dr K. C. Highnam, Society of Chemical Industry, at 14 Belgrave Square, London SW1.

Recent Advances in Medicine, Surgery and Therapy (6 p.m.) Sir Brian Windeyer, Royal Society of Arts, at John Adam Street, London WC2. (First of three Cantor Lectures. Further lectures on February 23 and March 2).

The Treatment and Disposal of Industrial Effluent (all-day symposium) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Tuesday, February 17

Acrylates, Their Production and Use (6 p.m.) Dr H. Willersinn, Society of Chemical Industry, at 14 Belgrave Square, London SW1.

Amplification Reactions and their Applications (4.30 p.m.) Professor R. Belcher, Society for Analytical Chemistry; and the Edinburgh University Chemical Society, in the Chemistry Department, University of Edinburgh, West Mains Road, Edinburgh 9.

Casting of Polyurethanes (2.30 p.m.) Plastics Institute, Processing Discussion Circle, at 11 Hobart Place, London SW1.

Cereal Growing in Britain (10 a.m.) Society of Chemical Industry, Agriculture Group, at 14 Belgrave Square, London SW1.

Discriminators for Broadcast FM Transmission (6.30 p.m.) Mr Hugh Mayo, Institution of Electronic and Radio Engineers, at Brighton College of Technology, Brighton.

Experimental Gallstones (5.30 p.m.) Dr I. A. D. Bouchier, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Twelfth of fifteen lectures on "The Scientific Basis of Medicine".)

Ionisation by Coordination (2.30 p.m.) Professor V. Gutmann (Vienna), University of London, in the New Chemistry Theatre, University College London, Gower Street, London WC1. (Further lecture on February 18).

Ocean Resources—Boom or Bubble? (1.20 p.m.) Professor L. J. Rydill and Dr A. J. Smith, University of London, in the Botany Theatre, University College London, Gower Street, London WC1.

System Identification with special reference to Respiration (5.30 p.m.) Dr I. Priban, Institution of Electrical Engineers; the Institute of Measurement and Control; and the Automatic Control Group of the I.Mech.E., at Savoy Place, London WC2.

The Management of Climatic Resources (5.30 p.m.) Professor T. J. Chandler, in the Chemistry Auditorium, University College London, Gower Street, London WC1.

The Volcano at Santorini and the Destruction of Minoan Crete (5.30 p.m.) Professor D. L. Page, University College London, in the Collegiate Theatre, 15 Gordon Street, London WC1. (Further lectures on February 19 and 24).

Wednesday, February 18

Cybernetics (7.30 p.m.) Dr A. M. Andrew, Institution of Electronic and Radio Engineers, at the University of Reading, Whiteknights Park, Reading.

Development and Marketing of New Chemical Engineering Equipment (2 p.m. symposium) Institution of Chemical Engineers, at the Royal Society, Carlton House Terrace, London SW1.

Factors Limiting the Application of Food Science and Technology in Developing Countries (2 p.m.) UK Coordinating Committee for Food Science and Technology, at the Society of Chemical Industry, 14 Belgrave Square, London SW1.

Gravitation and Geometry (5.30 p.m.) Professor P. C. Vaidya (Gijarat), University of London, at Queen Elizabeth College, Campden Hill Road, London W8.

Mass Communication (6 p.m.) Mr Stanhope Shelton, Royal Society of Arts, at John Adam Street, London WC2.

Microscopical Illustration (1 p.m.) Mr G. L'E. Turner, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Recent Advances in Radar Anti-Clutter Techniques (5.30 p.m.) Dr W. S. Whitlock, Institution of Electrical Engineers, at Savoy Place, London WC2.

Solute-Solvent Interactions (5.30 p.m.) Professor Viktor Gutmann, University of London, at King's College, Strand, London WC2.

Street Lighting (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Measurement of Ships' Velocity (5 p.m.) Mr B. W. Oakley, Institute of Navigation, at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London SW1.

The Presentation of Data from a Process Control Computer (7.30 p.m.) Institution of Chemical Engineers, in Room G44 Royal Fort, University of Bristol.

The Professional Engineer as an Industrial Tutor (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

The Role of The City University (1.10 p.m.) Sir James Tait, City University, at the Graduate Business Centre, Gresham College, Basinghall Street, London EC2.

Use of Computers in Designing Automatic Process Controllers (6 p.m.) Mr D. J. Norton, Institution of Electronic and Radio Engineers; and the Institution of Electrical Engineers, at the University of Bristol.

Thursday, February 19

Amino-Acids and Peptides in Gut and Kidney (5.30 p.m.) Professor M. D. Milne, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1.

Bridging the Gap—an Open Discussion of the Problems of Manufacturers and Users in the Field of Automatic Instrumentation (6.30 p.m. discussion) Society for Analytical Chemistry, Automatic Methods Group, at the Leicester Lounge, Glasshouse Street, London W1.

Carbon Fibres (7 p.m.) Mr R. Morgan, Plastics Institute, at Imperial Chemical Industries, Ltd., Britannia House, 50 Great Charles Street, Birmingham 3.

Gene Action and Its Control (10 a.m. two-day meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

Fragrances for the Paper Industry (7.30 p.m.) Mr A. F. Morris, British Society of Perfumers, at "The Havelock", Gray's Inn Road, London WC1.

How to Find a New Herbicide (7 p.m.) Dr J. A. Farrington, Society for Analytical Chemistry; and the Royal Institute of Chemistry, in the Department of Chemistry, University College of Swansea.

Manufacture of Small Electronic Components (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

People, Communications and Engineering (5.30 p.m.) Mr J. H. H. Merriman, OBE, Institution of Electrical Engineers, at the Central Hall, London SW1.

Phlebotomine Sandflies and Leishmaniasis in British Honduras (Belize) (7.30 p.m.) Dr Paul Williams, Royal Society of Tropical Medicine and Hygiene, at Manson House, 26 Portland Place, London W1.

Placental Localization Studies (5.45 p.m. discussion); **Plain Film Changes in Heart Disease** (7.15 p.m.) Dr R. G. Grainger, Dr K. E. Jefferson and Dr I. Kerr, British Institute of Radiology, at 32 Welbeck Street, London W1.

Polishes (7 p.m.) Mr E. M. Clough, Oil and Colour Chemists' Association, at the Manor Hotel, Dachet, Bucks.

The Oxford Biological Films Unit. Films and the Techniques of Scientific Film Production (5 p.m.) Linnean Society of London, at Burlington House, Piccadilly, London W1.

The Problems of Specifying Road Materials Mr J. H. Nicholas, Society of Chemical Industry, Road and Building Materials Group: and the Institute of Highway Engineers, in the Mechanical Engineering Department, Imperial College, Exhibition Road, London SW7.

The Use of Grease as an Engineering Component (two-day symposium) Institution of Mechanical Engineers, at 1 Birdcage Walk London SW1.

Your Environment at Stake (8.15 p.m.) Mr Dunstan Skillbeck, University of London, at Witheradane Hall, Wye College, near Ashford, Kent.

Friday, February 20

A Review of Image Intensification (10 a.m.) Dr F. H. Doyle, Dr T. H. Hills, Mr E. J. Steadman *et al.*, British Institute of Radiology, at 32 Welbeck Street, London W1.

Communications in the Pacific (1.15 p.m.) Mr E. Clunies-Ross, Institute of Rural Life at Home and Overseas, at Friends International Centre, Courtauld House, Byng Place, London WC1.

Corrosion Problems in Highway Research (6.30 p.m.) Dr R. R. Bishop, Oil and Colour Chemists' Association, at the Chamber of Commerce House, 75 Harbourne Road, Birmingham 15.

Field Store Standards Conversion (5.30 p.m.) Mr S. M. Edwardson, Institution of Electrical Engineers, at Savoy Place, London WC2.

Forensic Science (7.45 p.m.) Dr Hamish Walls, H. G. Wells Society, in the Electrical Engineering Department, Imperial College, Exhibition Road, London SW7.

Moon Dust (9 p.m.) Professor S. Tolansky, FRS, Royal Institution, at 21 Albemarle Street, London W1.

Some Recent Studies of Excited States by Pulse Radiolysis (1 p.m.) Dr E. J. Land, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

The Chemistry and Biological Activity of 1,4-Benzodiazepines (6.30 p.m.) Dr L. H. Sternbach, Society of Chemical Industry, at 14 Belgrave Square, London SW1.

The Core-Mantle Interface (2.30 p.m. geophysical discussion) Royal Astronomical Society, at Burlington House, Piccadilly, London W1.

The Relative Importance of Heredity and Environment in the Growth and Physique of Children in Sub-Saharan Africa (5.30 p.m.) Professor Jean Hiernaux (Brussels), University of London, at the Institute of Child Health, 30 Guilford Street, London WC1.

Saturday, February 21

Rapture of the Deep (3.30 p.m.) Mr Ley Kenyon, Inner London Education Authority, at the Horniman Museum, London Road, Forest Hill, London SE23.

HOW TO BUY NATURE

Volumes start in January, April, July and October, but subscriptions may begin with any issue.

The direct postal price per subscription for one copy of NATURE each week is:

12 MONTHS (52 issues and 4 indexes)

Great Britain and Eire	**£14.0.0** each
United States of America (by air freight)	**$48** each
Canada (by air freight)	**$52** each
Elsewhere overseas by surface mail	**£14.0.0** each

(Charge for delivery by air mail on application)

•Shorter periods *pro rata*. (Minimum three months.)

Monday, February 23

Terminal Units and Transmission in Electronic Telephone Exchanges (5.30 p.m.) Mr T. H. Flowers, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Mechanical Equipment for the Construction of the Victoria Line (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Thermoplastics in the Cable Industry (7.30 p.m.) Mr R. H. Greenhalgh, Plastics Institute, at the Stork Hotel, Queen Square, Liverpool.

Reports and Publications

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 265, No. 1161 (13 November 1969): A Discussion on Recent Research in Air Pollution. Organized by J. S. Forrest, FRS, and B. J. Mason, FRS. Pp. 139–318 + 3 plates. 99*s*.; $12.85. Vol. 265, No. 1162 (13 November, 1969): On Dual Approximation Principles and Optimization in Continuum Mechanics. By M. J. Sewell. Pp. 319–351. 17*s*.; $2.20. (London: The Royal Society, 1969.) [1711

Report of the Anti-Locust Research Centre for 1968. Pp. iii + 46. (London: HM Stationery Office, 1969.) 7*s*. net. [181

Natural Environment Research Council. The Nature Conservancy. Monks Wood Experimental Station—Report for 1966–1968. Pp. 88. (Abbots Ripton, Huntingdon: The Nature Conservancy, 1969.) 10*s*. net. [181

British Museum (Natural History). Economic Series, No. 2A: Lice. Fourth Edition. Pp. 23. (London: British Museum (Natural History), 1969.) 2*s*. [1911

Shirley Institute—Report and Accounts 1968/1969. Pp. 28. (Manchester: The Cotton Silk and Man-Made Fibres Research Association, 1969.) [1911

Mathematical Association. Computer Education for All. Pp. 4. Introduction of SI Units in Schools. Pp. 25. (London: Mathematical Association, 1969.) [2011

Ministry of Overseas Development. Report of the Anti-Locust Research Centre for 1968. Pp. iii + 46 + 4 plates. (London: HM Stationery Office, 1969.) 7*s*. net. [2411

Philosophical Transactions of the Royal Society of London, B: Biological Sciences. Vol. 256, No. 808 (20 November, 1969): The Complex of *Ficus deltoides*: a Recent Invasion of the Sunda Shelf. By E. J. H. Corner. Pp. 281–355. (London: The Royal Society, 1969.) 38*s*.; $5. [2411

Other Countries

US Department of the Interior: Geological Survey. Water-Supply Paper 1877: Feasibility Study of the Use of the Acoustic Velocity Meter for Measurement of New Outflow from the Sacramento–San Joaquin Delta in California. By Winchell Smith. Pp. vi + 54. $0.40. Water-Supply Paper 1879-E: Variations in Low-Water Streambed Elevations at Selected Stream-Gaging Stations in Northwestern California. By John J. Hickey. Pp. iii + 33 + plate 1. (Washington, DC: Government Printing Office, 1969.) [1711

Studia Forestalia Suecica. Nr. 68: Growth of *Fomes annosus* in the Presence of Host Material from Norway Spruce and Silver Fir. By Arne Hyppel. Pp. 16. 5 kr. Nr. 69: *Melampsora pinitorqua* (Braun) Rostr. on Progenies of *Pinus silvestris* L. and in Relation to Growth Regulating Substances. By Allan Klingström. Pp. 74. 12 kr. Nr. 70: A Summary of Meiotic Investigations in Conifers. By Enar Andersson, Inger Ekberg and Gösta Eriksson. Pp. 20. 5 kr. Nr. 71: Economic Planning of the Farm Forest Operating Unit. By Göran von Malmborg. Pp. 55. 10 kr. Nr. 73: Investigations on the Suitable Germination Duration for *Pinus silvestris* and *Picea abies* Seed. By S. K. Kamra. Pp. 16. 5 kr. (Stockholm: Skogshögskolan, Royal College of Forestry, 1969.) [1811

The Aeromagnetic Survey of Denmark, Finland, Norway, Sweden, 1965. (Contribution to the World Magnetic Survey.) Pp. 59. (Stockholm: Geological Survey of Sweden, Section of Regional Geophysics, 1969.) [1811

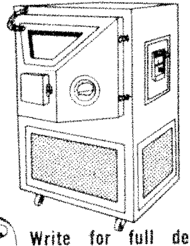
Fisheries Research Board of Canada. Technical Reports. No. 134. Preliminary Report on Parasites as Biological Tags in Atlantic Salmon (*Salmo salar*). 1: Investigations 1966 to 1968. By John H. C. Pippy. Pp. 44. No. 136: On the Origin and Distribution of Larval Swordfish *Xiphias gladius* L. in the Western Atlantic. By S. N. Tibbo and L. M. Louzier. Pp. 20. No. 139: Catch Statistics—Length and Age Composition, Gulf of St Lawrence Herring. By S. N. Tibbo, S. N. Messieh and C. D. Burnett. Pp. 5 + figures and tables. (St. Andrews, NB: Fisheries Research Board of Canada, 1969.) [1811

Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
4 LITTLE ESSEX STREET, LONDON WC2
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2
Subscription Department
MACMILLAN (JOURNALS) LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 5431
Advertisements only should be addressed to
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2
Telephones 01-242 6264/01-405 4743
Telegrams: Textualist London WC2
Registered as a newspaper at the Post Office

Copyright © Macmillan (Journals) Limited, February 14, 1970

the approved standard of preparing frozen tissue sections

LEHRER and WEDEEN MICROTOME CRYOSTATS



The WEDEEN Cryostat has the only wide range of controlled operating temperatures from 0°C to —80°C.

The LEHRER Cryostat has a main chamber with a controlled temperature to —40°C.

R/S Write for full details

Manufacturers of:
Lehrer and Wedeen Microtome
Cryostats and Atmo-Vac® Freeze Dryers.

Refrigeration For Science, Inc.
74 Alabama Avenue • Island Park, New York 11558

Represented by: AURIEMA INTERNATIONAL GROUP

'MICHROME'

STAINS & REAGENTS FOR BIOLOGY

Acridine orange, Aquamount, Fluormount, Lipid crimson, Matamount, May-Grünwald, Leishman, Giemsa Toluidine blue, Cresyl fast violet, Papanicolaou stains, Thioflavine S, Brilliant cresyl blue, Nile blue, Pyronin Y, Trifalgic acid, Rhodanile blue, Haplo, etc.

CATALOGUE AVAILABLE ON REQUEST

DYES FOR USE IN ALL INDUSTRIES

RESEARCH & PRODUCTION CHEMISTS: if you are seeking dyes with specific chemical and physical properties suitable for particular manufacturing and/or testing processes you are invited to contact us for supplies.

We hold stocks of an enormous variety of dyes, ready for immediate despatch, including:

Cyanine, Dicyanine, Fast Bordeaux O, Aurantia, Crystal violet, BPC-Scarlet R, BPC, Diazonium salts, Neutral violet, Lacmoid, Nile blue, Janus green, Brilliant green BPC, Rosolic acid, Methyl green, p-Ethoxy-chrysoidin, Methyl red, Nitrazine yellow, Pinacryptol green, etc.

INDUSTRIAL RESEARCH ESTABLISHMENTS

are invited to make use of our professional services as consultants on the selection and use of dyes as research and production tools.

EDWARD GURR LTD

Michrome Laboratories,
42 Upper Richmond Road West, London, S.W.14
Cables: Micromlabs London SW14. Telephone: 01-876 82289

QUALITY — RELIABILITY — SERVICE
PROMPT DELIVERY

wright ACRYLIC chromatography columns

versatile inexpensive laboratory columns for chromatographic and gel filtration procedures employing aqueous solvents

Simple to use—unique patented design in acrylic and nylon with sintered plastics bed support.
Minimal dead space with low-volume connections. Diameters selected to produce cross-sectional area of 2, 4, 8, & 16 sq. cms.
Fixed or adjustable columns with or without jackets.
Standard lengths of 30, 60 and 90 cms.
Columns easily lengthened by joining.
Prompt delivery of standard columns from stock.
Preparative columns with bed volumes up to 50 litres are available to special order

Prices from £5

Phone or write for detailed catalogue to Dept. CC1/N

wright SCIENTIFIC LIMITED 3 Lower Road, Kenley, Surrey, CR2 5NH *Tel:* 01-668 4611

MONASH UNIVERSITY

Graduate Research Scholarship for Research into Engineering Properties of Plastics (Civil Engineering)

Applications are invited from suitably qualified graduates for a graduate research scholarship which has been established by the State Rivers and Water Supply Commission for an investigation into the long term behaviour of plastics components in the field. The work involves both an examination of the materials involved and the conditions to which they will be subjected in service.

BENEFITS:

Stipend £2,500 p.a., plus dependants allowance £500 p.a. for a scholar with one or more dependent children; tuition fees covered by grant attached to scholarship. The scholarship is initially for 2 years and would enable a candidate to complete the requirements for the degree of Master of Engineering Science; a possible extension for a third year is envisaged to enable the scholar to transfer to Ph.D. candidature.

Further details may be obtained from Professor N. W. Murray, Department of Civil Engineering, Monash University with whom applications close on 28th February, 1970. It is hoped to hold interviews in Britain and Australia during March.

Application forms are available from the Agent General for Victoria, Victoria House, Melbourne Place, The Strand, London, W.C.2.

J. D. BUTCHART,
Academic Registrar

Monash University,
Clayton, Victoria, 3168.
Australia.

For further Classified advertisements see page vi

Railway Transport Research

MAJOR PHYSICS PROJECTS

The British Railways Board is engaged on a major expansion of its research activities involving a wide range of scientific and technological work. There are several major physics projects, some of which have special funding from the Ministry of Transport. This work has also attracted financial support from abroad.

This expansion has created a further three posts in the Electrical Research Division for work in the field of environmental physics and ergonomics. Candidates for the more senior posts should have research experience, good knowledge of basic physics and the ability to plan experiments. Familiarity with electronic techniques of measurement and information display, or a general knowledge of measuring techniques met in environmental studies is desirable.

The third post needs a keen practical experimentor with a good physics or electronic engineering background, interested in making and using apparatus for measuring the physical effects of environment on men and machines.

An Honours Degree or equivalent academic attainments will be necessary for all these posts. Salaries will be in the range £1810 to £2340 or £1700 to £2010 per annum depending on experience and qualifications. All posts will be located in the Railway Technical Centre, Derby.

The Board operates a contributory pension scheme and has arrangements with many employers for the preservation of pensions. There are also free and reduced rate rail travel facilities.

Applications giving age, education, qualifications, experience and present salary should be sent to the **Headquarters Staff Manager (quoting reference RD.E/U), British Railways Board, 222 Marylebone Road, London, NW1.**

(606)

Glaxo

IMMUNOLOGIST

A vacancy exists in the Biological Research Department of Glaxo Research Limited at Greenford, Middlesex, for an Immunologist to join a team engaged in a variety of problems connected with immunostimulation and immunosuppression.

The post is an interesting and challenging one, offering considerable opportunities for original work. Ideally, applicants should be between 25 and 35 years and should have a Ph.D. in Immunology, but graduates in Biological Sciences with post graduate experience in Immunology will also be considered.

An attractive salary will be offered to secure the right candidate. Conditions of employment include participation in the company's profitability, contributory pension scheme and assistance with removal expenses where appropriate.

Please write, quoting reference U.93, to the Senior Personnel Officer (GJB), Glaxo Laboratories Limited, Greenford, Middlesex. (607)

UNIVERSITY OF OXFORD

UNIVERSITY LECTURERSHIP IN GEOCHEMISTRY

University Lecturer in Geochemistry (in the Department of Geology and Mineralogy) required with effect from October 1, 1970.

Applicants may be graduates either in chemistry or in geology, and must be prepared to teach a course in inorganic and physical chemistry for the Preliminary Examination in Geology, and to be responsible for the organisation of, and some of the teaching for, the postgraduate Diploma in Geochemistry. Some assistance in teaching other relevant courses for the Honour School of Natural Science (Geology) may also be required. The person appointed will also be responsible for the working of the chemical laboratories within the Department.

The University Lecturership may be held in conjunction with a college Fellowship or Lecturership, particularly if the successful candidate were able to devote some of his time to college tuition in inorganic and physical chemistry for the Honour School of Natural Science (Chemistry).

The initial stipend will be according to age in the range £1,240 (at twenty-four and under) by £115 to £2,850 (at thirty-eight) by £90 to £3,120 (at forty-one), with F.S.S.U. benefits.

Four typed copies of applications (one only from overseas applicants), including the names of two referees, should reach Professor E. A. Vincent, Department of Geology and Mineralogy, Parks Road, Oxford, OX1 3PR (from whom further information may be obtained), not later than March 9, 1970. (628)

Searle Scientific Services

a division of G.D. Searle & Co. Ltd.

BIOCHEMIST

A Graduate Biochemist is required to work in the Biochemistry section of our Clinical Pathology Laboratory in London. The laboratory carries out a wide range of tests and is highly automated. Equipment includes Technicon SMA 12/60, 6/60, 7A, P.B.I. and single channel analysers and a B.T.L. Analmatic Clinical Analysis System. It is planned to put these on line to a computer and delivery of the first Digital PDP-12 advance Clinical Laboratory System in Europe is scheduled for early in March 1970. The post is concerned mainly with quality control and data processing and candidates should have an interest in this area in addition to a wide experience in a hospital biochemistry service. Emphasis will be placed on research and development and every encouragement will be given to publication of scientific work.
Salary will be attractive and negotiable; conditions of service are excellent.

Please write with brief details to:

The Personnel Director,
G. D. Searle & Co. Ltd.,
Lane End Road,
High Wycombe, Bucks.

SEARLE

Research in the Service of Medicine.

(572)

Scientific Writer Sweden

PHARMACIA

Leaders in the field of gel Filtration media are offering an excellent opportunity at their headquarters in Sweden. This post is based in Uppsala. The successful candidate should have a degree or equivalent qualification in Chemistry, Biochemistry or allied subjects.

Clear expression in the written word is essential with a high degree of accuracy and English as the Mother tongue.

He will be joining a young team in the Technical Information Department with responsibilities for the production of manuscripts for technical and scientific information and promotional literature. His duties will also include literature searching.

Preferred age 25–30. This is an important post which will command a salary commensurate with qualifications and experience and carries good social security arrangements.

Please apply in writing giving personal details, education, qualifications and experience. All applications will be treated in strict confidence.

To :

Pharmacia (G.B.) Ltd,
(Ref. Personnel F.C.),
75 Utbridge Road,
London W.5.

(655)

MONASH UNIVERSITY

MELBOURNE, AUSTRALIA

DEPARTMENT OF PHYSIOLOGY

Applications are invited for appointment to the position of Senior Teaching Fellow or Teaching Fellow in the Department of Physiology.

The department teaches courses in Physiology and Pharmacology for the degrees of B.Sc., B.Sc.(Hons.) and M.B., B.S., and accepts postgraduate students for the degrees of M.Sc., and Ph.D. Principal research interests are: brain function and ultrastructure, including learning mechanisms, the physiology of sense organs and their central pathways, spinal reflex mechanisms and control of movement by the brain, and biophysics and pharmacology of central and peripheral synapses and of smooth and cardiac muscle; endocrine and circulatory physiological control mechanisms. Increasing attention is being paid to the correlation of ultrastructure with physiological findings, and computer analysis of experimental data.

Salary ranges : Senior Teaching Fellow $A4,390 to $A5,290 per annum ; Teaching Fellow $A3,600 to $A4,275 per annum.

Benefits: Travelling expenses for appointee and family; removal allowance, initial subsidised housing ; repatriation after three years' appointment if desired.

Further general information including details of application procedure is available from the Academic Registrar, Monash University, Wellington Road, Clayton, Victoria 3168, Australia, or the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London, W.C.1. Information concerning research and teaching programmes may be obtained from Professor A. K. McIntyre, in the University.

Closing date for application : March 16, 1970.

The University reserves the right to make no appointment, or to appoint by invitation.

(624)

PAPUA AND NEW GUINEA INSTITUTE OF TECHNOLOGY

(Formerly Institute of Higher Technical Education)

PROFESSOR OF ELECTRICAL ENGINEERING OR PROFESSOR OF MECHANICAL ENGINEERING

Applications are invited from Mechanical and Electrical Engineers for the post of Head of the joint Electrical and Mechanical Engineering Department.

Salary: $A12,500 per annum. An additional $A360 is normally paid to married male members of staff.

The Chairman of the Council of the Institute is Dr. J. A. L. Matheson, M.B.E., M.Sc. (Manch.), Ph.D.(Birm.), F.I.C.E., M.I.Struct.E., F.I.E.Aust., who is Vice-Chancellor of Monash University. The Director is Dr. W. E. Duncanson, M.Sc.(Syd.), Ph.D.(Camb.), D.Sc. (Lond.), F.Inst.P., C.Eng., F.I.E.E., F.I.E.Aust.

Applicants should have a degree in the appropriate subject with experience in teaching, in research, or in industry. It is not expected that applicants will have had experience in both Electrical and Mechanical Engineering as there will be other senior staff in both disciplines. However, a broad interest in the subjects would be an advantage.

The Institute provides professional technological training for the whole of the Territory and has been charged with the responsibility of undertaking research and development in these subjects. A number of projects are already in hand and others are planned.

Further information about the Institute, method of application and conditions of appointment (including leave and leave passages, F.S.S.U. type superannuation, study leave, etc.), may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London, W.C.1.

Applications close on March 25, 1970.

The Institute reserves the right to make no appointment or to make an appointment by invitation at any stage. (591)

DEPARTMENT OF PHYSICS

UNIVERSITY OF MANITOBA

Offers generous assistance to highly qualified students wishing to do graduate work in physics. Extensive and modern facilities for work in nuclear structure and reactions, solid state, low temperature, atomic physics, and cosmic rays are available.

For information apply to: Head, Department of Physics, University of Manitoba, Winnipeg 19, Man. (X495)

NATURE

MACMILLAN (JOURNALS) LTD

No. 5234 SATURDAY FEBRUARY 21 1970 Vol. 225

CONTENTS

LOOSE EDGES IN THE GRAND DESIGN—CAN THE HEALTH SERVICE SURVIVE ?—100 Years Ago . . . 673

Old World—Drugs—Health—Social Medicine—Wild Life—Cell Biology—Avalanches—Atomic Physics—Rocket—Computers—Science Policy—Micro-organisms—Parliament in Britain . . . 675

New World—THE CRUSADE AGAINST POLLUTION—Oil Production—Meteorology—Standards—Nuclear Research—Spacecraft . . . 681

LOVELL CHARTS THE FUTURE . . . 686

News and Views—NEEDLES IN A HAYSTACK ARE HARD TO FIND—Planets—Many-body Problem—Fracture Mechanics—Electron Microscopy—Planetary Atmospheres—Entomology—Tumour Virology—Biochemical Thermodynamics—Gynaecology—Forestry—Plant Anatomy . . . 687

CASE HISTORIES OF INNOVATIONS by R. W. Cahn . . . 693

FORMATION OF THE INITIATION COMPLEX USING MUSCLE MESSENGER RNAs by Stuart M. Heywood . . . 696

ANTIGENIC PROPERTIES OF A HOMOGENEOUS TOBACCO MOSAIC VIRUS–HAPTEN CONJUGATE by L. I. Slobin . . . 698

EFFECTS OF BLACK WIDOW SPIDER VENOM ON THE FROG NEUROMUSCULAR JUNCTION by Herbert E. Longenecker, jun., William P. Hurlbut, Alexander Mauro and Allen W. Clark; Allen W. Clark, Alexander Mauro, Herbert E. Longenecker, jun., and William P. Hurlbut . . . 710

FAST SPECTROPHOTOMETRIC MEASUREMENT OF H^+ CHANGES IN *Chromatium* CHROMATOPHORES ACTIVATED BY A LIQUID DYE LASER by B. Chance, J. A. McCray and J. Bunkenburg . . . 705

LACK OF EYE TO EYE TRANSFER OF AN EARLY RESPONSE MODIFICATION IN BIRDS by H. Zeier . . . 708

Letters to the Editor (see overleaf) . . . 710

Book Reviews by John Watling, G. E. Eden, Richard Fitter, J. L. Cloudsley-Thompson, James Paton, P. F. Dale, Edward Phillips, K. L. Q. Read, D. V. Lindley, D. F. Brewer, G. T. Wright and J. A. Sharp . . . 767

Obituaries—Dr Anna Cosslett . . . 772

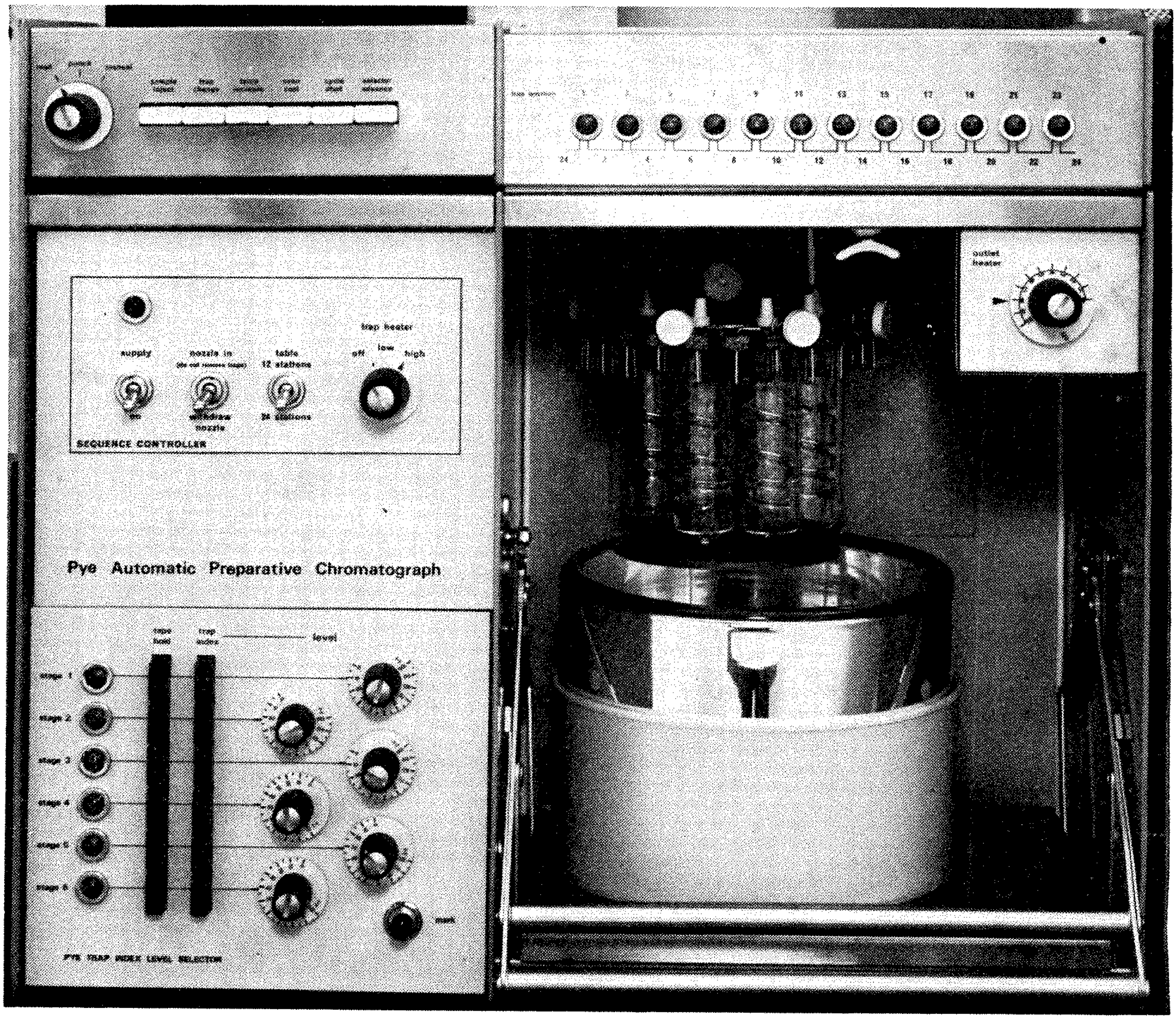
Correspondence—Science and Government by O. Wichterle; Attitudes to Conservation by Eliot Slater; The Third London Airport by W. R. P. Bourne; Goldfinger no Longer by J. R. Lewis; Sir William Grove by Sydney Gill; The Social Sciences by John Buettner-Janusch . . . 773

Nature in North America: The *Nature* office in Washington DC has opened and contributors in the United States and Canada are invited to submit manuscripts for publication either to the address given below or to the present London office. The intention is that each office will be able to answer inquiries about all manuscripts and other matters. This is one of several steps by which it is hoped to provide a better service for readers and contributors.

London Office
4 Little Essex Street,
London WC2.
01-826-6633

Washington Office
711 National Press
Building,
Washington DC 20004

(Continued overleaf)



An Index of Quality

The Pye Trap Index Level Selector is a brilliant new addition to the accessories available with the Series 105 Automatic Preparative Chromatograph. In conjunction with the instrument's punch tape control system, this highly sophisticated control unit, with an electric comparator circuit, achieves an exceptional degree of accuracy in component and fraction collection – even at the end of a separation lasting several hours. Trap rotation and sample collection at any level on the chromatogram are initiated independently of the recorder by the amplifier's signal. And up to six different actuation stages are available. Programming is straightforward – you press the command buttons either to synchronise the tape with the chromatogram and index a trap, or simply to index a trap, then dial the amplifier output level at which the operation is to be carried out.

Write or 'phone for full details of the Pye Series 105 Automatic Preparative Chromatograph and its Index of Quality.

PYE UNICAM

1966 1969
THE QUEEN'S AWARD TO INDUSTRY

Precision Instruments

Pye Unicam Ltd
York Street Cambridge, CB1 2PX, England
Telephone (0223) 58866 Telex 81215

LETTERS TO THE EDITOR

Page

PHYSICAL SCIENCES

Galaxy—Half the mass made up of black holes—TRURAN and CAMERON (Yeshiva and NASA) . 710
Astrophysics—More about gravitational collapse—LEIBOVITZ (Alberta) 711
Pulsars—Long term intensity variations—COLE, HESSE and PAGE (Cambridge) 712
Interplanetary space—Anisotropic distribution of neutral hydrogen—CHAMBERS, FEHLAU, FULLER and KUNZ (California) 713
Lunar rilles—A terrestrial analogue?—McCALL (Western Australia) 714
Apollo 11—Vesicle gas in rock samples—WELLMAN (NASA) 716
Meteorites—Microscopic spherules near terrestrial impact craters—HODGE and WRIGHT (Smithsonian Institution) 717
Retro-reflexion—Comments on increased luminance in the direction of reflex reflexion—MINNAERT (Utrecht) 718
Shock waves—Wall precursor effects in gaseous detonation—CAVENOR (London) 718
Lasers—Self-induced transparency and dispersion delays in potassium vapour—BRADLEY, GALE and SMITH (Queen's University of Belfast) . . 719
Electrons in solids—Electronic properties of well oriented graphite—COOPER, WOORE and YOUNG (London) 721
Surface chemistry—Interaction of atomic hydrogen with graphite crystal surfaces—McCARROLL and McKEE (General Electric) 722
Heavy ice—Vapour pressure of $H_2{}^{18}O$ ice and $H_2{}^{18}O$ water—JANCSO, PUPEZIN and VAN HOOK (Tennessee) 723
Viscoelasticity—Investigated by pulsable flow instrument for dilute polymer solutions—GADD (National Physical Laboratory) 724
Turbulence—Visualization of spiral motion in curved open channels of large width—FRANCIS and ASFARI (London) 725
Reaction mechanisms—Decomposition of isopropyl alcohol photosensitized by zinc oxide—MÜLLER and STEINBACH (Munich) 728
Band structures — Linear combination of molecular orbitals and poly-AT — FLURRY and BREEN (Louisiana State) 729

BIOLOGICAL SCIENCES

Immune response—Are phagocytes essential for induction of immunity and tolerance?—DIENER, SHORTMAN and RUSSELL (Melbourne) . . 731
Immunity—Properties of protective malarial antibody—COHEN and BUTCHER (London) . . 732
Hybrid cells—Evidence against "strong" histocompatibility antigens in Ehrlich cells—CHEN and WATKINS (Oxford) 734
Cytogenetics—Chromosome translocations causally related to spontaneous abortions—KADOTANI, OHAMA (Hiroshima), SOFUNI and HAMILTON (Atomic Bomb Casualty Commission) 735
Leukaemia—Specific metacentric chromosome in leukaemic cells of AKR/T1ALD mice—LEGRAND and DUPLAN (Laboratoire Pasteur, Paris) . . 737
Animal diseases—Signs of infectious bovine rhinotracheitis virus in Tanzania—RWEYEMAMU (Central Veterinary Laboratory, Dar es Salaam) . . 738
Pituitary gland—Removal limits conversion of noradrenaline to adrenaline in foetal lambs—COMLINE, SILVER and SILVER (Cambridge) 739

Page

Interactions of hormones—Two peptides can co-operate to inhibit a steroid hormone—HIPKIN (Liverpool) 740
Genetics of albumins—Need for standardized classification system—TÁRNOKY, DOWDING and LAKIN (Royal Berkshire Hospital and Reading) . . . 742
PMR spectrometry—Application to complex biological systems—KAUFMAN, STEIM and GIBBS (Brown) 743
Vitamin C—Ascorbic acid needed for formation of epithelial basement membrane—PRIEST (Colorado) . 744
(Na^++K^+)-ATPase—Allosteric interactions between Na^+ binding sites—TOBIN, BANERJEE and SEN (Toronto) 745
Exchange diffusion—Ambiguous usage—BRITTON (London) 746
Action potential—New method for measuring Na^+ and K^+—ROJAS, BEZANILLA (Chile) and TAYLOR (US National Institutes of Health) 747
Cytochemistry—Distinction of motor neurones into two classes—FERNANDEZ-CAMPA and ENGEL (US National Institutes of Health) 748
Biochemical psychiatry—Lithium and amino-acids of brain—DeFEUDIS and DELGADO (Yale) . . 749
Innate releasing mechanism—Two models compared for pied flycatchers—CURIO, BLAICH (Bochum) and RIEDER (Karlsruhe) 750
Learning without a brain—Chronic spinal rats learn leg actions—BUERGER and FENNESSY (Harvard) 751
Visual feedback control—Intermittency demonstrated in a repetitive motor task—BEGGS and HOWARTH (Nottingham) 752
Insect physiology—Water uptake from subsaturated atmospheres via anus of *Thermobia domestica*—NOBLE-NESBITT (East Anglia) 753
Fish behaviour—Alarm pheromone and avoidance conditioning in goldfish—KIMBRELL, WEINROTT, MORRIS, SCHEID and SANGSTON (Denison) . 754
Lipids of salmon—After spawning, migration serum has low lipid content—PATTON (Pennsylvania State), CROZIER (Alabama) and BENSON (California) . 754
Plant pathology—Host response of tobacco to *Verticillium dahliae*—MAHANTY (Cawthron Institute, Nelson) 755
Grasslands—Trends in vegetation on grazing land expressed as "relative entropy"—HUGHES and DALE (The Nature Conservancy) 756
Poisons—Creeper found to be antagonistic to jellyfish poison—WASUWAT (Applied Scientific Research Corporation of Thailand) 758
Algae—Introducing the Eustigmatophyceae—HIBBERD and LEEDALE (Leeds) 758
Cell walls—Where is the protein in the alga *Valonia*?—STEWARD, MOTT, ISRAEL and LUDFORD (Cornell) 760
Antibiotic activity—No increase of sensitivity after EDTA treatment of resistant bacteria—NEU and WINSHELL (Columbia) 763
Bacterial genetics—Complete mutagenesis induced in solid medium devoid of thymine—BRESLER, MOSEVITSKY and VYACHESLAVOV (Leningrad) . 764
Population growth—Describable by multi-type Galton-Watson process—SENETA (Cambridge) . 766

The titles on this page are prepared by the staff of *Nature* on the basis of the titles submitted by authors which appear in full on the pages indicated

University Life Assurance Society

Established 1825 *Incorporated by Royal Charter*

For nearly 150 years the Society has offered life assurance on attractive terms exclusively to past and present members of Universities and similar educational establishments within the United Kingdom.

The Society does not pay commission for the introduction of new business.

Special plans for Students or Young Graduates

The "Minimax" Policy **Reduced Premium Policy**
Young Graduate's Policy

Other plans include

Whole-life and Endowment assurance
Educational Policies **Children's Policies**
Pension Policies . Annuities . Family Protection
Unit-linked Assurances and Annuities

4 Coleman Street, London, E.C.2
Telephone: 01-606 6225

Annales de l'Institut National de la Recherche Agronomique

ANNALES AGRONOMIQUES (General agronomy, soil science), 6 issues annually.

ANNALES DE L'AMELIORATION DES PLANTES (Plant breeding), 4 issues annually.

ANNALES DES SCIENCES FORESTIERES (Forestry), 4 issues annually.

ANNALES DE PHYTOPATHOLOGIE (Mycology, bacteriology, virology), 4 issues annually.

ANNALES DE ZOOLOGIE—ECOLOGIE ANIMALE (Zoology, ecology), 4 issues annually.

ANNALES DE TECHNOLOGIE AGRICOLE (Agricultural and food technology), 4 issues annually.

ANNALES DE ZOOTECHNIE (Animal breeding and animal production), 4 issues annually.

ANNALES DE BIOLOGIE ANIMALE, BIOCHIMIE, BIOPHYSIQUE (Animal biology, biochemistry, biophysics), 4 issues annually.

ANNALES DE GENETIQUE ET DE SELECTION ANIMALE (Animal genetics), 4 issues annually.

APIDOLOGIE (Bee and Beekeeping) co-edited by I.N.R.A. and Deutscher Imkerbund, 4 issues annually.

Subscriptions should be sent to:

SERVICE DES PUBLICATIONS I.N.R.A.,
route de St-Cyr, 78-VERSAILLES, France.

Specimen copy on request

(Orders can also be sent through any bookseller)

BS **Precision Instruments**

Optical Elements— Manufacturing facilities for lenses, prisms and special optical elements in glass, silica, cultured and natural quartz. Polarising prisms up to 12 mm aperture usually ex stock.

Graticules— Circular and linear scales, masks, index lines, etc., on glass substrate, in photographic emulsion, etched or chrome deposited. Also clear apertures to precision form etched in metal foil for scanning discs, slits, etc.

Aluminising— Mirrors, fully or partially reflecting in aluminium and other metals by vacuum deposition.

Optical Calcite— A small quantity of high grade crystals in excess of our requirements is available for resale.

SOLE DISTRIBUTORS IN U.K. OF SCHOTT INTERFERENCE FILTERS

BELLINGHAM & STANLEY LIMITED BS
61, MARKFIELD RD. LONDON N.15. TEL. 01-808 2675

NATURE Volume 225 FEBRUARY 21, 1970

Loose Edges in the Grand Design

THE first thing to be said about the discussion so far on the proposed reorganization of the British Government's support for scientific research and development (see *Nature*, **225**, 219; 1970) is that too little of the discussion has been in public. No doubt the hierarchies of official committees are giving the matter their deliberate consideration, but that is unlikely to cut thick ice. A part of the reason is that the committees have usually had their say well in advance, and these opinions have been duly discounted by everybody concerned. But there are also a number of issues raised by Mr Wedgwood Benn's green paper where informed but detached opinion has a great deal to contribute. It will be a great pity if important matters like these are decided arbitrarily, in default of proper discussion.

The second thing to say is that there would be a good deal of merit in beginning public discussion with the question of what is in future to happen to the two parts of machinery of public science which are conspicuously absent from the green paper—the defence research establishments and the research associations. Since one of the more obvious defects of the British Research and Development Corporation which the Ministry of Technology proposes is that it will resemble all too closely what may be called, not with necessarily unkind intent, the rump of the Atomic Energy Authority, it must have taken some fortitude to omit these organizations from the proposed authority. Why, and is it too late to go back on this decision ?

The future of the Research Association is, unfortunately, something of a condominium. The associations have their origins in the pre-history, when research was in any case something of a mystery. Then, in the thirties, it may have seemed to many smallish companies that there would at least be profit in an attempt cooperatively to find out what research is all about. The tendency to cooperate was, however, reinforced by the somewhat shocked discovery, in the fifties, that a great many traditional industries were almost entirely innocent of scientific research and were even unprovided with trained technical people. (Jute manufacturing was one of the most scarifying illustrations of this, and practically, and almost predictably, it has not survived to tell the tale.) In the event, however, only a few of the research associations have become centres of original research. For most of them, the most urgent task is the dissemination of known information more widely and more efficiently. All of them are to some extent perplexed by the diversity of their membership—frequently a single member company may mount a larger effort, even in research on its own account, than the Research Association can support. Now that the annual government subvention has been pegged at £4 million, it is hard for any of them to see a brilliant future opening up ahead. Indeed, the chances are that if the research associations were transferred to the proposed research and development authority, their prosperity would be quickly diminished by the competition from other outlets for public money in research.

Against this background, the future of the research associations must necessarily rest with them. The first need is to define a function which is likely to persist. The most hopeful prospect is the provision of technical information to industries within which, for structural reasons, information does not flow easily. In due course, consultancy could follow. With such a goal, however, there is probably no reason why the research associations should remain as independent of each other as they are bound by their constitutions to be at present. All this suggests that there is a need for a unified organization for the research associations—a framework within which advice, ideas and people could be more readily interchanged than is at present possible. Paradoxically, the present system whereby government grants are channelled to individual associations is an impediment to this. Moreover, there is probably no reason why an organization formed by an amalgamation of the research associations, or at least by their integration into a single framework, could not play a useful part, providing technical advice for small businesses, within the kind of organization which the Government has proposed. To be sure, it is also possible that such an organization could support itself by levying fees on those who used its services. There is no certain choice between these two courses of development, but what is certain is that the research associations can only stay where they are, in limbo, if they are reconciled to extinction in the comparatively near future.

The defence research establishments are a harder nut to crack. The Royal Aircraft Establishment and the Royal Radar Establishment are the two most obvious omissions from the proposed arrangements, but several other smaller laboratories should belong as well. For one thing, it is fair seriously to doubt the viability of the new organization for research and development if these laboratories are not included. What will happen, for example, if under the new arrangements, the board of the British Research and Development Authority considers that it would be prudent to take on work in aerodynamics or telecommunications, thus threatening the defence laboratories with trespass ? Will somebody say no ? And if not, is there not a risk of serious muddle ? But, worse still, without the skill and special knowledge of these defence laboratories, is there not a danger that the British

Research and Development Corporation will be less well equipped than it should be to seize opportunities for which it is supposedly being created? Finally, it is also almost beyond dispute that the defence laboratories will suffer in the next few years the tribulations of finding that they have somehow outlived the purposes for which they were originally established. What will happen then? Will there have to be a second authority for research and development?

These questions call for urgent attention. If they are not answered sensibly, the new authority cannot itself make sense. But it is also only right to acknowledge that the whole fabric of the new proposals rests on the assumption that it will be possible, within the framework of the British Research and Development Corporation, to increase enormously the amount of useful work which government laboratories can carry out for industry, and that it will be possible within the public service to find means by which a public corporation can take financial risks with public money. Those are the questions on which the soundness of the new proposals must ultimately depend.

Can the Health Service Survive?

On the face of things, at least, Mr Richard Crossman seems to have made a set of sensible suggestions about the organization of the British Health Service in his discussion paper *The Future of the National Health Service* (HMSO, 5*s*), published last week. To the extent that the proposed arrangements will bring more closely together general practice, the hospital services and the responsibility for public health which has so far been vested in the great variety of local authorities throughout the country, the proposals are obviously to be warmly welcomed. One of the most serious defects of the working of the National Health Service in the past twenty years is that there has been no easy machinery by means of which medical people can decide among themselves whether, for example, an individual patient is best treated in hospital or at home. The new arrangements offer an opportunity, to say no more, for enabling general practitioners to follow their patients into hospital and, in due course, to retain some professional interest in some special branch of medicine. And who will doubt that the larger hospitals could make an important contribution to the working of the public health services if they were somehow more closely involved?

How well will the new arrangements work? What encouragement will there be for the tendencies which the government is commendably seeking to encourage? Everything will depend on the effectiveness of the proposed area health authorities, one for each of the units defined by the Royal Commission on Local Government in its report a year ago. On this basis, each of the new authorities will be responsible for the health services for between 200,000 and 1,500,000 people, and the intention is that they should consist of between 20 and 30 voluntary members superintended by a chairman who will eventually be paid a part-time salary. To the extent that this proposal will get rid of the anomalies in the present system, it is to be welcomed. Most probably, it is also wise that the new proposals do not follow the Royal Commission on Local Government in handing over the administration of the health services entirely to the local authorities. But is there not a danger that the new arrangements will fall awkwardly between the disadvantages of extreme centralization and excessive devolution?

Has enough thought been given to the possibility that the control of at least some parts of the health service should be entrusted not to voluntary committees of the kinds whose compromises have been traditionally an essential part of British public life, but to more clearly identified full timers who would be more obviously responsible to the community they serve for the efficient conduct of their operations? The dash of syndicalism in Mr Crossman's proposals—one third of the members of the new authorities will be nominated by the health professions (and one third by the local authorities)—is not a sufficient guarantee that the new arrangements will function well—to begin with, indeed, it may be sand in the works.

The potential weakness of the health authorities (with the intellectual dependence on the centre at Whitehall which that implies) is also a reason for anxiety about the proposals for closer links between the health service proper and the public health services at present administered by the local authorities. It is sensible enough that responsibility for matters such as vaccination, home nursing and family planning should be closely linked with the hospital services and with general practice, and there are evidently several valuable opportunities to be seized within the new framework, but it is also clear that the modest successes of the local authorities in the past few decades have depended on such facilities as they possess for carrying important messages to members of the public. What assurance is there that the new health authorities will be equipped in ways like these? Certainly there is nothing in the records of the regional hospital boards which administer the present system to suggest that these tasks will come naturally to the new people.

Public discussion about the organization of the National Health Service, valuable though it may be, is unfortunately only a minor contribution to the problems of public health in Britain which cry out urgently for solution. Mr Crossman's green paper says, quite properly, that the coming of the National Health Service has contributed enormously to the good health of the British population. That this should be is understandable enough. In the fifties, the health service was comparatively well provided with funds even if many of its facilities—hospitals, for example—left a great deal to be desired. Although the system has steadily improved since then, with commensurate increases of cost, the British system is now much less of an international boast than it used to be. The quality of medicine is good, even though it can sometimes cause wry smiles when compared with practice elsewhere. The quality of the service provided is variable, to say the best of it. But hanging over everything is the fear that it may only be a matter of months before there is a rapid deterioration of the efficacy of the system. Plainly the health services have exhausted their goodwill with the nurses. But would the framework which Mr Crossman now proposes be able to meet the strain of paying nurses and other medical ancillaries at rates which are competitive? With this anxiety, is it sensible of him to complain about the continued success of the private medical insurance schemes which are flourishing alongside the health service? Might it not be more prudent to devise some method of mixed financing for the new health service? That is at least a question which deserves serious consideration, quite independently of party politics and separate from the commitment to a totally free health service on which Mr Aneurin Bevan nailed the present government's colours to the mast just twenty years ago.

100 Years Ago

In fact, the Miocene Mammalian Fauna of Europe and the Himalayan regions contains associated together the types which are now separately located in the South African and Indian sub-provinces of Arctogæa. Now there is every reason to believe, on other grounds, that both Hindostan, south of the Ganges, and Africa, south of the Sahara, were separated by a wide sea from Europe and North Asia, during the middle and upper Eocene epochs. Hence it becomes highly probable that the well-known similarities and no less remarkable differences between the present Faunæ of India and South Africa have arisen in some such fashion as the following. Sometime during the Miocene epoch, possibly when the Himalayan chain was elevated, the bottom of the nummulitic sea was upheaved and converted into dry land, in the direction of a line extending from Abyssinia to the mouth of the Ganges. By this means, the Dekhan on the one hand, and South Africa on the other, became connected with the Miocene dry land and with one another. The Miocene Mammals spread gradually over this intermediate dry land, and if the condition of its eastern and western ends offered as wide contrasts as the valleys of the Ganges and Arabia do now, many forms which made their way into Africa must have been different from those which reached the Dekhan, while others might pass into both these sub-provinces.

That there was a continuity of dry land between Europe and North America during the Miocene epoch, appears to me to be a necessary consequence.

From T. H. Huxley's valedictory address to the Geological Society, of which he had been president for the past two years. Nature, **1**, 443, *February* 24, 1870.

OLD WORLD

DRUGS

More L-Dopa in Sight

INITIAL enthusiasm for the drug L-dopa in the treatment of Parkinson's disease seems to have been justified by the results of trials carried out so far. But the problem of supply is still formidable, although the Committee on the Safety of Drugs, in the thick of dealings with chemical and pharmaceutical companies, is doing its best to approve the use of new and reliable preparations.

Impressive evidence of the beneficial effects of the drug was seen at the first open meeting of the Parkinson's Disease Society in London on February 11. A film of two women sufferers whose unsteady gait and handwriting returned almost to normal after treatment was shown by Dr G. Stern, consultant neurologist at University College Hospital, London. This is one of five British centres where clinical trials of the drug are in progress under the auspices of the Medical Research Council. L-Dopa is, of course, the drug which was seen as a potential treatment for Parkinsonism when drugs which cause a decrease in amounts of monoamines, particularly dopamine, in the brain were found to cause the disease (*Nature*, **223**, 889; 1969).

Stern says that these patients are not his most successful cases; in some, for example, manual dexterity has apparently returned to normal from a very poor state. On the whole he finds that two-thirds of Parkinson patients treated with L-dopa during the trials have responded to varying degrees, while for the other third treatment has had no effect. These findings are similar to those reported in the *British Medical Journal* by Dr C. Mawdsley of the University of Edinburgh (1, 333; 1970). Of twenty patients treated for an average of six months he reported marked improvement in nine; this involved renewed ability to rise from a chair and walk comfortably without the need for aid. Fourteen patients experienced moderate improvement, in five it was only slight and a further five experienced no improvement.

Many Parkinson patients treated with L-dopa develop side effects—nausea and loss of appetite seem to be the commonest. Induced voluntary movements of the face and feet are also a frequent effect, but not usually distressing, disappearing as the dose is reduced. Psychiatric effects such as depression may pass off after a few days of treatment. Two of Mawdsley's patients developed hallucinations and paranoid delusions; one refused to take any more of the drug, but the other lost the symptoms when the dose was reduced.

There are clearly various possible side effects of L-dopa, and although many of them seem to be mild enough to accept for the sake of the benefits, neurologists involved in the trials must have great respect for the unknown element. As Stern told his audience on February 11, it is impossible to see yet how long the favourable effects of treatment will last. He was careful to stress that although L-dopa is the most effective form of treatment so far found for some patients, it is not a cure for Parkinsonism. When the drug becomes available for regular treatment patients who benefit from it will have to take it for the rest of their lives. Sufferers and their relatives who have heard so much about the new wonder drug may have been disappointed to hear Stern say that the most fascinating, and probably important, aspect of the work so far—much of it carried out in the United States—is that it has given an insight into the neurological basis of Parkinsonism.

Supplies of the drug are still a problem, and all patients taking part in the MRC trials know that they have to stop taking L-dopa afterwards, even if it has greatly benefited them. This is not as drastic as the Department of Health and Social Security implied when it warned physicians of the danger of suddenly taking a patient off L-dopa. When treatment stops, patients return to the condition they were in before taking the drug; they do not deteriorate further. It is unfortunate that there is insufficient L-dopa for everyone who could benefit from it, but those who have been blaming the Committee on the Safety of Drugs for dilatoriness in passing preparations as safe for use are being unfair: the committee is giving the matter priority treatment.

It is well known that the material used in the MRC trials is supplied by Roche of Switzerland, and until recently this was the only L-dopa approved by the committee. The other principal source of supply when the trials were set up was imported material from the Japanese company Sankyo, and criticism came from importers whose supplies were spurned by the ministry on the advice of the committee. But the committee points out that the companies concerned, primarily dealers in chemical reagents and not pharmaceuticals, were offering a preparation of doubtful quality.

Companies were asked to make a submission in the usual way, with details of manufacture, impurities and so on, together with details of other experimental studies carried out. This Roche had already done, and its preparation had been passed for clinical trials—the last stage before marketing. But in spite of a meeting at the end of last year between Professor Scowen and Dr Mansel-Jones of the committee and representatives of five firms no submissions were received, although the companies agreed to stop supplying L-dopa direct to patients, and only through neurologists. In these circumstances the committee was unable to do other than advise against the use of the preparations.

One company, Chemica Laboratories Ltd, announced publicly that it would sell in spite of the committee, complaining that it was not prepared to divulge manufacturing secrets. This was hardly a fair complaint when all such information is given in strict confidence. Chemica later retracted, however, announcing that it would cease to deal in L-dopa.

There is hope that this sad situation will be eased, for satisfactory submissions from two other companies, Brocades and Ward Blenkinsop, were dealt with at lightning speed two weeks ago. Their preparations have now been passed as safe for use by physicians, who will monitor the effects of treatment and send back detailed reports to the two companies. Ward Blenkinsop is producing L-dopa itself, while Brocades is initially importing the Japanese preparation and re-purifying it as well as making some of its own. Three more companies are actively engaged in research on L-dopa and are expected to make submissions to the committee soon.

HEALTH

Consultation to Curb Panic

ONE of the chief lessons of the cyclamate affair was the folly of one country taking precipitate action on a presumed health hazard without forewarning health authorities elsewhere. The obvious measure to prevent the recurrence of such fiascos (see *Nature*, **225**, 3; 1970) now seems to have been taken. Last week the Canadian minister of National Health and Welfare, Mr John Munro, was host in Ottawa to officials from the United States Department of Health, Education and Welfare and the British Department of Health and Social Security. The purpose of the meeting was to discuss future exchanges of information in areas related to public health. This presumably will include the results of toxicological tests on drugs, pesticides and food additives which, if circulated to other health authorities before the country of origin takes any public action, could avoid the embarrassing international consequences that have been the aftermath of the unilateral bans on cyclamates and other substances.

The next conference between the three countries will be held in London during April or May. Canada, the United States and Britain are three countries who have perhaps the most to gain from coordinating their action on drugs and additives, but if the consultation works well other European countries such as Sweden may well wish to join the club.

SOCIAL MEDICINE

Abortion Amendment

A BILL to amend the 1967 Abortion Act by limiting the number of doctors allowed to perform abortions was "talked out" during a debate in the House of Commons last Friday. This amounts to a defeat, although in principle the bill is not dead but goes to the bottom of the list of private members' bills. The amendment, proposed by Mr Godman Irvine and backed by the British Medical Association and the Royal College of Gynaecologists and Obstetricians, provided that abortions should be carried out "by or under the supervision of a consultant gynaecologist in the National Health Service (NHS) or a medical practitioner of equivalent status approved by the Secretary of State for Social Services" if he and another doctor agree that an abortion is necessary.

Mr Irvine claimed that the bill would make abortions safer and he believed that there would be no shortage of consultants to perform the operations. If half the 500 or so NHS consultants were prepared to carry out abortions, he calculated, the present demand would mean four abortions per week per gynaecologist, which was well within their capability.

The chief argument brought against the amendment was that it would reduce the effectiveness of the Abortion Act which has so far been working extremely well in reducing the number of criminal abortions (see *Nature*, **225**, 580; 1970). By cutting down the number of abortions carried out by doctors without specialist qualifications, the bill would make it more difficult for a woman to obtain an abortion legally, but would not really guarantee any more safeguards against abuse than the act already provides.

Speaking for the government, Dr Dunwoody said that the bill would result in a decrease in the number of private abortions and in consequence an increased demand on the already overburdened health service. An even more worrying effect might be an increase in the number of back street abortions. Because the operation becomes so much more dangerous after the first twelve weeks of pregnancy, a woman faced with delays in obtaining a legal termination might choose criminal abortion.

Under the present act the Secretary of State has extensive powers for inspecting clinics and insisting on an adequate standard of post-operative care. Dr Dunwoody explained that all but one of the private clinics have now been inspected at least once and steps taken to ensure that each patient stays in at least one night after the operation, that suitable autoclaves are installed and that there are facilities for dealing with emergencies. He made it clear that in cases of alleged professional misconduct, the Secretary of State has no power, or wish, to intervene, but that any evidence should be presented to the General Medical Council.

By requiring that the Secretary of State should approve a list of doctors of "equivalent status", Dr Dunwoody said, the bill would impose on him the unrealistic and invidious responsibility of drawing distinctions between individual doctors. Mr Houghton made the point that there is no reason to suppose that NHS gynaecologists, some of whom have done few, if any, terminations, would be any more skilled at carrying out abortions than professional abortionists. Private clinics have in fact a lower proportional death rate from abortion than the NHS.

Mrs Renée Short pointed out that recent public opinion polls show that most people are satisfied with the act or want to see it improved to make it easier for women to get abortions. A similar public opinion poll carried out among doctors shows that 66 per cent of general practitioners in the United Kingdom thought the act should be left as it is or changed in favour of easier abortions.

WILD LIFE

Italy Losing Friends

The Italian Government's decision to permit once again the unlimited netting of small birds (*Nature*, **225**, 494; 1970), three years after severely restricting the practice, has provoked strong reactions from elsewhere. Protests have been sent by the International Council for Bird Preservation (ICBP), the International Union for the Conservation of Nature and Natural Resources, and the European Committee for Bird Ringing; in Britain the Royal Society for the Protection of Birds, the Council for Nature and the British Trust for Ornithology are all completely opposed to the new legislation. The British secretary of ICBP, Miss Phyllis Barclay-Smith, has said that the law is "absolutely appalling"; and the World Wildlife Fund is "deeply concerned".

As a result of the new measure vast numbers of migrant birds will be trapped in mid-course between Africa and northern Europe—estimates of the annual kill are of the order of 20 million. What this means for the total population of the migrating species is hard to say, according to Mr Richard Porter of the Royal Society for the Protection of Birds, because the size of the annual movement is not known with sufficient accuracy. Mr Porter thinks that the warblers may suffer particularly because they congregate around bushes and small trees rather than in open country, and can be easily trapped by nets strung from tree to tree.

The official justification for the reinstatement of netting is that it made it easier to capture birds for scientific research, by which, apparently, was meant no more than ringing them. The European Committee for Bird Ringing nevertheless objects to the new law, and its secretary-general, Mr Robert Spencer, claims that the "scientific" label merely gave it "a veneer of respectability". Critics are united in thinking that the Italian Government is simply trying to make itself more popular with the large number of people who like to trap birds for food or sport. Much of the trapping is on a small scale, using single nets or sometimes more sophisticated methods triggered by decoys.

Italy is not the only European country that allows trapping, but elsewhere scope is more limited. Some birds are netted in Greece, Spain and Portugal. In south-west France some species, notably linnets, can be legally trapped. In Belgium starlings and thrushes can be trapped as a sport for six weeks of each autumn. Britain allows trapping only by ornithologists, who must obtain a licence from the Nature Conservancy.

Hostile public opinion and the possibility of damage to the Italian tourist industry are, it is suggested, the chief means by which the Italian Government could be persuaded to think again. Miss Barclay-Smith of the ICBP is urging people to write to embassies and tourist boards, and is herself compiling a dossier of angry views to send to the Italian Ministry of Tourism. But the basic problem remains the enthusiasm for netting that many Italians evidently have; and, as Mr Spencer gloomily noted, "it takes at least a generation to change people's minds".

CELL BIOLOGY

New European Organization

A new European Cell Biology Organization (ECBO) has been established at Leiden in the Netherlands. Its aims are to promote research in cell biology and to foster international cooperation through exchange of information and people. It intends to promote three types of activity—small working groups studying specific topics, two-day symposia, and exchange of individuals among institutions in Europe. But, unlike the European Molecular Biology Organization (EMBO), ECBO has no intention, as yet, of setting up its own laboratory.

There are other major differences besides that of size between the new organization and EMBO. ECBO will levy no subscription charges on its members, but its executive committee is not sure at the moment where the money to finance its activities is going to come from. It hopes that the symposia may be self-financing, and that some money will be forthcoming from other organizations and perhaps from scientific foundations, but it wants to avoid having to take money from government sources because this might limit participation from Eastern European scientists. Dr L. M. Franks, a member of ECBO's executive committee, said that the organization's field of interest, which will include all aspects of cell biology, will be wider than that of EMBO.

Membership of ECBO will be made up of registered cell biologists, together with representatives of other

European organizations, such as the International Society of Cell Biology, the International Cell Research Organization, EMBO and national societies. The president of the new organization is Professor P. J. Gaillard of the Institute for Cell Biology and Histology, Leiden, and the vice-president is M. Chèvremont of the Institute for Histology at Liège. Other members of the executive committee are W. Bernhard, Institute for Scientific Cancer Research, Villejuif; L. M. Franks, Imperial Cancer Research Fund, London; J. Paul, Beatson Institute for Cancer Research, Glasgow; M. Feldman, Weitzmann Institute of Sciences, Rehovot; and A. Lima-de-Faria, Institute of Molecular Genetics, Lund.

AVALANCHES

Swiss Example to France

Last week's avalanche disaster at Val d'Isère in the French Alps has provoked some searching questions on the adequacy of present monitoring and prevention techniques and on the amount of research that is being conducted on avalanches. Poor coordination of data is reflected in the almost total lack of statistics on avalanches in France, and the nine man enquiry set up by the French government to investigate the disaster may well feel compelled to suggest the setting up of a central institute for avalanches on similar lines to the one at Davos in Switzerland.

The Swiss research institute has an impressive record. More than a hundred major avalanches that have fallen in the last twenty years have caused only 24 deaths, and although no attempt is made to forecast individual avalanches a general pattern of snow conditions is formulated from statistics collected from fifty points throughout the country. Twenty or so physical quantities are monitored, including snow and air temperatures and snow hardness. All data are sent to the centre in Davos, which employs fifty people working full time to analyse and disseminate the information.

Although there are links between the warning services of the countries sharing the alpine land mass, it seems clear that research in France is much more fragmented than in Switzerland.

The CNRS (the government organ for scientific research) has a laboratory at Grenoble university for the study of snow, ice and avalanches, but funds seem to be too short for anything resembling a major programme. There is also a laboratory in the Haute Savoie which specializes in the melting of snow, and a group at the scientific university at Chambéry is carrying out a survey of all avalanches known. The possibility of diffusing avalanches with explosives is being investigated at the nuclear centre in Grenoble, but the Alps are probably too densely populated for such violent techniques.

ATOMIC PHYSICS

Anti-helium at Serpukhov

from our Soviet Correspondent

The Soviet Institute of High Energy Physics reports that nuclei of "anti-helium" have been successfully produced by a team of young physicists at Serpukhov, under the direction of Professor Yu. D. Prokoshkin.

The experiment was carried out using a beam of protons accelerated to 70 GeV, aimed at a target within the vacuum chamber of the accelerator. The negatively charged particles generated by the collision of the protons with the target were deflected by a magnetic field and formed into a beam of defined momentum. A special complex detection apparatus was developed by the Institute of High Energy Physics for the identification of the secondary particles in this beam (several million per second); this apparatus comprises more than 50 high-speed detectors and a nanosecond electronics system containing some 500 elements.

The anti-helium nuclei were identified in several different ways. The charge on each particle passing through the apparatus was determined by two methods: by the ionization produced by the particle and by the Čerenkov radiation. The velocity of the particle was determined in three ways: by means of differential and threshold Čerenkov counters and by direct measurement of the time of flight (this latter method had an accuracy of the order of ten nanoseconds).

The simultaneous application of these methods provided reliable means of recording any nuclei of anti-helium which might be formed. During the experiment, more than 200 thousand million particles passed through the apparatus—of these, five were identified as nuclei of anti-helium.

This discovery of the nucleus of an "anti-element" is, in the opinion of A. Loginov, director of the Institute of High Energy Physics, of great significance as a confirmation of the theoretical concept of anti-matter, and hence to the whole understanding of the nature of the space–time interaction of particles. It is understood from his report that further experiments in this and related fields will be carried out using the Serpukhov accelerator.

ROCKET

Fourth Member of the Space Club

The announcement on February 11 that Japan had succeeded in launching a small satellite by means of a Japanese rocket at the fifth attempt, and so become the fourth member of the "space club", prompts consideration of the space programmes and ambitions of a country whose situation in many respects is so comparable with Britain's. The Lambda 4S solid fuel rocket launcher is claimed to have cost only the equivalent of £138,000—a record in cheap space launchers.

Shortly before the successful satellite launching, the national Space Development Committee announced a programme for eight satellites to be nationally launched in the next six years. Six of these are for scientific purposes; one is described as ionospheric and there will be one communications satellite (largely for proving systems and feasibility, presumably). Further satellites for meteorological and navigational purposes are under consideration but have not yet been definitely adopted. There has been a 17 months delay in developing the Japanese satellite programme because of the shortage of American expertise as well as opposition from fishermen living near Japan's Pacific-facing rocket range who feared that the disturbance would spoil their livelihood.

This year for the first time the Japanese Antarctic expedition will launch rockets from their mainland base in support of their polar research programme. Two geophysical rockets are due for launch this month; next season 12 rockets are intended to be launched into aurorae. The S160 sounding rocket being used has been developed by the Institute of Space and Aeronautical Science of Tokyo University.

The Japanese Meteorological Agency has also taken up rocketry for its advanced high altitude investigations. A new rocket range near the coastal town of Sanriku has been built at a cost of £137,500. The first campaign comprising the launch of 40 4-metre long MT 135 meteorological rockets is due in July.

It is generally held that Britain could have launched her first small satellite by means of the small national space launcher *Black Arrow* at least 3 years ago. Lack of purpose—on which the Japanese cannot be faulted—has prevented this. The disadvantages of not having an alternative to US launchers when it comes to bargaining, as with the hardline Intelsat talks now resumed in Washington, is beginning to be apparent—as has frequently been predicted by the British aerospace industry; Britain has fallen between two stools—the muddles of European cooperation and the cost of going it alone.

COMPUTERS

Mintech's Right of Veto

LAST week's confrontation between subcommittee D of the Select Committee on Science and Technology and representatives of the UK Atomic Energy Authority produced a display of verbal acrobatics from the authority which Mr Ted Leadbitter said could be rivalled only by the Foreign Office. Mr Leadbitter and his colleagues on the subcommittee under the chairmanship of Mr Airey Neave are looking into the development of the British computer industry over the next ten years, and the UKAEA was giving evidence as a major user of computers. What inspired Mr Leadbitter's caustic comparison was the efforts by the witnesses to parry the question of who has the final say when the authority wants to buy a foreign computer—the UKAEA or the Ministry of Technology. This riddle took up much of the session and the answer never came, at least not during the part of the session open to the public. The best that could be done by Mr E. E. Newley, director of the Atomic Weapons Research Establishment, was to say that the view of the UKAEA so far has prevailed when they have wanted to buy machines from the United States.

But the subcommittee was successful in distilling from this knockabout stuff some odd definitions of what Mintech considers to be a British machine. The rules are bent so that the Honeywell computer, which is largely built in Britain, counts as British, but the comparable IBM computer is deemed to be foreign.

Much of the discussion centred on the software which has been developed by the UKAEA, and the subcommittee clearly felt that there was room for inprovement in making this material available to industry. Mr A. Gregory of the subcommittee mentioned complaints from industry about the poor flow of information from government departments such as the UKAEA, but Mr Newley and his colleagues thought that the subcommittee was tending to overstate the commercial returns. The UKAEA was looking at ways of disseminating the information, and marketing consultants had been called in to see how the multi-access system developed in conjunction with IBM might be made available.

The discussion moved to the future needs of the authority, and the highly secret project 52 which ICL is working on received a mention. But whether project 52 will cover the requirements of the UKAEA for the next ten years—the timescale covered by the subcommittee—depends on how the system is likely to expand. At present it is not clear what the upper range of project 52 is going to be, but there is little doubt that the UKAEA will need access to larger machines than the IBM 360/75 at Harwell.

Mr E. Lubbock was surprised that the UKAEA has no large machines on order considering that the doubling time for the growth of computing in the authority is three years. Mr Newley agreed that this was a question that the UKAEA was grappling with, and one solution might be to order more ICL 470s. The UKAEA already has an ICL 470, at Risley, and two more at Culham and Winfrith will be installed by the end of the year after delays which were admitted to be proving awkward for the UKAEA. What the UKAEA requires, the witnesses said, is a good multi-access system, although the need for equipment with a quick response for data acquisition from experiments is a problem. But it seemed clear that the UKAEA has no settled policy on computers with which to start the seventies.

SCIENCE POLICY

Dainton's Thoughts

THE new broom in the Council for Scientific Policy (CSP) does not intend to do much sweeping clean. Dr F. S. Dainton, who took over the chairmanship of the CSP on January 1, this week outlined some of his thoughts on the issues confronting the council during the next few years, and he said that he can see no reason for major changes in the workings of the committee in the near future. One great problem that science policy faces at the moment, said Dr Dainton, is a growing disillusionment with science, expressed in the attitude that science creates more problems than it solves. Such an attitude has grave consequences for future manpower resources, and can be reflected in the size of science budgets. But when asked whether he thought that the membership of the council, which consists almost entirely of pure scientists, tends to make the body rather inward-looking and therefore unable to come to grips with the problem of the swing away from science, Dr Dainton argued that its membership is not unbalanced. The council, he said, is especially well equipped to comment on the economic aspects of scientific research.

The CSP will continue to study various problems through its working groups. Studies already in hand include developing criteria for supporting scientific research in universities, international cooperation in science, especially molecular biology, the quantification of economic benefits of scientific research, and pollution. In addition, the CSP has recently established a study group to look into problems created by expansion of higher education. One of the main questions the council will be asking in this connexion will be whether research facilities need to grow at the same rate as student numbers, and what effects such an expansion would have on the quality of research. On pollution, Dr Dainton said that the CSP has invited the research councils to look at the scientific side of the question, but, because it is purely an advisory body, it cannot concern itself with legislation.

Dr J. C. Kendrew, who has just taken over the vice-chairmanship of the CSP, will be especially concerned with international cooperation and exchange. He indicated that the CSP is looking at the possibility of setting up international groups in existing laboratories, and is attempting to promote scientific exchange between European countries.

MICROORGANISMS

Cultures at Cambridge

by our Botany Correspondent

TEACHERS, pharmacologists and other biologists who use the collection of algae and protozoa at the Botany School in Cambridge can expect an even better service in the future. The Natural Environment Research Council (NERC) has just spent £106,000 on new buildings to house the collection, which it has taken under its wing as a culture centre of algae and protozoa. The NERC grant will allow both the staff and the collection to be expanded and has also provided an electron microscope which will aid the process of identifying protozoa.

The collection, now composed of about 1,500 different strains, is based on a few hundred cultures originally brought to England by Professor E. G. Pringsheim, who left the German University in Prague just before the 1938 invasion of Czechoslovakia. The director of the new centre, Mr E. A. George, began as Pringsheim's assistant in 1947, and has been the curator of the culture collection since 1952. He expects the size of the collection to more than double in the next few years in response to increasing demand.

About 6,000 cultures are dispatched every year from Cambridge for various purposes. Many customers are teachers who need material for their classes. One culture, which costs 10*s* within Britain and £1 if sent abroad, provides enough material for twenty-five students. Industry provides many customers too, for algae and protozoa make a convenient system for testing the effects of untried compounds on living organisms. Before proceeding to rats or human subjects, useful information can be gained from the responses of these microorganisms to potential drugs. Two algae in particular, *Oxomonas* and *Euglena gracilis*, are much used for estimating vitamins, which have a measurable effect on their rate of growth. The food industry and hospitals make frequent use of this method. The culture collection can also save microbiologists a great deal of trouble by supplying particular strains of known uniformity and purity.

Mr George hopes that in their new home the scientific officers on his staff will be able to devote half their time to research, apart from the work they have to do on the collection, which involves isolating new strains, developing new culture media and so on. The initial lines of research will include the study of marine flagellates by electron microscopy and the development of methods for preserving living cultures by freezing or drying.

Parliament in Britain

Lung Cancer

MR GERALD FOWLER, Minister of State, Department of Education and Science, said that the Medical Research Council's Air Pollution Unit is investigating the possibility of a link between lung cancer and air pollution. But no evidence has yet been found of any connexion between lung cancer and atmospheric sulphur dioxide. (Written answers, February 9.)

Fluoridation

EIGHTY-ONE local health authorities in England are opposed to fluoridation of water supplies, and ninety-eight are in favour. Dr John Dunwoody, Joint Under-Secretary of State, Department of Health and Social Security, who gave these figures, also said that the Health Education Council expects to spend about £16,000 in 1969–70 on education about fluoridation, and a similar sum in 1970–71. Dr Dunwoody did not comment, however, on the suggestion from Mr Frank Judd that the government should introduce new legislation on fluoridation, and that it should remove the present discretion given to local authorities. (Written answers, February 9.)

Scowen Committee

DR MICHAEL WINSTANLEY asked the Secretary of State for the Social Services whether he will make changes in the personnel of the Committee on the Safety of Drugs, to restore the medical profession's confidence in the committee following its announcements about adverse reactions to oral contraceptives. But Mr Richard Crossman could see no justification for changes. (Written answers, February 9.)

Radiological Protection

THE radiological protection bill received a second reading in the House of Commons. The bill, which seeks to transfer to a single statutory board the present functions of the Radioactive Substances Advisory Committee, the Radiological Protection Service (run by the Medical Research Council) and the health and safety branch of the UKAEA, received little opposition. The proposed National Radiological Board will undertake research and provide advice and services in connexion with radiation hazards. The estimated gross expenditure on these services for 1969–70 is £822,000, of which 45 per cent will be paid by the Department of Health and Social Services, 30 per cent by the Atomic Energy Authority, 20 per cent by the Medical Research Council, and the remaining 5 per cent will come from the Scottish Office. (Debate, February 10.)

Aircraft Smoke Pollution

SUITABLE locations for measuring the smoke pollution caused by aircraft flying on the glide path into London Airport have been established, and measurement tests should start within six to eight weeks. This assurance was given by Mr Goronwy Roberts, Minister of State, Board of Trade, in reply to a question from Mr Anthony Royle. (Written answers, February 11.)

L-Dopa

THE Committee on the Safety of Drugs has cleared two more preparations of L-dopa for clinical trials. But Dr John Dunwoody, who gave this information, said that it is too early to estimate the likely cost of the drugs. (Written answers, February 9.)

NEW WORLD

The Crusade against Pollution

by our Special Correspondent

THE battle against pollution has now become something between a crusade and an evangelical campaign. The newspapers are full of it. State governors are busy explaining how their plans for new sewage treatment plants are a contribution to the "environmental war", as Senator Edmund Muskie has called it. Administrators who appear before Congress to justify their claims on the public purse now seem instinctively to begin by explaining how their work will diminish pollution. And then, on February 10, President Nixon gave his blessing to the movement with a special message to Congress suggesting new ways of cleaning air and water. In the sharp, clear air of the past few days, it has occasionally been difficult to remember that Washington is now the centre of the anti-pollution movement.

The eloquence of Mr Nixon's message is a good illustration of the spirit in which the anti-pollution movement is being advanced. In the evangelical tradition, it begins with the acknowledgment of past error. "Like those in the last century who tilled a plot of land to exhaustion and then moved on to another, we in this century have too casually and too long abused our natural environment. The time has come when we can wait no longer to repair the damage already done and to establish new criteria to guide us in the future." The peroration is a clarion call—"No longer is it enough to conserve what we have—we must also restore what we have lost . . . The task of cleaning up our environment calls for a total mobilization by all of us . . . [This task] summons our energy, our ingenuity and our conscience in a cause as fundamental as life itself."

Rhetoric apart, Mr Nixon's actual proposals seem sensible enough. The most costly part of the plan is that for helping local authorities to install sewage treatment plants. The legislation for allowing the Federal Government to share in the cost of local treatment systems exists already, but in the past few years the Administration has chosen not to spend as much as it might have done on this good work. It is now estimated that something like $10,000 million will have to be spent to bring municipal treatment plants up to standard, of which the Federal Government's share would work out at $4,000 million. The intention is that this should be spent at the rate of at least $1,000 million a year, and that there should be a formal review of the programme in 1973.

The new proposals will also help to simplify responsibility for the control of inland water pollution. As things stand, the Federal Government is responsible for standards of purity only where waterways run from one state to another. It is now intended that there should be comprehensive plans for the treatment and control of water pollution in whole river basins, and that municipal authorities which use federal money to build treatment plants should be required to operate them properly. There is also a scheme for defining unambiguously the standards of purity to which water effluents should conform, with the intention that standards should be enforced by a uniform and stiffer series of penalties.

The essence of Mr Nixon's proposals on air pollution is a continuation of the war against the automobile. The most fierce of the new proposals is a set of regulations defining the admissible pollution in the exhausts of automobiles coming on the market in 1973 and 1975. In 1973, the output of oxides of nitrogen from motor vehicle exhausts will be restricted for the first time (to less than three grams per vehicle mile), and this limit will be reduced by a factor of three for 1975 models, when the permissible emission of particulate matter will be restricted for the first time (to less than 100 mg of solid matter per vehicle mile). In future, measurements shall be made not merely with an example of each new automobile submitted by the manufacturer but with samples taken from the production lines.

The new standards are a good deal more stringent than those now in force and are even more stringent than those being applied in California, the most adventurous of all states in the struggle with the automobile. It goes without saying that much will depend on how vigorously the new standards are enforced. One difficulty is that, so long as the enforcement of standards is applied to new automobiles, there can be no assurance that devices such as the crank-case ventilators being used to reduce the output of hydrocarbons in exhausts will remain efficient. It also appears that there will be a shortage of facilities and of people for testing automobile exhausts—it turns out that the responsible officials in Washington are being provided with Ringelman charts in the forlorn hope that these will allow them to prevent the emission of excess smoke from cars.

The other side of this coin is the part of Mr Nixon's message which promises "an unconventionally powered virtually pollution-free automobile within five years" or at least "an extensive federal research and development programme in unconventional vehicles". There is talk of legislation allowing government departments to buy such vehicles for actual service so long as their initial cost is not more than twice that of automobiles with internal combustion engines, but, in the meantime, both the manufacturers and the oil companies are parading their willingness to eliminate lead-based additives from automobile fuel. One difficulty here is that of designing engines of high performance which will run on 92 octane fuel—the highest octane number at which lead additives can be dispensed with. Another is that there appears to be very little evidence to show what happens to the lead which does manage to escape from motor vehicle exhausts.

No doubt these proposals, which include provision for the tighter and more closely integrated control of air pollution, a research programme aimed at producing more easily disposable packaging materials and the like and also a promise that land now occupied by the military and other government departments will be returned to public use, will help the great clean-up on its way. The fact that some of the extra funds for sewage plants will pay for certain works already built in anticipation of some relaxation of the purse-strings is probably no tragedy—these plants would in any case have had to have been built. In many ways, the manifest difficulty of applying the new regulations on motor vehicle exhausts may help to make a gentle mockery of some parts of the programme, but this is less serious than the danger that the evangelical overtones in the battle against pollution may raise expectations which cannot be fulfilled.

Senator Muskie, who is influential in these matters, not merely because he is chairman of the Senate subcommittee on Air and Water Pollution but also because he may well be a candidate for the Presidency in 1972, has at least been doing something to help people to appreciate that pollution is not the inevitable accompaniment of technology. Speaking in Pennsylvania last week, he said that "America must stop on a dime and make a 180 degree turn, not away from technological growth but towards new uses of that strength".

Among scientists, there has been a good deal of public speaking about the ways in which technology must help in the great pollution battle. Mr John T. Middleton, Commissioner of the National Air Pollution Control Administration, told the centenary conference for the Weather Bureau last week not merely that "quite simply, we have not been winning in the battle against pollution" but that it will take a sizable force of meteorologists to devise and administer more effective controls. A few days earlier, Dr Glen Seaborg, chairman of the United States Atomic Energy Commission, which has been vigorously attacked in the past year by those who fear that nuclear plants may cause severe damage to the environment, promised a programme of research to study the effects of neighbouring nuclear power stations on bodies of water such as Chesapeake Bay.

Elsewhere, the problem of the environment is regarded with more ferocity. The Graduate School of Fine Arts at the University of Pennsylvania seems to be one of the prime movers behind what is called "Earth Week" from April 16 to April 22, which will consist of three days of technical discussions "by experts on the environmental crisis" followed by a week-end when churches and synagogues in Philadelphia will be asked "to re-examine man's role in his environment". It will be interesting to see how many of them take as their texts passages like the following, from the leading article in the current issue of *Bioscience* :

> Over the past 200 years or so, man has been engaged in a positive feedback system with his environment in which ever higher levels of population encourage economic and technological growth at an ever-increasing expense to the diversity, complexity and stability of the whole world ecosystem. As with any other positive feedback system, this one will self-destruct [*sic*] if it continues long enough. Meanwhile, there have been real benefits from this process, which are manifest in the unfolding of the rich potentialities of the human species. The present concern with dehumanisation in a technicised society and the degradation of the quality of man's environment, however, may be cybernetic signals that are important in the conversion to a negative feedback system through which a viable homeostatic stability can be achieved. The time for conversion is now, and the changeover must take place rapidly. The momentum of the population explosion alone, assuming zero growth could be achieved overnight, is such that further degradation of the world ecosystem is unavoidable even with a rapid mobilisation of economic and human resources in the conversion process.

OIL PRODUCTION

Santa Barbara Spill Examined

A DETAILED geological explanation of the escape of petroleum and natural gas in January 1969 from an oilfield off the Californian coast near Santa Barbara has now been compiled by members of the United States Geological Survey (*Geological Survey Professional Paper 679*). As well as making it possible to understand why there should have been such a sudden escape of petroleum from the sea bed, the report will probably become one of the classic cautionary tales for petroleum engineers. What seems to have happened is that by drilling through a series of at least six oil reservoirs lying beneath a comparatively thin layer of unusually porous rock, the engineers of the Union Oil Company made it possible for fluid hydrocarbon at high pressure to enter and to fragment the thin layer of sandstone separating the uppermost reservoir from the sea.

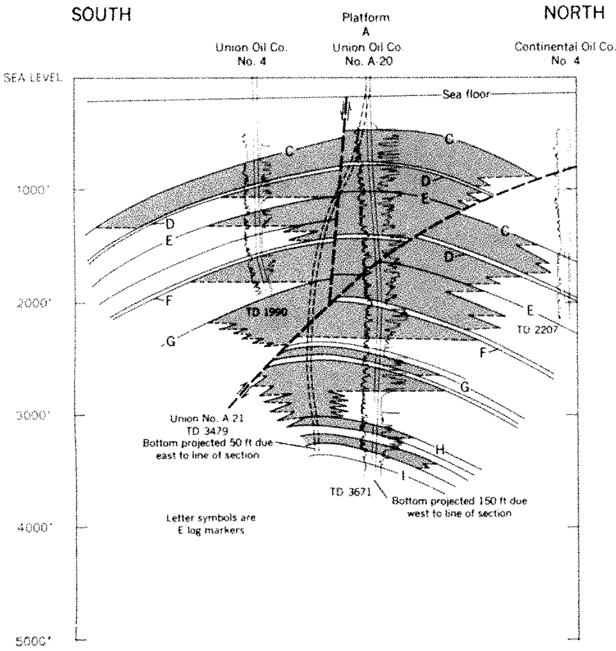
The sea bed off the coast at Santa Barbara has a long history of natural seepages of oil, and one of the earliest observations on record is apparently that of Captain Cook, who described a thick slimy substance floating on the surface of the sea in the Santa Barbara Channel one day in 1792. More recently, the submarine escape of oil and gas has been recorded over a wide area out as far as Santa Cruz Island, some 30 miles offshore. The well, which was being drilled immediately before the escape of oil and gas on January 28, 1969, is the farthest west so far on an antisyncline structure running for some thirty miles westwards towards the coast south of Santa Barbara and then for at least an equal distance out to sea. One part of the submarine structure has been exploited successfully since 1966, and now has close on 100 wells in it. The Dos Quadras oilfield from which the oil eventually escaped is a comparatively small domed structure about four miles long and half a mile across. The first wells were sunk in the formation and eventually closed off during 1968, but the well being drilled in the first few days of 1969 was intended to penetrate the superficial stratum of rock at almost its thinnest point, at the greatest height in the submarine reservoirs.

According to the description of this structure by T. H. McCulloh, the Dos Quadras offshore oilfield is "unique in one special way" among oilfields with reserves of 100 million barrels or more—the comparative thinness and permeability of the 300 feet of rock separating the uppermost part of the petroleum reservoir from the sea bed. There is a good deal to suggest that this covering is not a perfect seal for the petroleum beneath—for one thing, it contains substantial amounts of free hydrocarbons, while the comparatively high density of the oil in the topmost layers suggests that lighter and more mobile fractions have been lost progressively since the Middle Pliocene, when the anticline was formed.

The sequence of events during the blow-out of the well numbered A21 is hard to reconstruct because of the way in which the oil reservoir is intersected by at least two fault systems. First, there is a thrust fault by means of which the northern part of the antisyncline has already been driven beneath the southern part by about 500 feet. This is associated with an almost vertical tear fault. The line of well A21 manages to intersect both faults, thus making sure that the several zones of the oilfield were hydrostatically connected with each other, although it is only fair to say that the same effect would have been obtained if an unlined hole had been drilled through an uncomplicated part of the reservoir.

One of the most telling parts of the argument now published is the calculation which it has been possible to make of the hydrostatic pressure of the petroleum occurring in the successive layers of sandstone. Although it seems that the pressure of the hydrocarbons in the first 2,000 feet or so of the reservoir is that corresponding to an equivalent depth of water, the deeper strata contain hydrocarbons at greater pressure. Moreover, it appears that the lower layers contain larger quantities of gas, which means that the petroleum which they contain is less viscous and less dense than at lesser depths.

Mr McCulloh says that these relationships can account satisfactorily for the sequence of events following the blow-out. The first observation was that a "heavy condensate mist" followed the mud blown out of the hole, but that after the drill pipe had been dropped back down the hole, it seemed for a time that the well had been successfully closed off again.



Transverse section through anticline showing position of boreholes and faults (heavy broken line).

The first signs of trouble were the appearance of turbulent boiling of the sea something like a quarter of a mile from the drilling rig about two hours after the blow-out. Mr McCulloh says that the time lag would have been that required for the surface stratum to be fractured under the pressure of petroleum from the deeper layers of the reservoir, in exactly the way in which it is a part of normal petroleum practice to use large hydrostatic pressures for increasing the permeability of oil-bearing strata and thus the productivity of wells. He also points to the significant way in which drilling fluid was quickly lost from the well while cutting through the upper layers of rock, indicating unusually permeable material. The nearest Mr McCulloh comes to direct criticism, however, is his approving quotation of a statement that in "modern soundly engineered deep drilling" it is necessary accurately to predict the pressures likely to build up in the successive formations of the reservoir as well as the liability of some of these to fracture.

As things have turned out, it seems that the best cure has been more drilling. By now, more than a score of wells have been sunk into the formation, partly in the hope of drawing off enough oil to reduce the pressure and the risk of further seepages.

METEOROLOGY

Centenary for Weather Bureau

The United States Weather Bureau has been celebrating the centenary of its legal foundation on February 9, 1870, with an international conference on meteorology and other appropriate festivities. Although the conference itself may have been a somewhat sleepy occasion, the centenary does seem to have come aptly at a time when the new methods of meteorology are promising to transform the character of the work. This no doubt was a part of the motive for the way in which Dr Robert M. White, the administrator of the Environmental Science Services Administration, welcomed the coming of "big science" to meteorology, but it was clear last week that many of his listeners were still steeped in the old traditions of meteorology—the cataloguing of tornadoes in the prairies and the daring use of high-flying kites as ways of collecting upper atmosphere measurements.

The Weather Bureau began life as the Division of Telegrams and Reports for the Benefit of Commerce. Although the US Army seems to have been the first, in 1812, to persuade its scattered men to keep weather records, the coming of the telegraph allowed Professor Joseph Henry at the Smithsonian Institution to begin collecting observations systematically, chiefly with the help of telegraph operators. The need for a continuing organization was urged both by Henry and by Cleveland Abbe, the director of the Cincinnati Observatory, who began to set up his own system for correlating observations in 1869, after Henry's network had been partly dispersed during the Civil War. Eventually, the argu-

GRATITUDE

Welcoming the United States budget for 1971, Dr William D. McElroy, Director of the National Science Foundation, said: "It is a real pleasure for us that President Nixon, who is so deeply concerned with all of the Nation's problems, has recognized the importance of scientific research and has provided an increase over the amounts appropriated by the Congress last year. The National Science Foundation has, in turn, responded to the President's challenge to help reduce inflation while making progress in the most necessary Federal problems. Our budget will enable us to maintain a vigorous although austere programme of support for basic research and graduate education. . . ."

ment that a means of making weather observations could help to prevent disasters to shipping on the Great Lakes seems to have carried weight with Congress and with the president, Ulysses S. Grant.

The characteristic flavour of the United States Weather Bureau is its concern for meteorological hazards—which is only natural in the most advanced society which is regularly ravaged by hurricanes, floods, tornadoes and extreme fluctuations of temperature. There has been a flood warning service since 1873, and flood prediction was also one of the themes at the conference last week. There was also great interest last week in the great potential benefits of forecasting natural disasters of climate, from the floods which accompanied the hurricane Camille, which last year killed 320 people in the United States, to the foreknowledge of frost among the citrus crops, estimated to have been worth as much as $75 million on some occasions.

Better prediction was the most widely commended device for avoiding disaster like this, but there was also some talk of artificial weather control last week, not all of it enthusiastic. For the most part, the modifiers seem to have had very little to show for the past decades of endeavour. The most tangible new work was that described by Dr Joanne Simpson, who has been studying cumulus formation by a combination of the techniques of seeding actual clouds with silver iodide and mathematical modelling. She considers that recognizable changes, particularly reductions of windspeed, were brought about by the seeding of the eye of hurricane Debbie in August 1969. She also says that she can now identify cumulus clouds which are likely to grow explosively when seeded with silver iodide, and thinks it possible that the systematic use of this technique might make it possible to increase the summer rainfall over Florida by as much as a third.

Dr E. G. Bowen of CSIRO, Sydney, one of the first of the cloud seeders, was somewhat more perplexed to account for the way in which seeding sometimes seems to diminish the probability of rainfall, and suggested that there may be an unaccountable persistence of the effects of a single seeding in one restricted area. No doubt the dynamic theories of cloud formation and development on which Dr Simpson puts her faith will eventually help to make these points clearer, but there is probably most truth of all in a jibe due to Dr B. J. Mason, director of the Meteorological Office, that if people really want to modify the climate, why do they not begin by removing the Rocky Mountains?

Elsewhere the future for meteorology seems bright. At the conference last week, the promise of infrared sensors mounted in Earth satellites seemed to burn especially brightly. The point seems now to have been reached when some of the conventional programmes of meteorological observation—parts of the GARP programme, for example—should now be altered so as to take fuller account of the potentialities of advanced weather satellites, now able to provide a complete temperature profile throughout the atmosphere to a resolution of about 100 km in horizontal extent except in the tropics (where gradients are not sufficiently sharp to be measured accurately by the infrared devices). With this prospect ahead, people like Dr Mason are now saying that the time has come to concentrate on meteorological observations on a scale of about 1,000 km or even less.

STANDARDS

Error Comes to Light

A DISCREPANCY of 1·9 per cent in the United States secondary standards for the total luminous flux of gas filled tungsten lamps has now been revealed and corrected by the National Bureau of Standards. The bureau's sheepishness at having to admit such a comparatively large error is tempered by the knowledge that it was the first to notice it. The discrepancy first came to light during comparisons of American standard lamps with those used elsewhere, particularly in Canada and Britain. By all accounts, the American secondary standards were consistently found to give results at the extremes of the expected scatter. In the past two and a half years, according to Dr Donald McSparron, it has been possible to re-assess the absolute luminous flux of the standard lamps used at the laboratory by working upwards in the temperature scale from the basic black body standard by at least three different photometric routes. The work has, for example, involved the comparison of the energy output of tungsten lamps with those whose spectral characteristics have been deliberately distorted by the use of filters and the comparison of flux, wavelength by wavelength, using a spectroradiometer. The result is that previous calibrations carried out at the laboratory are now reckoned to have been 1·9 per cent too low.

The bureau is quick to point out that the error now revealed applies only to the secondary standards for tungsten filament lamps—mercury vapour lamps and fluorescent lamps seem to be correctly standardized. At the same time, however, it is hoped that some of the present uncertainties will be diminished when the bureau has completed its project for designing a new black body standard—an illumina crucible containing molten platinum. Dr McSparron explains that the design has been chosen so as to make possible an easier extrapolation from an actual to an ideal black body, but that so far it has turned out to be difficult to arrange for the manufacture of the illumina crucible, which has to be no more than 1 millimetre thick.

NUCLEAR RESEARCH

Swings and Roundabouts for AEC

IT now looks as if high energy physicists in the United States will have to pay for the new high energy accelerator being built at Weston, Illinois, with a reduction of the cost of operating high energy accelerators elsewhere. In the year ahead, it is planned to save $5·2 million on the operating budget by reducing the scale of effort at the Princeton, Pennsylvania, accelerator, the electron accelerator at Cambridge, Massachusetts, and the bevatron at Berkeley, California. The first of these, indeed, which produces a beam of protons at 3 GeV, will in fact be shut down during the year. At Cambridge, work in future will be confined to the use of colliding beams from the storage rings. Although the linear electron accelerator at Stanford (SLAC) and the Brookhaven accelerator will soldier on, as will the alternating gradient machine at the Argonne National Laboratory, the reduction of effort elsewhere will seem to many high energy physicists to be a cold douche of reality, predictable though it may have been. In practice, even the funds available for the construction of the 200 GeV accelerator are less than had been hoped for, but the director of the project, Dr R. R. Wilson, has apparently told the AEC that he will finish on time, in 1972, in spite of the cut. Altogether, high energy physics will get $1·1 million less in the coming year, while the AEC's expenditure on physical research will be $3·8 million less than in 1970 at $274·4 million.

Thermonuclear research has, however, come back into favour. During 1971, the AEC is planning to spend an extra $1·9 million on fusion work, and much of this will go on two new machines—a device incorporating some of the features of the old accelerator and the new Tokamak at Princeton and a new toroidal pinch machine at Oak Ridge. In his statement at the opening of the examination by the Joint Committee on Atomic Energy of the new budget, Dr Glenn T. Seaborg, chairman of the AEC, said that there had been "significant advances in this field of research in the past year".

As it happens, however, the joint committee is unlikely to swallow without protest the new budget which seems to the chairman of the committee at least to fall short of what is needed. Last week, Mr Chet Holifield said that he took a "dim view" of some features in the budget and that he was especially "disappointed" with the lack of funds for the cascade improvement programme by means of which it had been hoped that output of enriched uranium could be increased and by the lack of elbow room in the programme of development for the liquid metal fast breeder reactors (LMFBR)—it will be necessary during the coming fiscal year to make a decision between alternative designs for a prototype reactor. With all this, however, there is hardly a tear shed for the decline of interest in the Plowshare programme. With environment all the rage, who will defend a device for leaving accumulations of radioactive rock in potential gas wells or even Panama Canals?

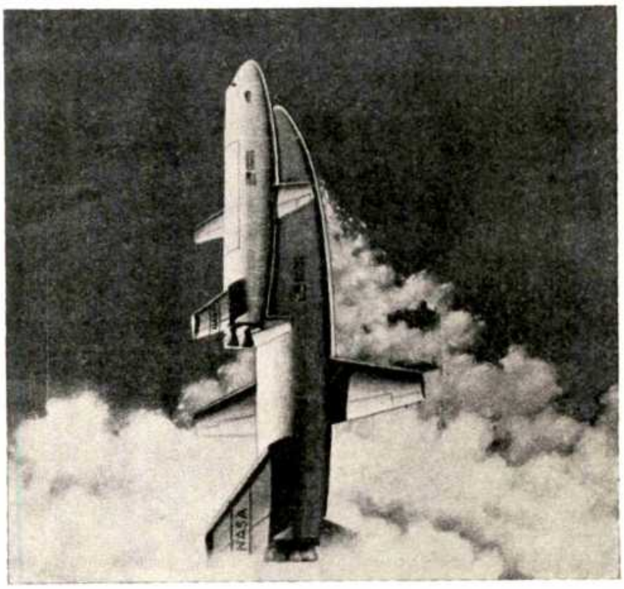
SPACECRAFT

Shuttles in Space

'I STAND before you a man who has just had his budget cut 12 per cent", jauntily remarked Dr Thomas O. Paine, administrator of the National Aeronautics and Space Administration, who was in London last week to receive various trophies commemorating the first manned lunar landing from the British Interplanetary Society. The general demeanour of his agency that he sought to put across in this predicament was one of buoyancy and optimism. Rationalization of programmes and ingenuity in place of blunt power were the means of economizing without compromising.

Central to NASA's revised thinking is the concept of re-usable vehicles and in particular the manned space shuttle for which definition studies are already in hand. It is calculated that fifty flights a year of the space shuttle could be carried out for less than the price of just one flight of the Saturn V. This reflects economy in ground support as well as of hardware.

The concept is for a craft that would take off vertically and land horizontally. Dr Paine indicated that the vehicle would be in two parts; a booster on the scale of a jumbo jet (such as the Boeing 747) and a detachable powered cabin about the size of a 707 or DC-10 which would carry the freight, whether passengers or supplies, and connect up with the orbiting space station for transfer. At the end of mission, each stage would return to base and land separately. Lift-off weight (3·5 million pounds) has been specified but not the payload. The payload compartment's size, however, has been defined; a volume of 15 feet diameter by 60 feet long. A number of configurations are possible but no choice between them has yet been made. They include fixed geometry and variable geometry craft; straight wing as well as delta wing types and lifting bodies. A design lifetime of not less than 100 re-uses is aimed for.



One of several possible configurations for the proposed space shuttle.

Economy in trained manpower and skill is also achieved by the concept. One of the points of the system is that it will get rid of special astronaut training for space visits. "Anyone, regardless of sex, with special reason to be in space—for instance, for scientific research—will be able to go this way", commented Dr Paine. "I would hope to see the first British astronautess fly before the end of the decade . . ." Inherent in the system is that the passenger and cargo environment will be comparable with that encountered in commercial airline practice. In the same way, of course, the craft will be flown by highly trained and specialized crew, but the passengers will not need to share this specialization.

The conventionally powered space shuttle for commuting to Earth orbital space stations is seen as a logical predecessor to a nuclear-powered shuttle for transporting visitors to lunar orbit and on interplanetary missions. The new promise of the space shuttle is so compelling that the British Aircraft Corporation, whose MUSTARD concept dates back to the early sixties, has been asked if it could revive it either for national or international consideration.

Lovell Charts the Future

by our Astronomy Correspondent

ONE of the traditions of the Royal Astronomical Society is that the presidential address should be devoted to the astronomical speciality of the president, but in a departure from convention last week Professor Sir Bernard Lovell spoke not on radio telescopes but on "The Prospects for British Astronomy", although some would say that an eye on the future also happens to be one of Sir Bernard's specialities. The occasion was the anniversary of the founding of the society 150 years ago, and Sir Bernard made clear that the celebrations have occurred at a crucial time for British astronomy. Since the war there has been a vast increase in the astronomy budget but this has brought important changes in the way British astronomy is run.

Sir Bernard cited the way the budget of the Astronomy, Space and Radio Board of the Science Research Council, which is responsible for almost all British astronomy, has risen from 20·6 per cent of the SRC budget in 1965–66 to about 25 per cent, or £11·7 million, now. He anticipated that by the mid 1970s the total annual budget of the SRC will have risen from its present £46·8 million to the region of £63 million, with the Astronomy, Space and Radio Board dealing with between £15 million and £17 million of that.

The share of ground-based astronomy might be expected to rise from its present £3 million to £4 million in 1975, and Sir Bernard reminded the society that this would be equivalent to the amount needed for about fifty per cent of the shelved Whitford programme for the development of ground-based astronomy in the United States. Any exhilaration at these figures should be tempered by the knowledge that astronomers can no longer run their affairs in the old way. They must now be prepared to spend more time on the committees of the SRC because if there is no policy, and no united front among astronomers, then the growth rate of astronomy will fall.

The address concentrated on observational astronomy, and Sir Bernard said this is where the financial and organizational weakness lies—theoretical astronomy is by comparison well nourished with money and talent. There was also a tinge of sadness that the role of the society in the running of British astronomy has diminished, officially at least. "The new radio telescope at Cambridge will be managed and directed by Sir Martin Ryle and the University of Cambridge on behalf of the SRC. If our Mark V radio telescope is ever built, this too will remain SRC property." The same goes for the optical telescopes, and although the Isaac Newton telescope is located at and maintained by the Royal Greenwich Observatory, its programme is essentially controlled by the Large Telescope Users Panel.

With the building of the 150-inch Anglo-Australian telescope at Siding Spring, the major British interest in the southern hemisphere is bound to shift from South Africa to Australia, Sir Bernard said. An urgent problem is to make sure that British astronomers are able to make full use of their half-share of the time on the 150-inch, and an admirable solution would be the establishment of a British supporting unit at Siding Spring equipped, for example, with a 48-inch Schmidt. This would do for the southern skies what the Palomar Schmidt has accomplished in the northern hemisphere.

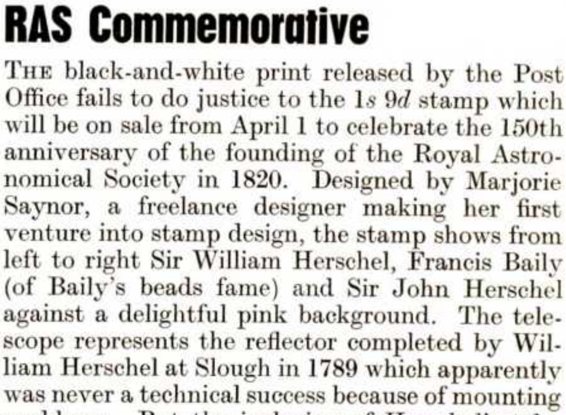
The problems of northern hemisphere astronomy centred on the UK are altogether more intractable, Sir Bernard said. "We have to develop advanced instrumentation, and that for use with the Anglo-Australian telescope is already becoming an urgent matter. We must stimulate the university astronomy departments to teach and train astronomers and we must provide UK observational facilities in the northern hemisphere under the best European seeing conditions to match those which we are developing in the south. Finally we must devise an organization for using the telescopes and directing the money which SRC may be able to provide to the appropriate research groups in the universities and elsewhere." The solutions to these urgent problems have to be based on what Sir Bernard called the somewhat incoherent structure of British astronomy in the northern hemisphere, founded on two royal observatories, one with a telescope still small by world standards working under sky conditions which are far from ideal, and several university departments heavily dependent on the SRC.

STAMP CORNER

RAS Commemorative

THE black-and-white print released by the Post Office fails to do justice to the 1*s* 9*d* stamp which will be on sale from April 1 to celebrate the 150th anniversary of the founding of the Royal Astronomical Society in 1820. Designed by Marjorie Saynor, a freelance designer making her first venture into stamp design, the stamp shows from left to right Sir William Herschel, Francis Baily (of Baily's beads fame) and Sir John Herschel against a delightful pink background. The telescope represents the reflector completed by William Herschel at Slough in 1789 which apparently was never a technical success because of mounting problems. But the inclusion of Herschel's telescope is appropriate because it features on the seal of the society.

A first-day cover service is being provided by the Royal Astronomical Society at a cost of 6*s* 6*d*. For 17*s* 6*d*, however, the deal includes an authentic signature of the president, and Sir Bernard Lovell claims to have made £40 for the society already.



NEWS AND VIEWS

Needles in a Haystack are Hard to Find

MANY of the most interesting physical phenomena have been first recognized as more or less minor departures from statistical regularity in a mass of observational evidence, which is why nobody will scoff and everybody will admire the great care which is being lavished by Professor J. Weber at the University of Maryland on the identification of what may eventually turn out to be gravitational radiation. Professor Weber has built the most elaborate equipment of its kind—perhaps even the only equipment of its kind—for the detection of the gravitational radiation which is predicated by Einstein's general theory of relativity. He has one detecting station at the Argonne National Laboratory and another at the University of Maryland. At each station there are massive metal cylinders which may be set vibrating mechanically either by the arrival of gravitational radiation from deep in space, or by more local phenomena—passing traffic and the like. In practice, comparison of the records of the two detecting stations distinguishes between spurious local phenomena and generalized influences which affect both stations similarly. Inevitably, however, disturbances of the detecting cylinders are so plentiful that purely accidental coincidences between the records of the detecting stations are common. Professor Weber's problem all along has been to find some way of distinguishing between these accidental coincidences and coincidences which may be genuine evidence of gravitational waves. The problem is familiar enough in fields as different as the detection of underground explosions and the hunt for quarks.

A year ago, it seemed very much as if Professor Weber's detecting equipment has indeed provided more coincidences than would be expected to occur by chance. In his first announcement (*Phys. Rev. Lett.*, **22**, 1320; 1969) of his success, Professor Weber appeared to be more firmly confident that he had actually recorded the arrival of packets of gravitational energy. The statistical analysis has turned out to be more difficult than seemed to be necessary at first sight. One difficulty, for example, was that of knowing how the simultaneous oscillation of several detecting instruments at one site should influence the statistical significance of a coincidence between that site and the distant one (see *Nature*, **224**, 411; 1969). Professor Weber is now going very deeply into these points and has put his statistical analysis in a form which is easier to follow, but which also suggests that the coincidences which may be evidence of gravitational radiation are by no means as exceptional as they seemed at first to be (*Phys. Rev. Lett.*, **24** 276; 1970). Thus, for disturbances at individual detecting sites so large that they occur by chance only 100 times a day, Professor Weber calculates that eighteen coincidences would have occurred in 214 days of observation—the effective time for which his equipment was functioning between January and the end of November last year. In practice, he observed 115 coincidences and he now suggests that "a considerable excess of coincidences over the expected number of accidental ones is observed and ascribed to gravitational waves".

No doubt Professor Weber, his colleagues and his critics, will be anxious to strengthen this tentative conclusion. One development has been to look at the rates at which coincidences occur when the records from the two sites are compared simultaneously and when one of them is delayed by two seconds. The result, that delay reduces the rate of coincidence to what would be expected, may again be clinching when more records have been accumulated. To be sure, there remains the possibility that some hidden systematic errors may account for the ten-fold enhancement of the coincidence rate—it could yet be, for example, that sending the records of the Argonne detector directly to Maryland for comparison may make it possible for some spurious influence to affect them both. Time, no doubt, will tell, but in the meantime everybody will wish Professor Weber well in his painstaking but exciting hunt.

PLANETS

Ammonia Ice in Saturn's Rings?

by our Astronomy Correspondent

EVIDENCE that seems to rule out H_2O ice as the chief constituent of the unknown material of the rings of Saturn has come from a comparison of the infrared spectrum of Saturn with that of Jupiter. This conclusion is part of a report by H. L. Johnson of the University of Arizona and the Universidad Nacional de México in the current issue of *Astrophysical Journal Letters* (**159**, L1; 1970). Johnson presents four spectra of Jupiter and one of Saturn in the infrared range 1·2 to 4·2 microns. At the same time, he suggests ammonia ice as a possible explanation.

At the time of the Saturn observation the rings occupied between 5 and 10 per cent of the area of the entrance diaphragm, and Johnson suggests that this factor may account for several differences between the Saturnian and Jovian spectra. At wavelengths around 1·4 microns, between 1·6 and 1·8 microns, and between 2 and 2·5 microns, Jupiter is black (that is, it reflects no light) and the spectral intensity drops to zero. The Saturnian spectrum is never quite zero at these wavelengths, however. Assuming that this contribution to the Saturnian spectrum comes from the rings it can be calculated that the rings contribute about 5 per cent of the reflected light, which agrees with their projected area on the diaphragm.

Granted that this is the explanation of the discrepancies, and this assumes that the planet Saturn is as black as Jupiter in the three wavelength intervals, what does it say about the composition of the rings? The clue here is a fourth band from 3·3 to 4 microns where both Jupiter and Saturn are black; there is no sign of reflected light from the rings of Saturn. According to Johnson, if the rings consist of H_2O ice they ought to contribute in the 3·3 to 4 micron band, and there ought to be an ice absorption band at 3 microns as well. Johnson suggests that this makes ammonia ice a more likely explanation. The point here is that ammonia ice has been put forward as the explanation of the absorption which makes Jupiter black between 3·3 and 4 microns, and therefore might explain why the rings of Saturn fail to register at these wavelengths.

MANY-BODY PROBLEM

Pairwise Harmonic Forces

from a Correspondent

FROM a microscopic point of view all problems in physics and chemistry are many-body problems. The problems become interesting when the particles of the system interact with one another, usually via two-body forces. There are very few systems of interacting particles for which exact solutions are known, and so it is always exciting to study a model of interacting particles which is exactly soluble.

Storer (*Phys. Rev. Lett.*, **24**, 5; 1970) has given a quantum statistical mechanics treatment of a system of particles interacting through pairwise harmonic oscillator forces. Working in terms of the grand partition function he shows that this interacting system behaves like a system of independent particles moving in an external harmonic well with, however, an important modification which implies a greatly reduced density of states. Not only is the density of states reduced but the spectrum of a system of particles interacting through the pairwise forces is shifted down by $\frac{3}{2}\hbar\omega$ (where ω is the oscillator frequency) relative to that of a system of independent particles in an external well.

The problem of describing N interacting particles is usually stated in terms of the N coordinates of the particles $x_1, x_2 \ldots x_N$. In the absence of external forces the centre of mass of the system, with coordinate $\vec{X} = \frac{1}{N}\sum_{i=1}^{N} \vec{x_i}$, has its motion conserved, that is, if we fix the centre of mass there remain only $(N-1)$ independent coordinates. This distinction between N independent coordinates and $(N-1)$ independent coordinates is lost in a statistical treatment.

It follows immediately from the definition of the centre of mass coordinate that the energy of the system of particles interacting through pairwise harmonic forces is equal to the energy of a system of independent particles moving in an external harmonic well the origin of which is attached to the centre of mass, which itself describes harmonic motion, plus the energy associated with the motion of the centre of mass.

When we consider the full spectrum of the system of independent particles moving in an external harmonic well, many of the configurations will correspond to excited states of the centre of mass motion. If we neglect the centre of mass motion the density of states will be decreased as observed by Storer. Further, because the centre of mass undergoes harmonic motion the zero point energy associated with this will be $\frac{3}{2}\hbar\omega$, and so when the centre of mass motion is ignored the whole spectrum will be shifted down by this amount.

The many-body system in which particles interact through pairwise harmonic forces has frequently been examined in the context of nuclear structure theory and the results quoted here have long been the basis for the standard method used to remove states containing spurious centre of mass motion from nuclear shell-model calculations (Elliott and Skyrme, *Proc. Phys. Soc.*, A**232**, 561; 1955).

FRACTURE MECHANICS

Brittle Fracture by Thermal Shock

from our Materials Science Correspondent

ONE of the many obstacles in the path of the design engineer who would like to make use of the high strength of brittle ceramics such as alumina or silicon nitride is their sensitivity to thermal shock. Thermal gradients induced by sudden heating or cooling cannot be relieved by plastic flow (which is why these materials are strong) and so cracks readily form and propagate. Quantitative treatment of this process is difficult and so it is of considerable importance that a unified theory has been published recently by D. P. H. Hasselman (*J. Amer. Ceram. Soc.*, **52**, 600; 1969).

Hasselman has considered three distinct stages in thermal shock: the thermal condition required to initiate fracture (assuming a pre-existing population of small Griffith cracks of uniform size), the length of crack which results and the thermal condition required to cause the arrested crack to start propagating again. It turns out that the temperature difference between surface and core—rather surprisingly, it is the difference and not the gradient that enters the equations—required to initiate fracture is independent of the density of cracks. But it varies slowly with crack size, while the size of crack at which propagation is arrested depends steeply on initial crack size and also on population density. It is particularly noteworthy that there is a particular initial crack size (depending somewhat on the population density) for which the temperature difference to initiate crack propagation is a minimum: that is, as far as initiation is concerned, the material is then at its most sensitive. In these circumstances, however, crack propagation is least favoured; propagation is quasi-static instead of being dynamic. As a result of his calculations (which agree in detail with some careful measurements by Davidge and Tappin of thermal shock behaviour of alumina spheres), Hasselman recommends that, for a given material, sensitivity to thermal shock should be minimized by adjusting the grain size, or population of artificial cracks, so that fracture is easily initiated but unlikely to propagate.

One incidental feature of interest which emerges from the survey is the asymmetry of thermal shock fracture: alumina is much more apt to shatter on heating than on quenching. The difference is connected with differences in the number of nucleation sites in the two cases.

ELECTRON MICROSCOPY

Ion Erosion for Non-metals

from our Materials Science Correspondent

REPLICAS have had their day. Probably nine-tenths of electron micrographs nowadays are made from thinned foils, and nine-tenths of those from metals. Many ceramists, mineralogists and glass scientists would gladly make more use of electron microscopes if the problems of thinning down specimens were less daunting. With inorganic compounds that are lamellar in form and cleave easily, there is no problem, but run-of-the-mill oxides have been troublesome. Some people have used mechanical grinding, others have experimented with chemical thinning—often with indifferent success—and some in desperation have become crystalloclasts and taken to using a pestle and mortar.

Several investigators have profited from the current interest in ion erosion to develop a new method of thinning non-metallic samples for electron microscopy. It has been described independently by D. J. Barber (*J. Materials Sci.*, **5**, 1; 1970) and H. Bach (*J. Non-Cryst. Solids*, **3**, 1; 1970), who concerns himself largely with glasses. Both use focused beams of argon ions at 5–7 kV energy to erode material from the sample surface at nearly glancing incidence. The principal difference between the two scientists is that one erodes one side only, while the other simultaneously bombards from both sides, using a cold finger to inhibit redeposition of sputtered atoms. The apparatus is simple—Barber's is the simpler of the two, and equally effective—and there appears to be no problem about heating of the sample, or controlling the process. The two reports contain several micrographs of glasses and crystalline compounds such as olivine, alumina, quartz and lithium fluoride, as well as some thin films sectioned transversely.

Glasses do not give good micrographs if they are truly vitreous, for lack of contrasting features, but with many samples it is possible to see incipient or advanced breakdown into two glassy phases or into a crystalline phase dispersed in a vitreous matrix. These features show up well, while larger inclusions are revealed by a sort of self-shadowing in the ion beam. Barber reports no problems with crystalline materials, but Bach has found that in some cases the surface layers lose their crystallinity because of radiation damage by the ions. This was clearly revealed by electron micrographs made of ion-eroded quartz, but lithium fluoride was immune. It will plainly be important in this respect to separate the sheep from the goats.

Ion erosion has also been used to remove successive layers from the surfaces of samples in a scanning electron microscope, to permit examination of structures in depth. D. Kynaston, working with a Stereoscan (*New Scientist*, **45**, 256; 1970), has obtained micrographs with the help of a small ion source built into the microscope's specimen chamber; he has applied the technique to both inorganic and biological specimens.

Barber, Bach and Kynaston have all used compact cold cathode sources operating with a glow discharge, which represents a cheap and simple way of obtaining focused beams of ions at moderate energies. It is to be hoped that some enterprising manufacturer will soon market an inexpensive device for conventional electron microscopists—it seems that scanning electron microscopists will soon be provided for.

PLANETARY ATMOSPHERES

Stain in the Clouds of Venus

by our Astronomy Correspondent

THE clouds which perpetually obscure the surface of Venus have been reported to be tinged with yellow, but the notion arising therefrom that the clouds are essentially carbon suboxide, C_3O_2, is scotched in the current number of the *Astrophysical Journal* by W. T. Plummer and R. K. Carson of the University of Massachusetts (**159**, 159; 1970). The possibility that carbon suboxide plays an important part in the atmosphere of Venus has been entertained at various times since the 1950s, but Plummer and Carson show that this is at variance with the infrared evidence.

They have prepared carbon suboxide frost in the laboratory and shown that its infrared reflexion spectrum differs from the spectrum of Venus. Although in the atmosphere of Venus carbon suboxide is likely to be liquid rather than solid, Plummer and Carson say that frost spectra are a good substitute for the spectra of a cloud of droplets. Their measurements show that between 1 and 3 microns the reflexion spectrum of carbon suboxide in general agrees with the Venus spectrum, but there are some important differences.

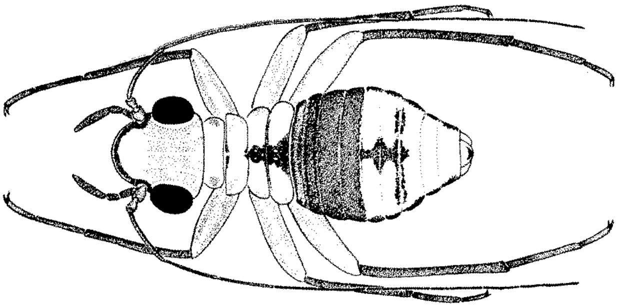
For example, a prominent carbon suboxide absorption feature at 2·25 microns is absent from the Venus spectrum, and Plummer and Carson say that this could only happen if the size of the droplets is suitably small, less than 0·1 micron across. Particles this small are eliminated by polarization measurements of Venus.

Although this seems to rule out carbon suboxide as an important component of the clouds, the point about carbon suboxide is that the monomer which is white in the solid state gradually polymerizes to a yellow, red or brown variety, with the possibility that the clouds might be made up of a polymer, thus explaining the yellow tinge. It is clear, however, that a faint yellow polymer of carbon suboxide cannot form the bulk of the clouds. For one thing, the chemical evidence is that all the polymers have a high reflectivity in the infrared region contradicting the region of low reflectivity in the Venus spectrum which begins on the low wavelength side at about 2·6 microns. What is more, Plummer and Carson say that the effect of ultraviolet light is to continue the polymerization until the product is a deep brown colour which is not likely to go unrecognized. To maintain the whiteness of the clouds the monomer would have to be continually replenished, but this is forbidden by the absence of the 2·25 micron feature. Clearly carbon suboxide is now properly ruled out as a major constituent of the enigmatic clouds—it was never a strong contender—but it is still feasible that a small amount of a polymer of low molecular weight might be responsible for the yellow stain, possibly in clouds which are composed of ice particles.

ENTOMOLOGY

Another Louse

THIS larva of an African louse, which is 1·3 mm long, has been assigned to the species, *Epipsocopsis cincta*, by A. Badonnel in a study of the Psocoptera, or lice, of Angola and neighbouring countries. It appears in one of the lavishly produced publications from the Museum at Dundo in Angola (Companhia de Diamantes de Angola, *Publicaçoes Culturais*, No. 79; 1969). The material for this study was a collection made by A. de Barros Machado and his colleagues, and Badonnel identified 2,220 adult lice.



TUMOUR VIROLOGY

Chasing a Minus Strand

from our Cell Biology Correspondent

WORKING up an experimental system to the point at which it can be used to answer interesting questions is a job most people prefer to leave to someone else, and tumour virologists, especially those who aspire to molecular biology, are no exception. Today it is simpler, and more immediately rewarding, to work with DNA tumour viruses, polyoma and SV_{40}, than to work with the RNA tumour viruses even though, as far as the viral aetiology of cancer is concerned, RNA tumour viruses seem to be the more pertinent of the two groups.

The RNA tumour viruses unfortunately present daunting technical problems. For one thing they have too much genetic information to make analysis of gene functions straightforward and for another they wrap themselves up in a large amount of host cell lipoprotein, which can comprise more than 99 per cent of the total mass of the virions. But in spite of their apparent complexity the RNA tumour viruses may prove at bottom to be very similar to the simplest of all viruses, the RNA bacteriophages. And certainly the RNA phages and small non-oncogenic RNA viruses, such as polio virus, are still valuable models for the RNA tumour viruses.

No one will be surprised, for example, if the replication of the single stranded RNA genomes of the tumour viruses proves to follow the same pattern as replication of the RNA phage and polio genomes. Several groups are busily searching for a tumour virus-specific RNA replicase similar to the well characterized Qβ phage replicase, and Watson and Beaudreau (*Biochem. Biophys. Res. Commun.*, **37**, 925; 1969) have made some progress in this direction. They have isolated a crude enzyme fraction from blood cells of chicks infected with avian myeloblastosis virus which may well contain the virus replicase. The crude enzyme preparation is not template-specific for the virus RNA, although of the several RNA templates tested avian myeloblastosis viral RNA was most effective; the RNA made with the virus RNA as template has yet to be characterized and the amount of RNA made is much less than the amount of template added. Nevertheless, the preparation probably contains the viral replicase, and although Watson and Beaudreau have a long way to go to prove that, the properties of their extract parallel in many respects the properties of crude extracts of Qβ phage replicase in the early days of its characterization.

If RNA tumour virus replication is similar to replication of other single stranded RNA genomes a minus strand complementary to the plus strand in the virion should be made during replication. Biswal and Benyesh-Melnick (*Proc. US Nat. Acad. Sci.*, **64**, 1372; 1969) have claimed recently to have detected such a minus strand in rat cells infected with murine sarcoma–leukaemia virus. They find, after extraction by a phenol–*m*-cresol procedure which could be the secret of their success, two RNA species restricted to the nuclei of infected cells. These sediment at 31–36S and 18–22S and, after heat denaturation, hybridize with RNA from murine sarcoma–leukaemia virions. On the other hand, none of the RNA from the cytoplasm of infected or uninfected cells, or the nuclei of uninfected cells, hybridizes with virion RNA. Biswal and Benyesh-Melnick conclude that the 31–36S and 18–22S RNA species are minus strands or fragments of minus strands, which presumably act as templates for synthesis of progeny virus genomes. The smaller of these two RNAs, the 18–22S RNA, is, moreover, resistant to ribonuclease and therefore probably double stranded. It may be part of a replicative form, but if so it is surprisingly small.

On the face of things these results are precisely as anticipated—the fact that only about 22 per cent of labelled virion RNA hybridizes even with large excesses of nuclear RNA may be a technical artefact—and the way seems open for the characterization of tumour virus RNA replication.

BIOCHEMICAL THERMODYNAMICS

Counting the Calories

from our Molecular Biology Correspondent

THERE is, of course, nothing new about the use of calorimetry to follow chemical and even biochemical processes. Nonetheless, all the indications are that this technique has only just arrived so far as molecular biology is concerned, and that it will not be long before the present trickle of papers becomes a flood. This growth of interest must be presumed to be at least partly a consequence of the development of good commercial instruments, which have gone some way to making the method experimentally foolproof. Two papers in the current literature—both in fact by long-standing practitioners of the art—illustrate well the scope of microcalorimetry in the study of enzymes.

Shiao and Sturtevant (*Biochemistry*, **8**, 4910; 1969) have studied the interaction of chymotrypsin with its inhibitors, and its dependence on protein concentration. In the first place, the heat of dilution of chymotrypsin depends on the initial concentration, and is found to follow it in a manner consistent with a simple dimerization of the enzyme. Correcting for this effect, it is possible to determine the heats of binding of specific inhibitors, by using large molar excesses of the ligand, so that the binding sites are always saturated. It emerges that the heat of binding is in all cases dependent on enzyme concentration, and it can be inferred that the dimer has little or no affinity for the inhibitor. These results, which refer to *p*H 7·8, at which the enzyme slowly autolyses, differ in various respects from those reported by other workers, using different methods, all, however, in more acid conditions. Shiao and Sturtevant suggest that at their *p*H they are observing cannibalistic enzyme–substrate complexes, and the inhibitors are in competition with this interaction. The affinity of the monomeric enzyme for the inhibitors is compounded of a large negative enthalpy and a sizable negative entropy, even when the ligand is uncharged. It is likely that a change in enzyme conformation (induced fit) is responsible for a considerable part of both.

A more complex system has been studied by Ross and Ginsburg (*ibid.*, 4690). This involves the binding of feedback inhibitors, which control its activity *in vivo*, to the twelve-subunit enzyme, glutamine synthetase. Twelve molecules each of tryptophan and 5′-AMP are bound by each protein molecule, indicating that all subunits are functionally equivalent. The heats of binding of the two ligands separately were measured, and also that of both together. The contributions of the two interactions are strictly additive, and this is good evidence that the sites for tryptophan and AMP are separate entities. The binding of either ligand is accompanied by a large entropy change, but one (AMP) is positive, the other negative. This curious phenomenon may be related to the induction of conformational changes by one or other ligand.

An interesting application of microcalorimetry in the polynucleotide field has also appeared (Scruggs and Ross, *J. Mol. Biol.*, **47**, 29; 1970). This concerns the binding of monomeric adenine derivatives to poly-U, a process that always has a 2 : 1 stoichiometry. The principal finding is that the enthalpy is always the same, irrespective of whether the monomeric species is adenine, adenosine or deoxyadenosine. This eliminates any involvement of the 2′-hydroxyl group, or any other part of the sugar, in the stabilization of the helix, at least in this prototypic system. The binding of a derivative containing an extra amino group in the purine ring, so that three hydrogen bonds can be formed with the pyrimidine, causes the enthalpy to become 3 kcalories/mole more negative. Knowing the melting temperatures of the complexes, the entropies of association can be derived, and are large and unfavourable, as one might expect for the immobilization of monomers.

GYNAECOLOGY

Test for Spontaneous Abortion

from our Medical Biochemistry Correspondent

If it is true that a gynaecologist's practice is made up of women desperately anxious either to have babies or to avoid them, they may all be helped by some recent biochemical investigations. Genazzani *et al.* have shown how women whose pregnancies are likely to end in spontaneous abortion can be distinguished by measuring the concentrations of human placental lactogen in their serum (*Lancet*, ii, 1385; 1969).

Human placental lactogen, discovered in 1962, is a polypeptide hormone produced by the placenta, and similar in structure and in some of its effects to pituitary growth hormone. Radioimmunoassay carried out on seventy-four women showed that concentrations of the lactogen steadily increase between the seventh and thirty-first weeks of normal pregnancies. This probably reflects the growth in size of the placenta at this time. In two cases the increase during several weeks was very similar to the mean increase for the group.

Of thirteen women with threatened abortion, who had been admitted to hospital with pain and vaginal bleeding, five experienced an increase in human placental lactogen comparable with the increase in the normal pregnancies. These women all completed their pregnancies successfully. Five of the thirteen admitted to hospital, however, had barely detectable concentrations of human placental lactogen and rapidly aborted. The other three had relatively low concentrations when admitted to hospital, and these rapidly decreased until the women aborted.

In cases when the human urinary chorionic gonadotrophin was also measured it turned out to be a very unreliable indicator of abortion. But the test for placental lactogen, in the early weeks of pregnancy at least, seems to be a good guide to the likelihood of spontaneous abortion, pointing out those patients for whom active therapeutic measures are necessary.

FORESTRY

Trees in Trouble

by our Botany Correspondent

Butt rot is a problem that often occupies the plant pathologists of the Forestry Commission. Their latest manoeuvres against *Fomes annosus*, the fungal cause of the trouble, are described in *Forest Research* 1969 (HMSO, £1.12½). Resistant species of forest trees are being sought to counteract the problem of butt rot, which seems to loom on most acid mineral sites in Britain. A survey of the spread of the fungus in stands of different species has confirmed that infection is less extensive in grand fir, *Abies grandis*, than in some other species. This is a hopeful sign for those who thought that this species might prove to be resistant to butt rot. The aim of the work in progress is to identify species which can be recommended for planting on heavily infested soils.

Another disease which has made surprising progress in the past year is beech bark disease, which had been thought to be of little importance except in mature trees. But there have been two serious outbreaks in young crops in Britain, in the Queen Elizabeth Forest (Hampshire and Sussex) and in Savernake Forest (Wiltshire and Berkshire). Preliminary investigations have indicated that drought is not after all an important causal factor. The cause seems to be a combination of the beech coccus insect and fungi of the genus *Nectria*.

The enquiries which the Forestry Commission pathologists dealt with in 1969 have shown them that elm disease, caused by *Ceratocystis ulmi*, is on the increase, but there was a striking decline last year in damage to weeping willows by *Marssonina salicicola*, which causes leaf spot, shoot canker and dieback. *Cristulariella depraedans*, a fungus previously unrecorded by the commission, caused spectacular damage to sycamore leaves in the south last year.

Larger pests are the problem of the commission's mammal and bird experts. Last year saw the continuation of trials of chemical repellants to prevent severe browsing by roe deer. A Dutch repellant, 'Aaprotect', was sprayed onto the shoots of Norway spruce, and three months after the beginning of the trial about a third of the shoots had been damaged. This represents a marked reduction in the damage done by the deer, but the chances are that a slow accumulation of damage will reach economic proportions in one or two seasons where browsing is a severe hazard to young plantations.

PLANT ANATOMY

New Cells Described

by our Botany Correspondent

A NEW category of plant cells has been recognized after an extensive survey of living material and literature. Drs B. E. S. Gunning and J. S. Pate of Queen's University, Belfast, have suggested that cells with marked ingrowths on their walls, which give them a large ratio of surface to volume, have evolved to cope with the transfer of solutes across the cell membrane. For this reason the cells are called transfer cells (*Protoplasma*, **68**, 107; 1969).

In spite of considerable morphological variations, transfer cells, which are found throughout the higher plants, are always characterized by distinctive, irregular ingrowths of the cell walls. Gunning and Pate have defined five situations in which transfer cells are found, and each case involves transfer of solutes across the membrane. First, there is absorption from the external medium. The ability of the submerged leaves of water plants to absorb and accumulate ions has often been investigated, and there is clear evidence in several species of ingrowths on the outer faces of epidermal cells, which are not covered by a waxy cuticle. Gunning and Pate think that these epidermal transfer cells will be found to be widespread in aquatic plants, facilitating the uptake of solutes by the provision of a large area of cell membrane.

Transfer cells are also common when solutes have to pass to the external medium; for example, in the glands of insectivorous plants. And there are transfer cells in the outer layers of the haustoria of parasitic plants such as *Cuscuta*, dodder, which penetrate into the vascular system of the host to absorb food materials. A similar situation is the placental connexion between the sporophytic generations of mosses and ferns and the gametophytic generations on which they develop. Transfer cells of both generations are found adjacent to each other, and Gunning and Pate assume that this means that there is specialization related to both influx of solutes to the parasitic sporophyte and efflux from the host gametophyte.

When it comes to reproduction in the higher plants, it is common to find situations in which an increased surface area of membrane would enhance absorption from the surrounding nutritive cells to the developing parts of the plant. There is discontinuity of contact between, for example, developing pollen grains and the surrounding tapetum and between the embryo and the embryo sac. Transfer cells always seem to be there to facilitate the passage of nutrients from one to the other.

The final context in which transfer cells have been reported is the transport systems of plants. The cells may be associated predominantly with the xylem or the phloem (Fig. 1*a*) or with both (Fig. 1*b*). Evidently the cells pass solutes between the conducting elements of xylem and phloem, or between the cells of xylem and phloem themselves.

Speculating about the development of transfer cells, Gunning and Pate have pointed to evidence that some secretory cells do not form ingrowths until secretion begins; perhaps the specialization of the wall will be found to be synchronized with the onset of intensive transport of solutes. The frequent localization of ingrowths on the cell walls considered to be most active in transfer suggests that the interaction with neighbouring structures is a determining factor in development. But the mechanism of development of the ingrowths is still a mystery. They will provide botanists with some intriguing problems, and it will be interesting to see how eagerly the name transfer cell is greeted.

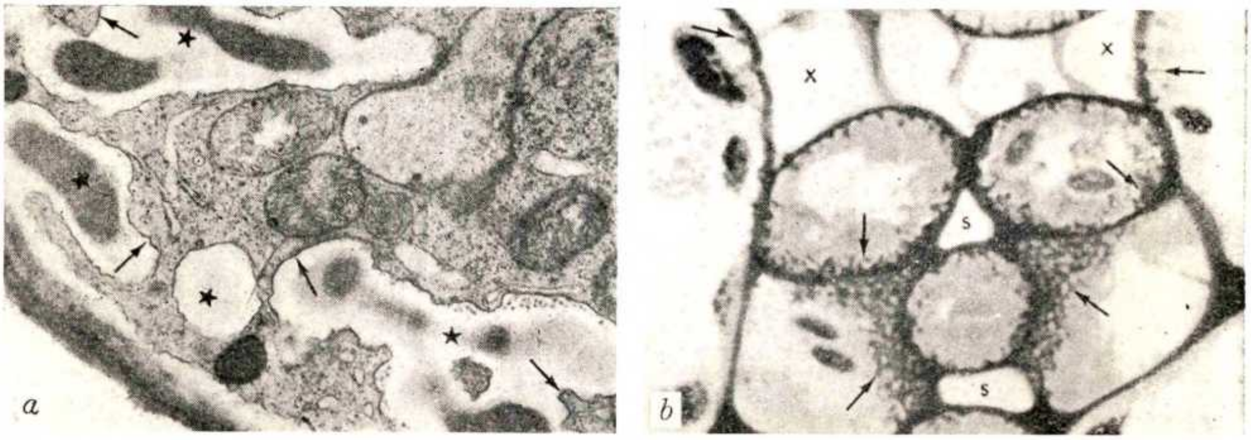


Fig. 1. (*a*) Electron micrograph showing the wall and membrane of transfer cells in phloem of *Vicia faba*. Stars mark the irregular ingrowths, which are associated with plasma membrane, arrowed (×20,000). (*b*) Photomicrograph of transfer cells surrounding sieve elements (s) and xylem (x) of *Anacyclus*. Some of the ingrowths of transfer cell walls are arrowed (×2,100).

CURRENT TOPICS IN DEVELOPMENTAL BIOLOGY

VOLUME 4

edited by **A. A. MOSCONA,** *Department of Biology, University of Chicago, Chicago, Illinois,* and **ALBERTO MONROY,** *Institute of Comparative Anatomy, University of Palermo, and Research Unit for Molecular Embryology, National Council of Research, Palermo, Italy.*

This book presents concise, topical reviews of research and concepts fundamental to the field of developmental biology. The emphasis is on cellular, biochemical, molecular and genetic approaches to the processes of development and differentiation. It provides an interdisciplinary forum for the presentation and discussion of facts and views, and aims to bridge the "interdisciplinary information gap" in this field.

CONTENTS: CLIFFORD GROBSTEIN: Genetics and Genesis. ALEX KEYNAN: The Outgrowing Bacterial Endospore. DAVID YAFFE: Cellular Aspects of Muscle Differentiation in Vitro. BERNARD ROIZMAN and PATRICIA G. SPEAR: Macromolecular Biosynthesis in Animal Cells Infected with Cytolic Viruses. MAX HAMBURGH: The Role of Thyroid and Growth Hormones in Neurogenesis. JACK GORSKI, G. SHYAMALA, and D. TOFT: Interrelationships of Nuclear and Cytoplasmic Estrogen Receptors. JACK E. LILIEN: Toward a Molecular Explanation for Specific Cell Adhesion. LEONARD WARREN: The Biological Significance of Turnover of the Surface Membrane of Animal Cells. Author Index. Subject Index.

THE STRUCTURE AND FUNCTION OF NERVOUS TISSUE

VOLUME 3: BIOCHEMISTRY AND DISEASE

edited by **GEOFFREY H. BOURNE,** *Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia.*

1970, 645 *pp.*, 308*s.* ($33.00),
subscription price 261*s.* ($28.05)

FISH PHYSIOLOGY

edited by **WILLIAM S. HOAR** and **DAVID J. RANDALL,** *both at Department of Zoology, University of British Columbia, Vancouver, Canada.*

Volume 1: EXCRETION, IONIC REGULATION, AND METABOLISM

1970, 465 *pp.*, 215s. ($23.00),
Subscription price 168s. ($18.00)

Volume 2: THE ENDOCRINE SYSTEM

1970, 447 *pp.*, 215s. ($23.00),
Subscription price 182s. ($19.55)

Volume 3: REPRODUCTION AND GROWTH: BIOLUMINESCENCE, PIGMENTS, AND POISONS

1970, 485 *pp.*, 224s. ($24.00)
Subscription price 187s. ($20.00)

EARLY DIABETES

Supplement 1 to **Advances in Metabolic Disorders**
Proceedings of the First International Symposium

edited by **RAFAEL A. CAMERINI-DAVALOS,** *Director Diabetes Center, New York Medical College, New York* and **HAROLD S. COLE,** *Department of Pediatrics, New York Medical College, New York.*

April, 1970, 478 *pp.*, 215s. ($23.00)

A New Journal . . .

CELLULAR IMMUNOLOGY

edited by **H. SHERWOOD LAWRENCE.** *Infectious Diseases and Immunology Division, Department of Medicine, New York University Medical Center, New York, New York.*

Cellular Immunology will provide an international medium devoted exclusively to publication of original investigations concerned with the immunology activities of cells in experimental or clinical situations. The scope of the journal will encompass the broad area of in vitro and in vivo studies of cellular immune responses. These include, for example, delayed type hypersensitivity of cellular immunity; immunologic surveillance and tumour immunity; transplantation immunology; autoimmunity, resistance to intracellular microbial and viral infection; immunologic deficiency states and their reconstitution; antigen receptor sites; thymus and lymphocyte immunobiology, etc. The chief criteria for acceptance of papers submitted for publication are the originality, clarity, and quality of the work reported.

All manuscripts should be submitted to the editor. The publisher will announce prices for personal and institutional subscriptions in the near future. Interested parties should contact the publishers to ensure prompt receipt of details. A limited supply of sample issues will be made available by the publishers to honour individual requests.

Anticipated publication date: Second quarter of 1970

CELLS AND TISSUES BY LIGHT AND ELECTRON MICROSCOPY

VOLUME 1

by **EDMUND B. SANDBORN,** *Department of Anatomy, Faculty of Medicine, University of Montreal, Montreal, Quebec, Canada.*

This book is a coordinated study of animal cells and tissues as seen in light and electron microscopy. It shows the organization of cells and tissues in unusual detail. Those tissues most frequently encountered in the studies of histology, cytology, and pathology are thoroughly covered. It is the first attempt at providing a study of biological structure that includes both light and electron microscopy. the book also contains examples of cytochemical analyses of membrane and organella function. Legends are in both French and English.

April, 1970, 364 *pp.*, 135*s.* ($14.50)

TRANSPORT PHENOMENA IN MEMBRANES

by **N. LAKSHMINARAYANAIAH,** *Department o, Phamacology, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania*

1970, 517 *pp.*, 205s. ($22.00)

INTERNATIONAL REVIEW OF CYTOLOGY

VOLUME 28

edited by **G. H. BOURNE,** *Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia,* **J. F. DANIELLI** and **K. W. JEON,** *both at the Center for Theoretical Biology, State University of New York at Buffalo, Buffalo, New York.*

May, 1970, 384 *pp.*, 196s. ($21.00)

Academic Press AP
111 Fifth Avenue, New York 10003
Berkeley Square House, London, W.1

Academic Press announces
a new quarterly journal

Physiological Plant Pathology

An international journal of experimental plant pathology

Editors B. J. Deverall, *London*, and T. F. Preece, *Leeds*

Editorial board *Chairman:* P. W. Brian, *Cambridge*

C. H. Beckman, *Rhode Island*
J. M. Daly, *Nebraska*
J. Dekker, *Wageningen*
J. E. DeVay, *Davis*
J. Friend, *Hull*
R. N. Goodman, *Missouri*
R. Heitfuss, *Göttingen*
J. Kuć, *Indiana*
R. D. Preston, *Leeds*
K. J. Scott, *Brisbane*
M. Shaw, *Vancouver*
I. Uritani, *Nagoya*
R. L. Wain, *Wye*
M. S. Wolfe, *Cambridge*
R. K. S. Wood, *London*

An understanding of host-parasite interactions is now essential to a wide range of biologists. This journal will publish work on physiological, biochemical, ultrastructural, genetic and molecular aspects of host-parasite relationships in plant disease, on the effects of environment on these relationships, and on modifications of host metabolism induced by infection with pathogens. Work involving any kind of infective agent will be included as well as research based on organisms in pure culture having a direct bearing on host-parasite interactions. Particular attention will be given to the publication of high quality plates and photomicrographs.

Aims To present this work to all plant scientists, plant pathologists and to physiologists, biochemists, geneticists and molecular biologists.

Level Undergraduates specializing in plant pathology, postgraduate students and research workers.

Frequency and price One issue quarterly; four issues per volume of 400 pages
Annual subscription: 126s. UK ($17.00 overseas)

Academic Press
London and New York

Berkeley Square House
Berkeley Square
London WIX 6BA

111 Fifth Avenue
New York
N.Y. 10003, USA

Case Histories of Innovations

by
R. W. CAHN
Materials Science Division,
School of Applied Sciences,
University of Sussex

Technologically slanted case histories of the development of industrial products are valuable supplements to more general analyses of technological innovation, which usually centre on economic and organizational considerations. This survey of available case histories shows that several lessons can be learnt from them.

EMERSON said that "if a man . . . make a better mousetrap than his neighbour, tho' he build his house in the woods, the world will make a beaten path to his door". He lived in a confident age, and today we are not so sure. A good deal of anxious soul-searching is going on, chiefly in government committees and at high powered conferences, to discover how a research director may recognize a better mousetrap; how he may persuade his managing director to supply the means to perfect and manufacture the new model; how, alternatively, he can fulfil his strategic function to recognize the need for the new mousetrap, and so constitute and guide his research team that they may as rapidly as possible develop it; and, finally, how to cope with the problems that face him when, with his nice new mousetrap just in the shops, a new breed of supercats is imported from America and the trap gathers dust on the shelf.

The inventors of the jet engine, hovercraft, swing-wing aircraft have all testified that the world was sceptical and slow to be convinced. Many people have accordingly been seeking to discover the reasons why, and what distinguished these inventions from others which were promptly taken up, developed and brought into successful production. Others have analysed the factors that favour the genesis of inventions in the first place, quite apart from the question of their effective use. Much discussion has been in terms of patterns of organization, or analysis of the financial implications of scale, lead-time and discounted cash flow, or else in psychological or social anthropological terms applied to the thought processes and motivational patterns of innovators. All too little attention has been devoted to the detailed scientific and engineering history of individual technological innovations —there is much discussion in general terms as to how research and development is best organized and financed, but little as to the particular detailed circumstances and steps which led to the perfection of a new product or process.

Justification for Case Histories

The justification for expending talent and money in assembling case histories, and time in studying them, has been aptly summarized by Conant in the introduction to his collection of case histories in experimental pure science[1]: "Almost all that a trained scientist has to go on when he passes judgment on the prospects of a new venture far removed from his own speciality is his knowledge of the methods by which science has progressed in his own experience. To a considerable degree a non-scientist may come to have a similar basis for his opinions. An intimate acquaintance with a relatively few historic cases should assist him in finding his way through the complexities of modern investigation as he listens to those who tell him what is being proposed". Conant was concerned only with scientific and not with technological events; yet the educational ends for which he assembled his collection are equally valid for those who guide the advantageous interplay of science and technology—that is, technological innovation.

The kind of case histories which I am recommending cast light on the mechanism of "coupling" between scientists and technologists, to use the term favoured by Price and Bass in their recent survey of scientific research and the innovation process[2]. The decisions which an industrial research manager has to make in organizing his laboratory include such matters as: Should there be a measure of fundamental research, not mission-orientated, so as to provide informal contact between technologists who are mission-orientated and those who can start them off on a new tack, either because of their own research or because of the reading they necessarily do—or is interaction too rare to warrant the cost ? Is it financially more advantageous to make use of external consultants, academic or otherwise, and, if so, should it be *ad hoc* or as a matter of routine ? Should the manager seek to identify what Price and Bass call "gatekeepers", human catalysts who are gifted scientists but devote their energies to seeking out possible applications for scientific discoveries ? Are scientific conferences more likely to foster coupling than frequent in-house colloquia combined with other social devices to foster interaction ? The accretion of case histories alone is not enough, and must be complemented by analysis, but any historian would confirm that analysis depends entirely on the availability of primary source material. This is the value of case histories: they constitute primary source material.

Economically Based Surveys

Many general surveys of organization, information flow and finance have been published recently, especially in the United States. The National Science Foundation arranged a conference on technology transfer and innovation[3], NASA commissioned a report[4] on assessing technology transfer (between different national sectors), the US Department of Commerce invited a panel to discuss the environment and management of technological innovation[5]. In Britain, a concise but illuminating report on technological innovation has recently been issued by the Central Advisory Council for Science and Technology[6], while large scale economic, political and managerial arguments have also been set out by Shanks[7] and Layton[8]. The last of these particularizes to the extent of concentrating on particular industries in separate chapters, whereas all the others make their analysis at a general level. Another study, devoted to a particular industry, is Beer's history of the German dye industry[9]. Particularly effective examples are the analysis of innovation in the plastics[10], electronic capital goods[11] and chemical process plant[12] industries. Although their primary emphasis is economic and international, these reports devote considerable attention to purely technological and organizational factors that have influenced the rate at which innovations have been brought to fruition in different countries and firms.

Freeman's reports come quite close to tackling the problem of managing research and development at the level of the individual industrial firm or laboratory. Analysis at this level is gradually growing: thus Mencher[13] and Blake[14] have reported briefly on two American studies, and Lord Rothschild has published a short survey of some of the research and development problems of Shell[15]. Lord Rothschild's survey, unlike Mencher's and Blake's, deals in part with specific episodes. So does Gowing[16] in her historical analysis of the development of British activity in atomic energy during the war, which is not less instructive for dealing with government research in wartime constraints.

Case Histories

The most thorough attempt until recently to map out the case histories of several inventions was made by Jewkes *et al.*[17], who emphasized the individual inventor; he is often seen as the sole motive force, pushing through his ideas against institutional apparatus and inertia, though institutional facilities play an important part in many of Jewkes's sixty-one case histories. Langrish (talking at the British Association meeting at Leeds in 1967) questioned the set of assumptions which he terms the "innovation myth", which includes the assumption of the preponderant role of the gifted individual, following his own private demon. He analysed five recent British innovations in some depth, treating them in their economic setting and attempting to trace some of the key conversations and contacts between the principal participants. Individual lessons are drawn from these case histories, and his lecture (which reports work in progress) represents a new standard in this form of analysis.

A chapter in a recent book on the zinc smelting industry in Britain[18] traces in detail the steps in the development of the important Imperial Smelting (blast furnace) process for extracting zinc from its ores, taking the story all the way from conception to large scale application. Economic aspects of the innovation are accorded considerable weight. A particularly useful example of this kind of analysis is Allen's[19] survey of the discovery and industrial introduction of polythene, terylene and oxygen steelmaking. Allen traces in considerable detail the intellectual stages of the discoveries, including the important accidents of the stimuli to which the principal protagonists were exposed and the papers they read and responded to. This "micro-innovative" approach is complemented by macroeconomic arguments.

The publication of a report on applied science and technological progress, prepared by the Committee on Science and Science Policy of the National Academy of Sciences for the Committee on Science and Astronautics of the US House of Representatives[20], is an important step forward. This contains seventeen signed articles and a coherent set of conclusions and recommendations, including a set of conclusions under the sub-heading "with respect to individuals who conduct applied research". (The committee emphasizes the importance of the individual technical entrepreneur, not only for his inventiveness but also for his determination; but it recognizes that he is not a *sine qua non* for technological innovation.) Harvey Brooks, of Harvard University, wrote a stimulating introduction, in which he refers to a number of case histories collected by the Materials Advisory Board of the National Research Council[21] and also to the US Defense Department's Project Hindsight[22], which is a continuing attempt to analyse the organization and progress of defence projects in the United States. Brooks particularly emphasizes the fact that most historical studies of innovation have "generally pointed up the fact that the development of technology has been surprisingly independent of the development of science, at least in detail". But he points out that this conclusion emerges from studies of events of fifty years or more ago, and that it is not valid for recent events. He emphasizes that the current intellectual style is towards abstract analysis, in technological innovation no less than in pure science, and suggests that a man like Willard Gibbs would not today start his career with a thesis on the design of gear trains. A critical analysis, in the light of some specific episodes, of the change of emphasis from the empirical to the abstract in modern technological innovation is a worthwhile task for a historian with deep engineering and mathematical knowledge.

The most useful case histories are to be found in the chapter by Suits and Bueche, of the General Electric Company, and they all refer to work done in the laboratories of that company. The projects include the development of man-made diamond, a vacuum circuit-breaker, two new polymers, a new form of an old ceramic, a new form of fuel cell, uses of etched particle tracks in thin films, and some others, including a new form of heat insulation which, though effective, was not a technological success—a rare example of frankness ! Each case history is accompanied by a list of key personnel with their antecedents and qualifications, and lists of patents and of related publications. The story of the vacuum circuit-breaker, for example, spells out the technological need which spurred the research effort, the scientific reasons for hoping that a new effort at a particular time might well be successful, and some of the specific inventions which together constituted the essential background. One of the polymer case histories sets out in some detail the role of chance conversations and more formal colloquia between the members of a laboratory in promoting a vital experiment or new way of looking at a problem. The story of the etched particle tracks (see also ref. 23) is a classic case of a wholly basic piece of research, which, through the freemasonry of the scientific profession, led to a totally unexpected but commercially viable application; the stages of this episode are recounted in detail.

All the General Electric case histories emphasize the critical role of the interaction between investigators, both those primarily involved with the project and those who merely join in conversations. This type of case history must be difficult to assemble, and because it depends in part on the recollection of conversations, no doubt it is subject to the occasional error of memory; nevertheless, it is peculiarly valuable for the lessons it brings for laboratory management. It is plain that someone at General Electric must have made it his business to keep records of some at least of the less formal aspects of research programmes, even before success was assured; laboratory notebooks, patents and papers are a necessary but not a sufficient component of the sort of intimate case history at which the General Electric writers excel. If this is accepted, then it follows that an enlightened organization which wishes to help itself—and people concerned with research and development everywhere—by assembling case histories, will need to pay a good salary to an observer who combines the gifts of scientist, engineer, psychologist, historian and writer.

Price and Bass[2] have already made good use of the case histories assembled by Suits and Bueche and by Frey and

Goldman[20], together with those published by Tanenbaum[21], to analyse the frequency of different patterns of interaction between scientists and technologists.

The Individual Investigator

Suits[24], in his book on the art of research management, accords primacy to the role or the interactions of individual investigators. Several detailed biographical studies of American industrial scientists and innovators are listed in the bibliography to W. D. Lewis's brief chapter on industrial research and development in a recent historical textbook[25]. Some of these studies undoubtedly emphasize the political aspects of innovation, and in particular the role of the determination and advocacy of one man pushing an idea to fruition against opposition. Such studies may refer to events in the distant past and yet carry lessons for the present day: one example is Manuel's essay on Isaac Newton as autocrat of science[26].

The collection of case histories seems to be gathering further momentum in the United States. One interesting experiment is the Suny/Albany seminar on technological change, organized by Professor J. W. Corbett at the Suny/Albany Center for Science and the Future of Human Affairs, at the State University of New York at Albany. This seminar brings about contact between a group of students, on the campus, and active applied scientists, entrepreneurs and research administrators. Much of the teaching is through the medium of case histories, of which Corbett has accumulated a substantial collection. He hopes to publish these and also to film the seminar itself.

Again in America, a further collection of case histories has recently been prepared for the National Science Foundation[27]. These case histories break new ground in that they take into account the discoveries in pure science which, during many years, created a body of fundamental knowledge from which the innovation could spring. The histories (they are acronymically called TRACES) are set out in the form of genealogical diagrams; a distinction is made between non-mission research, mission-orientated research and development and application, and the chronology of the individual "events" in these three categories is plotted backwards from the date of first commercial production, taken as time zero. For full measure, the various events are classified into American and foreign. The chief criticism one can level at this approach is the failure to distinguish major discoveries from more trivial ones; in fact, the authors fight shy of value judgments.

Allen's[19] study of the discovery of terylene and polythene incorporates an attempt to trace the web of antecedents, similar to the TRACES study. Indeed, Allen goes further in one valuable respect: he traces the role of the moves and personal contacts and influences of the scientists principally involved.

Current Developments

A. B. Robertson and his colleagues at the Science Policy Research Unit at the University of Sussex are engaged in a systematic attempt to collect case histories of technological innovation in several British firms; a special feature of this project is that failures are receiving as much attention as successes, in the conviction that only by this means can useful conclusions be drawn for the future. The inclusion of failures in future collections of case histories is particularly to be encouraged, for the sake of the lessons to be derived from them. McLaughlin's[28] study of the demise of the steam-driven motor car is a rare example, even though it may eventually prove not to have been a failure after all.

Clearly case histories of technological innovation can fruitfully be presented and analysed in several quite different ways; primacy can be accorded to economic arguments, to questions of laboratory management, to personal characteristics and interaction, or to long term sequences of scientific and engineering events. It is necessary now for more studies to build on the few precedents that are available, and this can only be achieved with the willing cooperation—indeed, the initiative where possible—of industrial firms. It is asking much of a firm to spell out in detail its own technological workings, even well after the event, and it is asking even more for it to analyse its own failures. Nevertheless, the whole of science-based industry can only benefit from the results of such studies.

A sceptic may well object that the anatomy of an innovation, however conscientiously dissected, will offer little insight into the structure of another innovation, the bones and nerves of which are differently arranged. There is force in this objection, but the force diminishes when case histories are regarded as effectively primary source material, from which science humanists of the future will create an illuminating synthesis.

I thank P. M. Knowlson, A. B. Robertson, Professor C. Freeman, Mrs J. K. Fuller and Professor J. W. Corbett for advice, information and comments.

[1] Conant, J. B., and Nash, L. K., *Harvard Case Histories in Experimental Science* (Harvard University Press, Cambridge, 1957).

[2] Price, W. J., and Bass, L. W., *Science*, **164**, 802 (1969).

[3] National Science Foundation, *Technology Transfer and Innovation* (NSF 67–5, Washington, 1967).

[4] National Aeronautics and Space Administration, *Assessing Technology Transfer* (NASA SP–5067, Washington, 1966).

[5] US Department of Commerce, *Technological Innovation: Its Environment and Management* (Washington, 1967).

[6] Central Advisory Council for Science and Technology, *Technological Innovation in Britain* (HMSO, London, 1968).

[7] Shanks, M., *The Innovators* (Penguin, London, 1967).

[8] Layton, C., *European Advanced Technology—A Programme for Integration* (Allen and Unwin, London, 1969).

[9] Beer, J., *The Emergence of the German Dye Industry* (Urbana, Illinois, 1959).

[10] Freeman, C., Young, A., and Fuller, J. K., *The Plastics Industry: A Comparative Study of Research and Innovation* (National Institute Economic Review, London, No. 26, 1963).

[11] Freeman, C., Harlow, C. J. E., and Fuller, J. K., *Research and Development in Electronic Capital Goods* (National Institute Economic Review London, No. 34, 1965).

[12] Freeman, C., Robertson, A. B., Whittaker, P. J., Curnow, R. C., and Fuller, J. K., *Chemical Process Plant: Innovation and the World Market* (National Institute Economic Review, London, No. 45, 1968).

[13] Mencher, A. G., *Sci. J.*, **5**, 81 (June 1969).

[14] Blake, S. P., *Sci. J.*, **5**, 84 (June 1969).

[15] Rothschild, G. M., *Proc. Roy. Inst. Great Britain*, **41**, 454 (1967).

[16] Gowing, M., *Britain and Atomic Energy 1939–45* (Macmillan, London, 1964).

[17] Jewkes, J., Sawers, D., and Stillerman, R., *The Sources of Invention*, second ed. (Macmillan, London, 1969).

[18] Cocks, E. J., and Walters, B., *A History of the Zinc Smelting Industry in Britain* (Harrap, London, 1968).

[19] Allen, J. A., *Studies in Innovation in the Steel and Chemical Industries* (Manchester University Press, 1967).

[20] National Academy of Sciences, USA, *Applied Science and Technological Progress*, Report to US House of Representatives, Washington (Government Printing Office, Washington, 1967).

[21] Tanenbaum, M., *et al.*, *Materials Advisory Board of the National Research Council, Report* (Washington, ARPA, MAB-222-M, 1966).

[22] Sherwin, C. W., and Isenson, R. S., *First Interim Report on Project Hindsight* (Summary) (Washington, Office of the Director of Defense Research and Engineering, 1966); also *Science*, **156**, 1571 (1967).

[23] Fleischer, R. L., Walker, R. M., and Price, P. B., *Sci. Amer.*, **220** (6), 30 (1969).

[24] Suits, C. G., *Speaking of Research* (Wiley, New York, 1965).

[25] Kranzberg, M., and Pursell, jun., C. W., *Technology in Western Civilisation, Vol. 2, Technology in the Twentieth Century* (Oxford University Press, 1967).

[26] Manuel, F. E., *Daedalus*, 969 (summer 1968).

[27] Illinois Institute of Technology Research Institute (for National Science Foundation), *Technology in Retrospect and Critical Events in Science (TRACES)*, **1** (Chicago, 1968) (**2** to be published).

[28] McLaughlin, C. C., in *Explorations in Enterprise* (edit. by Aitken, H. G. J.), 259 (Harvard University Press, 1965).

Formation of the Initiation Complex using Muscle Messenger RNAs

by
STUART M. HEYWOOD
Genetics and Cell Biology Section,
Biological Science Group,
University of Connecticut,
Storrs, Connecticut

In eukaryotes, as in prokaryotes, the smaller of the two ribosomal subunits binds mRNA during the initiation of protein synthesis. The formation of the complete initiation complex, sedimenting at 75S, requires GTP, tRNA and initiation factors.

STUDIES on the initiation of protein synthesis have provided evidence that ribosomal subunits are essential for the initiation of protein synthesis in bacteria. The subunit model proposed by Nomura and his group[1-3] has been confirmed in other laboratories[4-6]. According to this model the 30S ribosomal subunit, in conjunction with initiation factors, interacts with mRNA; this complex in turn binds initiator fMet-tRNA and associates with the 50S ribosomal subunit to form the completed initiation complex. The importance of the initiation factors in the formation of this complex is well documented[7-9]. Ribosomes lacking initiation factors are incapable of translating natural mRNA, and in the absence of these proteins, neither mRNA nor initiator fMet-tRNA is bound to the ribosome[10-12]. Although direct evidence is not available, studies involving the polysome–ribosomal–subunit cycle in eukaryotes suggest that the subunit model for initiation of protein synthesis may also apply to higher organisms[13-15]. Knowledge of the role of initiation factors in eukaryotes is fragmentary and so far only suggests that factors removed from ribosomes by washing with a high concentration of salt are necessary for the *de novo* synthesis of proteins[16-18].

We have recently reported that the "factors" removed from embryonic chick muscle ribosomes by washing with 1·0 M KCl were essential for the *de novo* synthesis of the 200,000 molecular weight subunit of myosin[18]. These "factors" could not be replaced by the 0·1 M KCl wash from reticulocyte ribosomes when 26S myosin mRNA was used to direct the synthesis of myosin on reticulocyte ribosomes. These "factors" were judged to be initiation factors because of their ability to bind mRNA to ribosomes attached to nitrocellulose filters[18]. The initiation factors, in combination with ribosomes washed with 1·0 M KCl, demonstrated a specificity for binding mRNA, that is, only initiation factors from muscle ribosomes were found to bind muscle mRNA. Here are presented observations which suggest (*a*) that native 40S subunits (not 75S ribosomes) are involved with initiation of protein synthesis by binding mRNA, (*b*) that both GTP and tRNA are essential for the association of the 40S and 60S ribosomal subunits into the completed initiation complex, and (*c*) that binding of mRNA to the 40S subunit requires specific initiation factors.

Unwashed ribosomes, 1·0 M KCl washed ribosomes and initiation factors (1·0 M KCl wash of the ribosomes) were

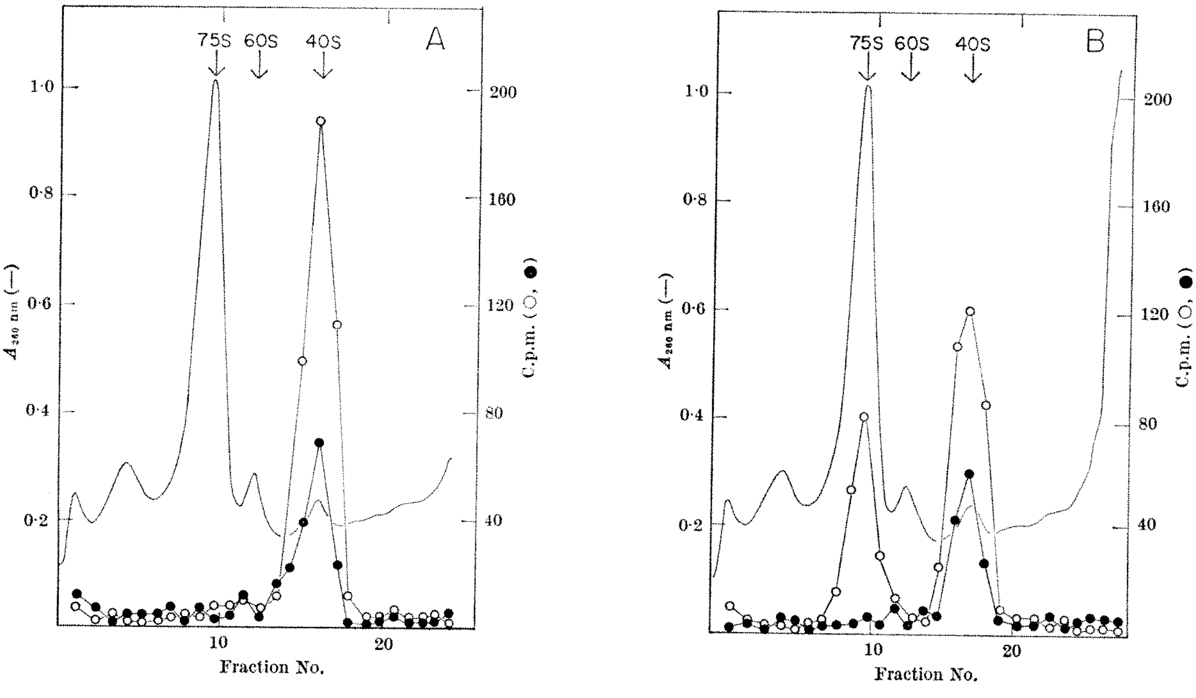


Fig. 1. Requirements for the binding of myosin 26S mRNA to unwashed muscle ribosomes. ^{32}P-mRNA (700 c.p.m.) was incubated in the conditions described in the text. *A*, GTP and tRNA were omitted (●); tRNA was omitted (○); *B*, GTP was omitted (●); complete system with 0·4 mg unwashed ribosomes, 0·25 µmole GTP, 100 µg tRNA (○).

prepared as previously described[19]. NaF treatment of the ribosomes was omitted, for it seemed to decrease the amount of native ribosomal subunits recovered. The ribosomes were stored at $-20°$ C for up to a week without loss of activity; the initiation factors were, however, less stable and were therefore prepared freshly before each experiment. Before being used, the initiation factors were dialysed against 0·15 M KCl–0·005 M $MgCl_2$–0·02 M Tris-HCl (*p*H 7·6)–0·006 M mercaptoethanol. Messenger RNA was prepared from various size classes of muscle polysomes as reported previously[19]. Fourteen day embryonic chicks were injected with 1 mCi $^{32}PO_4$ and incubated for 90 min in order to label mRNA. In this period maximum radioactivity is incorporated into 26S myosin mRNA, a minimum amount being incorporated into ribosomal RNA[19]. Unstripped tRNA was prepared from adult chicken muscle by the method of Von Ehrenstein[20]. ^{32}P-mRNA was bound to ribosomes in 0·5 ml. of 0·02 M Tris-HCl (*p*H 7·6)–0·005 M $MgCl_2$–0·15 M KCl–0·006 M mercaptoethanol containing 0·4 mg ribosomes. 0·25 µmoles GTP and 100 µg initiation factors were added individually or in combination as indicated in the legends. The reaction mixture was incubated for 10 min at 25° C, rapidly cooled, and layered on a 27 ml., 10–30 per cent sucrose gradient containing 0·02 M Tris-HCl (*p*H 7·6), 0·15 M KCl, 0·005 M $MgCl_2$, and 0·006 M mercaptoethanol. The mixture was centrifuged for 5 h at 25,000 r.p.m. in an IEC 'SB110' rotor. Twenty-eight drop fractions were collected and the absorbance was continuously recorded by a Gilford spectrophotometer. The samples were filtered through nitrocellulose membrane filters ('Millipore, HA', 0·45 µm). The filters were then washed with 3 ml. buffer and dried, and radioactivity was determined by liquid scintillation counting.

The conditions used for testing the binding of myosin mRNA to ribosomes were identical to those in which it was demonstrated that the 26S RNA associated with myosin synthesizing polysomes could direct the synthesis of myosin when added to a cell-free amino-acid incorporating system[18,19]. As shown in Fig. 1, these conditions allow for the attachment of myosin mRNA to the ribosomes. The mRNA sediments with the 40S ribosomal subunit and the binding is stimulated or stabilized by the addition of GTP to the reaction mixture (Fig. 1*A*). Addition of tRNA did not result in an increased binding of mRNA to the 40S subunit (Fig. 1*B*), and neither GTP nor tRNA when added singly resulted in the formation of the initiation complex as judged by the absence of ^{32}P-mRNA sedimenting with the 75S ribosomes. But when both GTP and tRNA were present in the reaction mixture a significant amount of ^{32}P-mRNA sedimented with the 75S ribosomes. The results suggest that initiation of protein synthesis in eukaryotes involves the binding of mRNA to the 40S ribosomal subunit followed by a GTP and tRNA dependent association of the 40S and 60S subunits to form the initiation complex. If, in addition, an 'S150' muscle supernatant fraction and an energy source are added to the reaction mixture, radioactivity is also observed in the polysome region of the sucrose gradient (unpublished results).

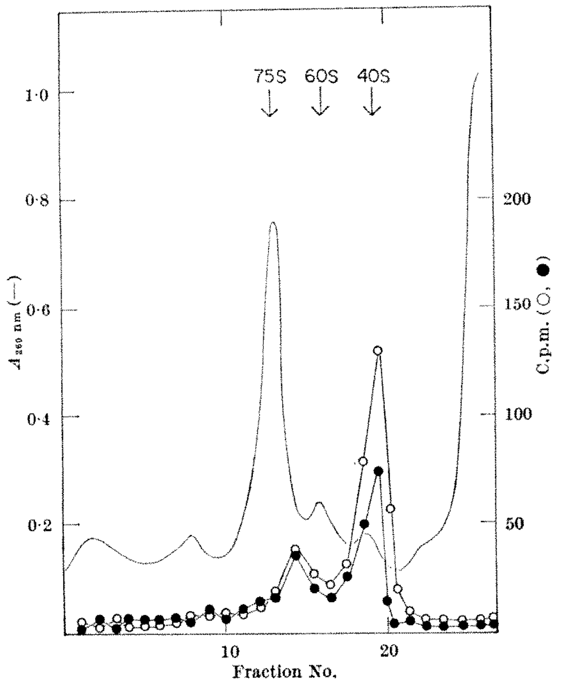


Fig. 2. Ribosomal RNA (28S) binding to unwashed muscle ribosomes. ^{32}P-ribosomal RNA (1,200 c.p.m.) was incubated as described in the text and Fig. 1. Complete system (○); GTP and tRNA omitted (●).

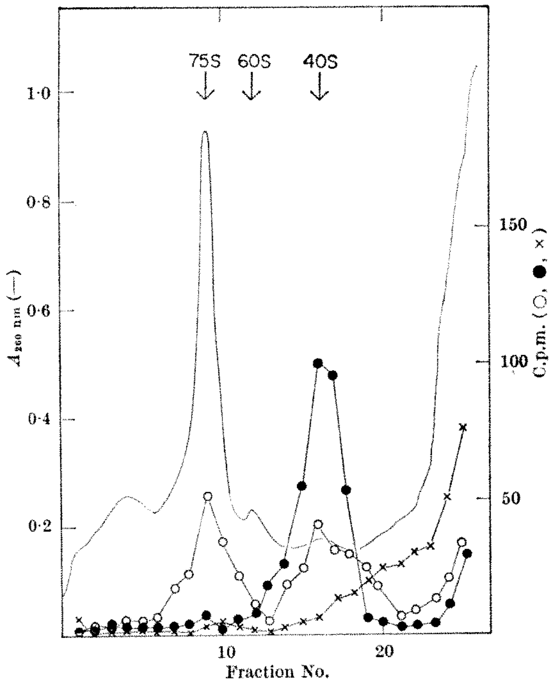


Fig. 3. Initiation factor dependent binding of 10–15S muscle ^{32}P-mRNA to 1 M KCl washed muscle ribosomes. RNA was prepared from small muscle polysomes[19]. ^{32}P-mRNA (500 c.p.m.) was incubated in the conditions described in the text. Complete system with 0·4 mg of 1 M KCl washed ribosomes, GTP, tRNA and 100 µg of initiation factors (○); initiation factors omitted (×); tRNA omitted (●).

Although a minimum of radioactivity is incorporated into the 28S ribosomal RNA of the myosin synthesizing polysomes during the labelling period, it is necessary to ascertain that the binding observed is characteristic of mRNA and not a result of 28S ribosomal RNA contamination. 28S ribosomal RNA was therefore obtained from smaller polysomes and tested for its ability to bind ribosomes and ribosomal subunits. 28S ribosomal RNA sediments with the 40S ribosomal subunit (Fig. 2), and a smaller, faster sedimenting peak, 65S, is routinely observed. The addition of GTP and tRNA results in an increase of radioactivity sedimenting with the 40S ribosomal subunit (GTP alone is sufficient to cause this increase), but no radioactivity is found to sediment with the 75S ribosomes. 18S ribosomal RNA was found to behave in a similar manner except that the 65S peak of radioactivity was absent. The reasons why ribosomal RNA should bind to the 40S subunit and why this is enhanced in the presence of GTP are not clear. Unlike mRNA, ribosomal RNA binds the 40S ribosomal subunit even in the absence of initiation factors. It is clear, however, that ribosomal RNA does not form the initiation complex (sediment with the 75S ribosomes in the presence of GTP and tRNA) as does myosin mRNA. 10–15S

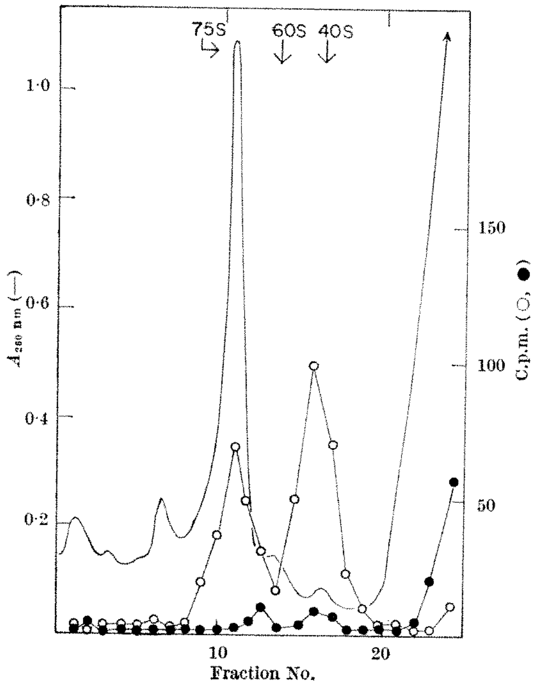


Fig. 4. Muscle initiation factor dependent binding of 26S myosin mRNA to reticulocyte ribosomes washed with 1 M KCl[18]. ^{32}P-mRNA (700 c.p.m.) was incubated in the complete system as described in the text and Fig. 3. 100 μg of reticulocyte initiation factors (●), 100 μg of muscle initiation factors (○).

RNA from the smaller muscle polysomes[19], containing actin and tropomyosin mRNA (unpublished results), binds ribosomes in a similar manner as 26S myosin mRNA. The GTP, tRNA dependent binding reaction may therefore be used as a test for mRNA.

If ribosomes washed with 1·0 M KCl are used in the binding experiments, no ^{32}P-mRNA is found to sediment with the ribosomal subunits (Fig. 3). If the initiation factors—removed by the 1·0 M KCl—are added back to the ribosomes, the mRNA again binds the 40S ribosomal subunit. Addition of tRNA, in the presence of GTP, results in the formation of the initiation complex sedimenting at 75S. The initiation factors are therefore essential for binding mRNA to the 40S ribosomal subunit and the subsequent formation of the initiation complex.

It has been reported that initiation factors, responsible for binding 26S myosin mRNA to ribosomes, are necessary for the *de novo* synthesis of myosin[18]. When myosin mRNA was translated on reticulocyte ribosomes, it was essential to add the initiation factors washed from muscle ribosomes to the washed reticulocyte ribosomes. Myosin mRNA does not bind reticulocyte ribosomes unless the initiation factors from muscle ribosomes are present (Fig. 4). These results extend our previous observations[18] which suggested that the messenger binding factors recognize only those mRNAs derived from the same cell type as the binding factors.

We suggest that the mechanism for the initiation of protein synthesis involving ribosomal subunits is similar in both prokaryotes and eukaryotes. Further, the requirement of GTP, tRNA and initiation factors for the formation of the initiation complex is the same for both prokaryotes and eukaryotes. The identity of the initiator tRNA(s) involved in the initiation of protein synthesis in higher organisms and the precise role of the initiation factors in the formation of the complex are still not clear.

I thank Mrs Janice Jasnos for assistance. This work was supported by a grant from the US National Institute of Child Health and Human Development.

Received December 1, 1969.

[1] Nomura, M., and Lowry, C. V., *Proc. US Nat. Acad. Sci.*, **58**, 946 (1967).
[2] Nomura, M., Lowry, C. V., and Guthrie, C., *Proc. US Nat. Acad. Sci.*, **58**, 1487 (1967).
[3] Guthrie, C., and Nomura, M., *Nature*, **219**, 232 (1968).
[4] Grubman, M. J., and Nakada, D., *Nature*, **223**, 1242 (1969).
[5] Kaempfer, R., Meselson, M., and Raskas, H. J., *J. Mol. Biol.*, **31**, 277 (1968).
[6] Schlessinger, D., Mangiarotti, G., and Apirion, D., *Proc. US Nat. Acad. Sci.*, **58**, 1782 (1967).
[7] Brown, J. C., and Doty, P., *Biochem. Biophys. Res. Commun.*, **30**, 284 (1968).
[8] Salas, M., Hille, M. B., Last, J. A., Wahba, A., and Ochoa, S., *Proc. US Nat. Acad. Sci.*, **57**, 357 (1967).
[9] Brawerman, G., Revel, M., Salser, W., and Gros, F., *Nature*, **223**, 957 (1969).
[10] Anderson, J. S., Dahlberg, J. E., Bretscher, M., Revel, M., and Clark, B., *Nature*, **216**, 1072 (1967).
[11] Revel, M., Herzberg, M., Becarevic, A., and Gross, F., *J. Mol. Biol.*, **33**, 231 (1968).
[12] Greenshpan, H., and Revel, M., *Nature*, **224**, 331 (1969).
[13] Hogan, B. L., and Korner, A., *Biochim. Biophys. Acta*, **169**, 139 (1968).
[14] Kabat, D., and Rich, A., *Biochemistry*, **8**, 3742 (1969).
[15] Joklik, W. K., and Becker, Y., *J. Mol. Biol.*, **13**, 496 (1965).
[16] Miller, R. L., and Schweet, R., *Arch. Biochem. Biophys.*, **125**, 632 (1968).
[17] Godin, C., Kruh, J., and Dreyfus, J. C., *Biochim. Biophys. Acta*, **182**, 175 (1969).
[18] Heywood, S. M., *Cold Spring Harbor Symp. Quant. Biol.* (in the press).
[19] Heywood, S. M., and Nwagwu, M., *Biochemistry*, **8**, 3839 (1969).
[20] Von Ehrenstein, G., *Methods in Enzymology*, **12**, 588 (1968).

Antigenic Properties of a Homogeneous Tobacco Mosaic Virus–Hapten Conjugate

by

L. I. SLOBIN
Division of Biological Sciences,
Cornell University,
Ithaca, NY 14850

Anti-hapten antibodies may only be induced if the hapten modifies the conformation of the protein carrier.

Although much is known about the structural factors necessary to produce an effective haptenic determinant[1,2], there has been little inquiry into the reason(s) why antibodies can be successfully produced against haptens in the first place. It has generally been assumed that the introduction into an animal of a "foreign" chemical grouping is sufficient to elicit an immune response. Thus the problem has been treated as part of the larger question of why "foreign" macromolecules are immunogenic. By definition, however, haptens are not immunogenic by themselves. They are completely dependent on the structure of the carrier protein in order to produce their effect.

It is our thesis that the immunogenic character of haptens, when attached to protein carriers, is controlled by the degree to which they alter the native conformation of the carrier molecule. As a corollary, the introduction of haptens which have little or no effect on the secondary or tertiary structure of a protein carrier is likely to produce little or no specific immune response.

To understand the experimental basis for this proposal it is necessary to consider the part that protein conformation plays in determining antigenic specificity. The dependence of antibody reactivity on the structure of antigens has been the subject of several recent reviews[3-5]. It seems now generally to be accepted that the conformational integrity of native proteins is essential for the maintenance of their immunological specificity. Three types of experiments lend strong support to this view. (1) It has long been known that antisera to native and denatured proteins exhibit marked differences in immunological reactivity[1]. Two recent examples that clearly illustrate this finding are afforded by work on the antigenic properties of chemically modified ribonuclease and myoglobin[6-8]. (2) It has not in general been possible to inhibit quantitatively a homologous antigen–antibody reaction by using small peptide fragments (of the size of a hypothetical antibody-binding site), derived from antigen by proteolytic digestion[9-11]. These findings include studies on antibodies specific for tobacco mosaic virus (TMV), where Anderer and co-workers[10,12] found that isolated amino-acid sequences from TMV protein only partially inhibited the homologous precipitin reaction. The lack of additivity of the inhibition values given by individual peptides further suggests that specific anti-TMV antibodies are not directed against isolated amino-acid sequences. (3) Using synthetic polypeptides it has been possible to show that the specificity of antibodies produced against L-Tyr–L-Ala–L-Glu is controlled by the conformation of this tripeptide determinant[13]. Studies with antibodies to bradykinin have demonstrated that alterations in residues which had an obligatory effect on conformation, such as changes in glycine or proline, had a profound effect on the binding of homologous antibody[14]. In addition, the results with bradykinin suggest that the conformation of the entire bradykinin peptide was antigenically important.

Consequences of Hapten Attachment

By contrast with antibodies against proteins and polypeptides, antibodies with antihapten specificity are usually elicited by chemical modification of pre-existing native structures. The attachment of the hapten to a macromolecule usually entails significant modification of the charge balance of the protein (for example, by substitution at lysine residues) or alteration of other residues (such as tyrosine or histidine) that are likely to play a critical part in protein conformation. Although little direct information is available on this subject, it seems reasonable to assume that haptenation generally results in conformational change in carrier protein, at least in the region of hapten attachment.

An apparent consequence of the structural changes induced by reaction with hapten is that anti-hapten specificity shows little, if any, dependence on protein conformation. Although it has been established that amino-acid residues adjoining a haptenic group can have a detectable influence on binding reactions[15], this is essentially a second order effect, corresponding to only a small percentage of the total binding energy. In most cases, the conjugate of the hapten with an appropriate side chain can adequately account for the total ΔG of a specific anti-hapten antibody reaction.

Several other observations also lead us to assign minor importance to the role of the conformation of carrier structure in the control of an anti-hapten antibody response. It is possible to obtain an anamnestic anti-hapten response by challenging sensitized animals with hapten–heterologous protein conjugates[16]. Similar carrier-independent effects can be obtained in delayed hypersensitivity reactions[17,18].

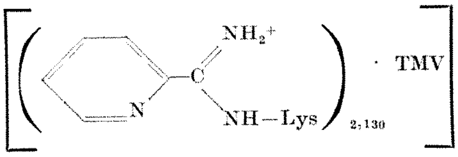
By contrast with protein antigenic determinants, the immunological recognition of haptens seems to involve conformation-independent loci on the surface of the carrier protein. I suggest that addition of a hapten at a single amino-acid residue within a potentially antigenic locus, which did little to alter the overall conformation of that site, would have little immunological consequence. Only by significantly changing the conformation of a protein segment—for example, in going from α helix to a random coil at an antigenic site—or by shifting the contact residues in the tertiary folding of the polypeptide chain, is a new immunological specificity likely to arise. Thus it is not the "foreignness" of a hapten *per se* but rather its effect on protein conformation which determines its ultimate fate as an antigenic determinant. If this proposal has any validity it should be possible to prepare hapten–protein conjugates in which a potentially powerful haptenic determinant is either totally masked or only weakly immunogenic as a result of negligible effect on protein conformation. Support for this idea comes from an examination of the antigenic properties of a homogeneous TMV–hapten conjugate.

The Picolinimidyl–TMV System

It has been shown recently[19,20] that lysine-68 (each TMV subunit contains one additional lysine residue at position 53) reacts preferentially with a variety of nucleophilic reagents, including organic acid anhydrides and imido esters. In particular, methyl picolinimidate has been shown to react exclusively with all 2,130 subunits of the TMV protein coat to form a mono-substituted derivative of each subunit, without apparently disturbing the structural integrity of the virus. Such a derivative presumably places 2,130 potential haptenic groups on or near the surface of the virion, each modified lysine being equivalently related to its neighbouring amino-acid residues by the fixed tertiary structure of the subunits in the virus superhelix[21]. The native virus structure does not seem to be disrupted by this modification as judged by electron microscopy and ultracentrifugation[20].

It is known that amino-acid substitutions adjacent to lysine-68 (at residues 65 and 66) alter the immunological specificity of the intact virus[22], indicating that alterations in this region of the protein subunit can have detectable immunological consequences.

Intact TMV (10 mg/ml.) was treated with methyl picolinimidate[23] (0·1 M) in 0·1 M N-ethylmorpholine–acetic acid buffer (*p*H 8·2) at 22° for 12–14 h[20]. The modified virus



was isolated by several alternate low (10,000*g* for 10 min) and high (50,000*g* for 1 h) speed centrifugations. The final pellet was suspended in distilled water to give a 1 per cent solution and stored at 4° C.

The extent of reaction was determined by the trinitrobenzenesulphonic acid (TNBS) assay for free amino groups[24] using the purified virus coat protein (TMVP)[25]. An equally reliable method for measuring the number of modified lysines, which also gave useful information on the specificity of the amidination reaction, was to subject the protein coat of the modified virus to amino-acid analysis. It was found that in the standard conditions of acid hydrolysis (6 M HCl at 110° C) the half-life for the conversion of an ε-(N-picolinimidyl)lysyl residue to free lysine was greater than 48 h. Extrapolation of lysine values found after various hydrolysis times (24 and 72 h) to zero time therefore permitted a reliable estimate of unreacted lysine in TMVP. Both TNBS determinations and amino-acid analyses gave values of 0·9 ± 0·1 for the number of substituted lysine residues per 17,500 daltons of coat protein. Amino-acid analysis revealed no other alteration in the composition of the protein.

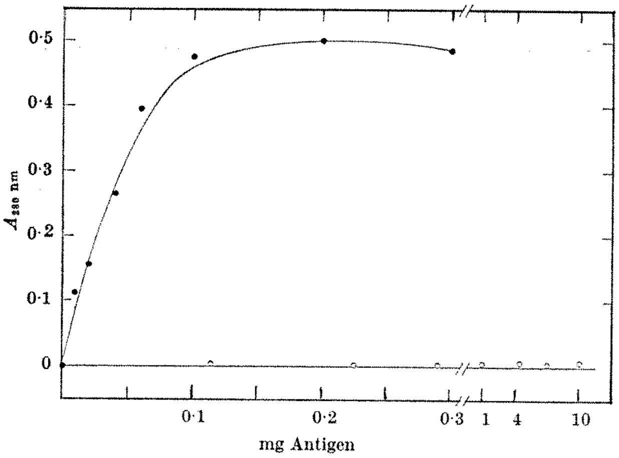


Fig. 1. Precipitin curves of anti-(P-BSA) reacted with P-γG (●—●) and P-TMV or P-TMVP (○—○). Ascitic fluid (0·4 ml.) containing specific antibodies was incubated with varying amounts of test antigen (total volume 0·9 ml.) for 1 h at 37° C followed by overnight incubation (16 h) at 4° C. The precipitates were recovered by low speed centrifugation (2,500*g* for 30 min), washed three times with 1 ml. portions of phosphate-saline buffer, and dissolved in 1 ml. of 0·1 M NaOH. The absorbance was read at 280 nm using a Gilford model 240 spectrophotometer.

Picolinimidated derivatives of bovine serum albumin (P-BSA) and equine γ-globulins (P-γG), containing 11 (out of 57) and 62 (out of 78) moles of substituted lysines per mole of protein, respectively, were prepared essentially as described for P-TMV, except that the reaction time with methyl picolinimidate was reduced to 2 h. After dialysis against phosphate–saline buffer (0·15 M NaCl, 0·01 M sodium phosphate, *p*H 7·4); the protein derivatives were stored as frozen solutions. ε-(N-Picolinimidyl)aminocaproic acid hydrochloride (P-ACA) was prepared by reacting ε-aminocaproic acid with a two-fold molar excess of methyl picolinimidate in conditions identical to those described for TMV. The reaction mixture was acidified with concentrated HCl (to *p*H 1) and taken to dryness on a rotary evaporator. The product was crystallized from ethanol–ether (m.p. 171–173) and was characterized by an absorption maximum at 262 nm ($\varepsilon_M = 5{\cdot}3 \times 10^3\ \mathrm{M}^{-1}\ \mathrm{cm}^{-1}$) (ref. 26).

Two six year old 500 kg geldings underwent a series of intramuscular injections of P-TMV in distilled water (the immunization and bleeding protocol will be published elsewhere), each animal receiving a total of 450 mg of virus derivative in distilled water over the course of 8 months. During this time, although large amounts (titres greater than 1 mg/ml.) of anti-TMV antibodies were produced in both animals, I was unable to detect any antibodies with specificity toward the picolinimidyl group by the following criteria. (1) None of the test sera precipitated either P-BSA or P-γG. (2) An immunoadsorbent was prepared by coupling P-ACA to carboxymethylcellulose using N-3-dimethylaminopropyl-N′-ethylcarbodiimide as the coupling agent. Antisera from various bleedings were passed over columns of immunoadsorbent, and after washing with phosphate–saline buffer, the column was developed with the same buffer containing 3 M KSCM (ref. 27). By this technique no picolinimidyl-specific antibody could be detected (< 20 μg antibody/ml. antiserum). (3) An attempt was made to elute anti-picolinimidyl antibodies by the techniques of hapten elution[28]. Incubation of a washed precipitate of P-TMV, and equine anti-(P-TMV) antibodies (containing more than 1 mg anti-TMV γ-globulin) with 0·1 M P-ACA in phosphate–saline buffer at 37° C for 1 h, failed to elute any globulin (< 20 μg antibody protein in 1 ml. of hapten–buffer solution).

Several trivial explanations of the failure to elicit anti-picolinimidyl antibodies are possible. The two animals may fortuitously have been genetic non-responders[29]. Alternatively, for some unknown reason, the picolinimidyl group might be ineffective as a haptenic determinant. (I know of no attempt to prepare antibodies to picolinimidated proteins.) To resolve these questions, groups of mice (WRAIR albino mice, 2–4 months old) were immunized with P-TMV or P-BSA. The animals received a total of three intraperitoneal injections of antigen, spaced one week apart (1 mg per injection), in complete Freund's adjuvant. Five days after the last injection the mice were inoculated with Ehrlich ascites tumour cells[30]; the tumours were tapped and ascitic fluid was removed 10 days later.

As was the case with equine antisera, it was found that mice immunized with P-TMV, although they produced a vigorous antibody response to the virus, did not make any antibodies with anti-picolinimidyl specificity. As may be seen in Fig. 1, however, the mice responded to treatment with P-BSA by producing substantial quantities (titres approached 1 mg/ml.) of anti-hapten antibody. Somewhat surprisingly, anti-picolinimidyl antibodies failed to precipitate either P-TMV or P-TMVP. To test whether antibody was capable of combining with the picolinimidyl determinant on TMV, increasing amounts of either P-TMV (or P-TMVP) were incubated with a fixed amount of antibody and P-γG. The precipitates that formed were treated as described in the legend to Fig. 1, and the results (Table 1) are based on the percentage of the control (minus TMV derivative) $A_{280\,\mathrm{nm}}$ found in the presence of P-TMV.

Table 1. INHIBITION OF PRECIPITATION OF ANTI-PICOLINIMIDYL ANTIBODIES BY P-TMV AND P-TMVP*

Exp.	Inhibitor	Inhibitor (mg)	(Inhibitor)/ P-γG†	Per cent inhibition
1	P-TMV	1·2	2·8	29
2	P-TMV	6·0	14	54
3	P-TMV protein	1	2·8	33
4	TMV	3·5	—	0

* Each tube contained 0·4 ml. of ascitic fluid and the appropriate amount of inhibitor in a total volume of 0·9 ml. Because of the tendency of P-TMV protein to precipitate when incubated at 37° C, the tubes were maintained at 4° C for 44 h before analysis. Per cent inhibition values represent the average of duplicate determinations.

† The ratio of the concentration of picolinimidyl groups present on inhibitor to that on P-γG, the precipitating antigen.

Clearly P-TMV derivatives behave as simple univalent haptens, capable of inhibiting a homologous precipitin reaction, but unable to cause precipitation directly. As a further check on the specificity of the inhibition it was found that TMV by itself produces no effect. Thus it seems as if the picolinimidyl group is exposed on the surface of the virus, allowing it to interact with an antibody combining site.

In the course of this investigation, it was discovered that Malkiel[31], working with a chemically uncharacterized virus–azoarsanilic acid conjugate, was also unable to detect specific haptenic antibody when rabbits were immunized with modified TMV. The modified virus did, however, react (precipitate) with an antiserum to a heterologous azoarsanilic acid derivative. Furthermore, no appreciable difference was seen in the serological reaction between native tobacco mosaic virus and various chemically altered virus derivatives when tested with antiserum to the native virus.

Modification with Retention of Conformation

The failure to elicit anti-hapten antibody by chemical modification of TMV can readily be interpreted by the idea that such modifications produced no significant alteration of either the tertiary structure of the protein subunits or the quaternary structure of the intact virion. The amidination reaction, which is specific for lysine residues, does not alter the net charge of a protein at neutral *p*H. Extensively amidinated proteins have been found to maintain their biological function with only slight alteration of their physical properties[32,33].

An alternative explanation for the failure to produce anti-picolinimidyl antibodies with a P-TMV conjugate is that because of the homogeneity of hapten attachment only an insignificant number of specific antigen sensitive cells are capable of responding to the picolinimidyl determinant. The chemical homogeneity of a hapten conjugate, however, has not so far been found necessarily to eliminate immunological responsiveness, as witnessed by the success in eliciting hapten-specific antibodies by immunizing with α-DNP-oligolysines[34]. Alternatively, the *in vivo* concentration of haptenic determinant may have been too small to elicit a response. This argument would appear to be vitiated by the positive results obtained with P-BSA, which contained only about twice as many picolinimidyl groups as P-TMV on a weight basis.

Although the details of the mechanism(s) for the recognition of antigenic determinants are still unknown, it is possible that haptenic systems are "recognized" only after being metabolized by macrophages[35], because of an increased susceptibility of the "denatured" protein conjugate to proteolytic digestion[36]. This model would then contrast with native protein systems where the conformational requirements for the maintenance of antigenic structure preclude extensive protein hydrolysis. Whatever differences may exist in the recognition mechanism, it should be possible to examine the validity of my hypothesis by observing what effects, if any, denaturation of "native" conjugates, such as P-TMV, has on the production of anti-hapten antibodies. Studies on the antigenicity of chemically altered immunogens produced by using organic modifications that have only slight effect on protein conformation, such as amidination, should also provide further tests.

This work was supported by a grant from the US Public Health Service. I thank Professor F. Ross for providing infected tobacco leaves, Drs D. Stark and D. Erickson for help with the immunization and bleeding of horses, Dr J. M. Fessenden-Raden for providing WRAIR mice and Miss B. Greenberg for aiding in the immunization of mice. The assistance of Mr D. Hawley is also acknowledged.

Received August 5; revised November 17, 1969.

[1] Landsteiner, K., *The Specificity of Serological Reactions* (Harvard Univ., Cambridge, 1945).

[2] Haurowitz, F., *Immunochemistry and the Biosynthesis of Antibodies* (John Wiley, London, 1968).

[3] Kabat, E. A., *J. Immunol.*, **97**, 1 (1966).

[4] Sela, M., *Adv. Immunol.*, **5**, 29 (1966).

[5] Goodman, J. W., *Immunochemistry*, **6**, 139 (1969).

[6] Brown, R. K., *Ann. NY Acad. Sci.*, **81**, 524 (1959).

[7] Mills, J. A., and Haber, E., *J. Immunol.*, **91**, 536 (1963).

[8] Atassi, M. Z., and Caruso, D. R., *Biochemistry*, **7**, 699 (1968).

[9] Crumpton, M. J., and Wilkinson, J. M., *Biochem. J.*, **94**, 545 (1965).

[10] Anderer, F. A., and Handshuh, D., *Z. Naturforsch.*, **18**b, 1015 (1963).

[11] Kraus, L. M., *J. Immunol.*, **99**, 894 (1967).

[12] Anderer, F. A., *Z. Naturforsch.*, **18**b, 1010 (1963).

[13] Sela, M., Schechter, B., Schechter, I., and Borek, F., *Cold Spring Harbor Symp. Quant. Biol.*, **32**, 537 (1967).

[14] Spragg, J., Schroder, E., Stewart, M. J., Austen, K. F., and Haber, E., *Biochemistry*, **6**, 3933 (1967).

[15] Parker, C. W., Gott, S. M., and Johnson, M. C., *Biochemistry*, **5**, 2314 (1966).

[16] Rittenberg, M. B., and Campbell, D. H., *J. Exp. Med.*, **127**, 717 (1968).

[17] Leskowitz, S., *J. Exp. Med.*, **117**, 909 (1963).

[18] Leskowitz, S., Jones, V. E., and Zak, S. J., *J. Exp. Med.*, **123**, 229 (1966).

[19] Fraenkel-Conrat, H., and Colloms, M., *Biochemistry*, **6**, 2740 (1967).

[20] Perham, R. N., and Richards, F. M., *J. Mol. Biol.*, **33**, 795 (1968).

[21] Caspar, D. L. D., *Adv. Prot. Chem.*, **18**, 37 (1963).

[22] Van Regenmortel, M. H. V., *Virology*, **31**, 467 (1967).

[23] Schaefer, F. C., and Peters, G. A., *J. Org. Chem.*, **26**, 412 (1961).

[24] Habeeb, A. F. S. A., *Anal. Biochem.*, **14**, 328 (1966).

[25] Fraenkel-Conrat, H., *Virology*, **4**, 1 (1957).

[26] Benisek, W. F., and Richards, F. M., *J. Biol. Chem.*, **243**, 4267 (1968).

[27] Dandliker, W. B., and De Saussure, V. A., *Immunochemistry*, **5**, 357 (1968).

[28] Farah, F. S., Kern, M., and Eisen, H. N., *J. Exp. Med.*, **112**, 1195 (1960).

[29] Benacerraf, B., Green, I., and Paul, W. E., *Cold Spring Harbor Symp. Quant. Biol.*, **32**, 569 (1967).

[30] Hiramoto, R., Bernecky, J., and Jurand, J., *Proc. Soc. Exp. Biol. and Med.*, **110**, 895 (1962).

[31] Malkiel, S., *J. Immunol.*, 533 (1952).

[32] Wofsy, L., and Singer, S. J., *Biochemistry*, **2**, 104 (1963).

[33] Ludwig, M. L., and Hunter, M. J., *Methods in Enzymology*, **11**, 595 (1967).

[34] Schlossman, S. F., Levine, H., and Yaron, A., *Biochemistry*, **7**, 1 (1968).

[35] Fishman, M., and Adler, F. L., *Cold Spring Harbor Symp. Quant. Biol.*, **32**, 343 (1967).

[36] Ruply, J. A., *Methods in Enzymology*, **11**, 905 (1967).

Effects of Black Widow Spider Venom on the Frog Neuromuscular Junction

Black widow spider venom causes exhaustion of miniature end-plate activity and depletes the nerve terminal of vesicles.

Effects on End-plate Potential, Miniature End-plate Potential and Nerve Terminal Spike

BLACK widow spider venom (BWSV) renders the stretch receptor of the crayfish inexcitable[1] by depolarizing the cell body (unpublished results of S. Obara and A. M.). The venom seems to act on the nerve cells of the sixth abdominal ganglion of the cockroach in a similar manner[2]. Early studies with the sciatic nerve–sartorius muscle preparation of the frog and toad[3,4] indicated that BWSV had little effect on either the nerve, the muscle or the neuromuscular junction. But Cantore[5] has found evidence of neuromuscular blockade in the rat diaphragm. Moreover, one of us (A. M.) recently noted that occasional fibrillations could be seen in the muscle about 30 min after BWSV had been applied to a frog sciatic–sartorius preparation, and that neuromuscular transmission was completely blocked 2 h later. These observations led us to study the effects of BWSV on single neuromuscular

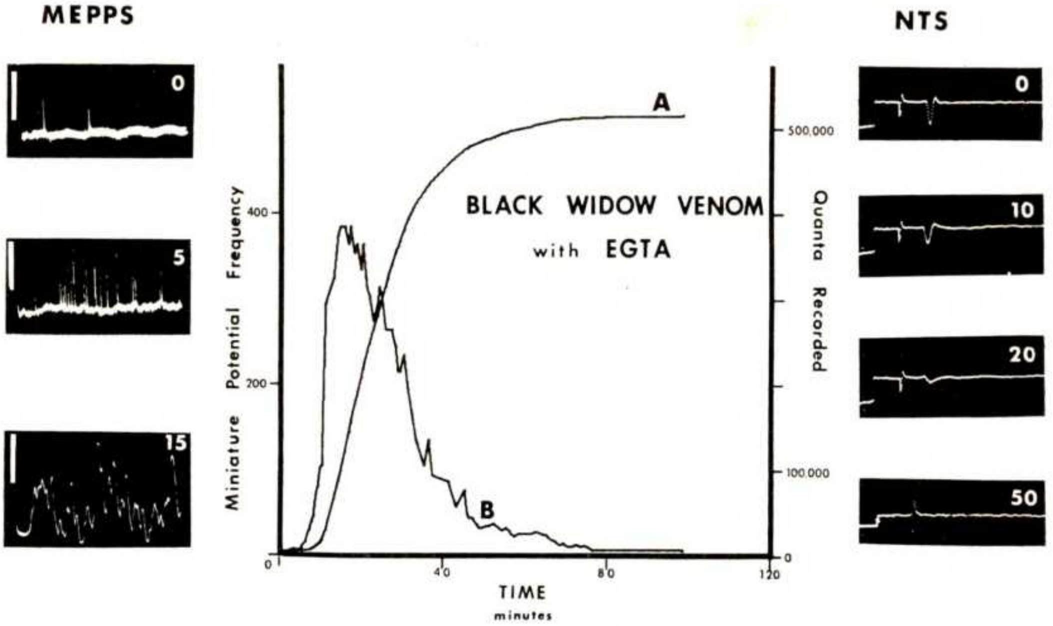


Fig. 1. Effect of BWSV on MEPP frequency and on the NTS in a calcium free solution with EGTA. Numbers in the upper right corner of the left and right columns of pictures are times in minutes after addition of 0·1 ml. of BWSV solution to the muscle chamber. Pictures on the left are intracellularly recorded MEPPs. The calibration bar represents 500 μV. Durations of sweeps from top to bottom are 1 s, 1 s, and 0·1 s. Curve *B* in the central figure is the MEPP frequency per second versus time after addition of BWSV, while curve *A* represents the total number of MEPPs or quanta recorded. Pictures at the right from a different experiment are computed average ($n=20$) nerve terminal spikes. The calibration pulse at the left of each record represents 100 μV and 1·7 ms.

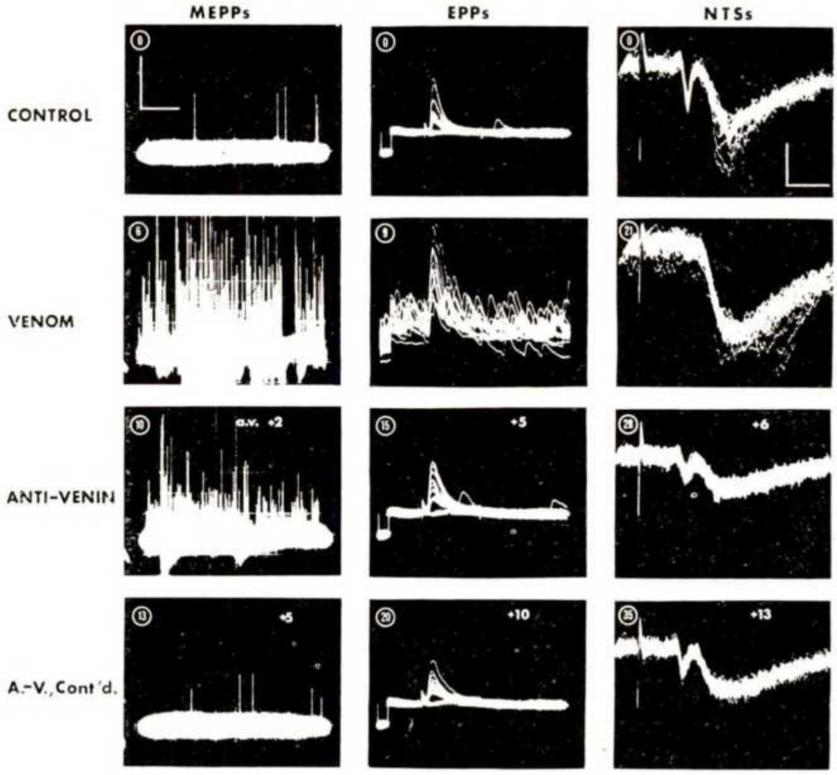


Fig. 2. Reversibility of effects of BWSV on MEPP frequency, EPP amplitude and NTS amplitude produced by addition of antivenin. Circled numbers in the upper left-hand corner of each picture are times in minutes after addition of BWSV. Numbers in the middle of each picture in the bottom two rows are times after addition of antivenin. Intracellularly recorded MEPPs in the left column and EPPs in the middle column are from the same experiment, while the externally recorded NTSs and EPPs (mainly nonspecific) are from a separate experiment. The left column shows single sweeps. Calibration is 500 μV and 2 s. Pictures in the right two columns are composed of 15 traces each. The calibration pulse for the EPPs is 1 mV and 5 ms. Calibration for the NTSs is 100 μV and 2 ms.

junctions of the frog sartorius, using methods of intracellular[6] and extracellular[7] recording.

A sartorius muscle, with its nerve intact, was dissected from *Rana pipiens* and mounted in a 'Lucite' chamber at 20°–25° C. Muscle twitch evoked by nerve stimulation was blocked by bathing the preparation in a solution containing 0·5 mM $CaCl_2$, 4 mM $MgCl_2$, 2·5 mM KCl, 110 mM NaCl, and 5 mM Tris-buffer (*p*H 7·4). Intracellular records of end-plate potentials (EPP) and miniature end-plate potentials (MEPP) were obtained by impaling the end-plate region of surface muscle fibres with glass micropipettes (4–15 MΩ) filled with 3 M KCl. Extracellular records of the nerve terminal spike (NTS), EPPs, and MEPPs were made with micropipettes (0·5–1·2 MΩ) filled with 4 M NaCl. A LINC-8 digital computer was used to determine the mean amplitude and quantum content of the intracellularly recorded EPP and the amplitude of the NTS. Amplitude and frequency of occurrence of MEPPs were monitored with the computer throughout each experiment. If MEPP frequency was low (< 100 s^{-1}), individual MEPPs could be readily discriminated, and the computer was used for continuous recording of the mean MEPP frequency (determined from averages over successive one second intervals). When the MEPP frequency was high (100–1,000 s^{-1}) samples of 0·1 s duration were taken and displayed by the LINC-8 once every 5 s. The responses occurring in each sample were counted manually to estimate the mean MEPP frequency over that 5 s interval. Venom was prepared by dissolving the lyophilized extracts (supplied by N. Toschi, A. Grasso and S. Bettini, Istituto Superiore di Sanità, Rome) of eight poison glands from four spiders (*Latrodetus mactans tridecimguttatus*) in 1·0 ml. of distilled water, and this solution was stored at 3° C. Dissolved venom remained active for at least three weeks. In most of our experiments, 0·1 ml. of this venom solution was added to 5·0 ml. of Ringer's solution in the muscle chamber.

After addition of BWSV, and a lag of several minutes, the MEPP frequency rose during the next 5–10 min from control values of 0·5 to 1·5 s^{-1} to peak values of 300–1,000 s^{-1} (Fig. 1). After reaching a peak, MEPP frequency declined to low levels (less than 1 s^{-1}) along a roughly exponential time course with a time constant of about 5 min. We estimated that about 4×10^5 MEPPs occurred after application of BWSV. (The mean of sixteen experiments was $4{\cdot}6 \times 10^5$ MEPP, *S.D.* $= 2{\cdot}8 \times 10^5$.) As the MEPP frequency increased, the amplitude of the EPP first increased several-fold and then decreased to zero concomitant with the attainment of the peak MEPP frequency. The NTS disappeared at roughly the same time, presumably because the nerve terminal was depolarized. MEPP amplitude remained constant throughout most experiments. Occasionally, MEPP amplitude declined by as much as 50 per cent after several hours. There was no significant change in membrane potential of the muscle fibre after application of BWSV. We infer from these observations that the venom displayed little postsynaptic action.

MEPP frequency may be increased by depolarizing the nerve terminal either with electric current or by increasing the external K^+ concentration. This increase in MEPP frequency requires Ca^{2+} in the external solution[8]. We observed that as the K^+ concentration of the external solution was increased from 2·5 to 20 mM the MEPP frequency rose from 1 s^{-1} to several hundred per second, if the solution contained 1·0 mM Ca^{2+} and no Mg^{2+}. This increase in MEPP frequency was absent in Ca^{2+}-free solution with 4 mM Mg^{2+}. In these experiments the osmotic pressure of the solution was held constant by substituting K^+ for Na^+. In marked contrast to the Ca^{2+}-dependence of the effect of external K^+ on MEPP frequency, the effects of BWSV were fully developed in Ca^{2+}-free solutions with 4·0 mM Mg^{2+}, with or without EGTA[9] (Fig. 1). It is therefore unlikely that the effect of the venom on MEPP frequency was caused entirely by depolarization of the nerve terminal. Black widow spider venom is not the only agent that causes a marked increase in MEPP frequency in Ca^{2+}-free solutions. Hypertonic solutions[10] or tetanic stimulation[11] of the nerve also cause a large increase in the MEPP frequency, in the absence of Ca^{2+}. We have confirmed these observations.

We obtained a discontinuous dose-response curve for the venom. When 0·02 ml. of venom solution was used, no effects on the neuromuscular junction were seen, even after an hour; if 0·03 ml. was used, the results were similar to those described above. Addition of more than 0·03 ml. of the venom reduced the lag time for venom action, but peak MEPP frequency and the number of quanta recorded were similar.

Once the action of the venom had been initiated, it could not be reversed by washing the preparation with venom-free Ringer's solution, but an antivenin preparation obtained from horse serum (Lyovac, donated by D. A. Press, Merck Sharp and Dohme) was very effective in reversing all the effects of the venom, provided that it was applied during the early stages of the venom action (Fig. 2). Antivenin alone or antivenin and BWSV had no effect on the preparation.

Our interpretation of these events is that the venom may react with the nerve terminal membrane, thereby inducing the release of transmitter. This release of transmitter occurs independently of the presence of Ca^{2+} and independently of the depolarization of the terminal. Release proceeds until the total pre-existent store of transmitter has been exhausted. If our counting procedures are adequate and if replenishment of the transmitter due to metabolic mechanisms is not significant, this store of transmitter amounts to about 4×10^5 quanta per nerve terminal. A similar figure has been given by Elmquist and Quastel[12] in their investigations of the rat diaphragm nerve terminal.

HERBERT E. LONGENECKER, jun.*
WILLIAM P. HURLBUT
ALEXANDER MAURO
ALLEN W. CLARK†

The Rockefeller University,
New York.

Received September 15, 1969; revised January 19, 1970.

---

* Present address: Department of Pharmacology, Cornell University School of Medicine.

† Present address: Department of Anatomy, University of Wisconsin.

[1] Grasso, A., and Poggi, P., *Toxicon*, **5**, 1 (1967).
[2] D'Ajello, V., Mauro, A., and Bettini, S., *Toxicon*, **6** (in the press)
[3] D'Amour, F. E., Becker, F. E., and van Riper, W., *Quart. Rev. Biol.*, **11**, 123 (1936).
[4] Sampayo, R. R. L., *J. Pharmacol. Exp. Ther.*, **80**, 309 (1944).
[5] Cantore, G. P., *Riv. Parass.*, **19**, 297 (1958).
[6] Fatt, P., and Katz, B., *J. Physiol.*, **115**, 320 (1951).
[7] Katz, B., and Miledi, R., *Proc. Roy. Soc.*, B, **155**, 455 (1962).
[8] del Castillo, J., and Katz, B., *J. Physiol.*, **124**, 586 (1954).
[9] Hubbard, J., Jones, S. F., and Landau, E. M., *J. Physiol.*, **194**, 355 (1968).
[10] Blioch, Z. V., Glagoena, I. M., Lieverman, E. A., and Nemashev, V. A., *J. Physiol.*, **199**, 11 (1968).
[11] Miledi, R., and Thies, R., *J. Physiol.*, **192**, 54P (1967).
[12] Elmquist, S., and Quastel, D. M. J., *J. Physiol.*, **177**, 463 (1965).

Effects on the Fine Structure of the Frog Neuromuscular Junction

FOR a long time, the synaptic vesicle has been correlated theoretically with the quantum of chemical transmitter[1–5], but experimental evidence for the equivalence, especially in the neuromuscular junction, has not been decisive[5–7,12]. The large number of vesicles and quanta in a single nerve terminal[5,10] complicates conventional methods[5,7] of demonstrating their simultaneous depletion. Black widow spider venom (BWSV) and hypertonic solutions of sucrose can eliminate this difficulty. Because neuromuscular junctions in the sartorius are difficult to find with the electron microscope, we chose instead to look for structural alterations of junctions treated with these substances in the cutaneous pectoris[8], a very thin muscle whose junctions can easily be found.

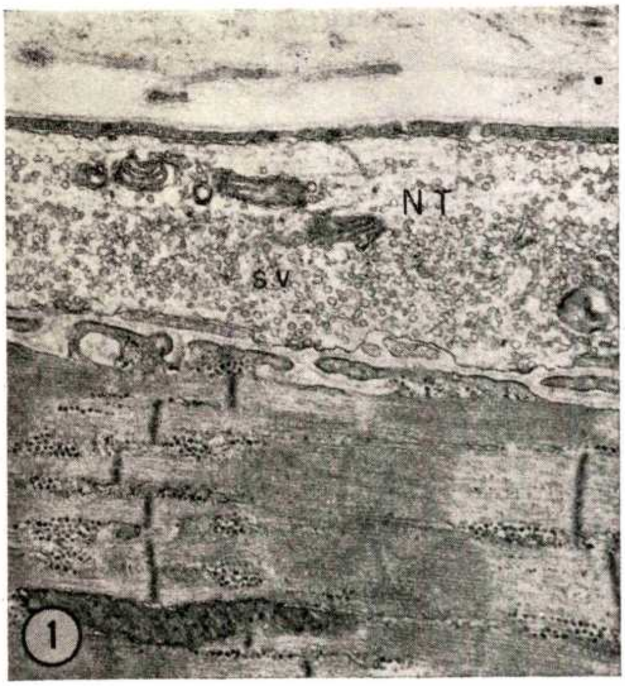


Fig. 1. Electron micrograph showing a portion of an end-plate from cutaneous pectoris muscle of the frog, a control preparation to which inactivated BWSV was added. The nerve terminal (NT) is characterized by a large number of synaptic vesicles (sv) massed along the presynaptic membrane. The nerve terminal also contains mitochondria, neurofilaments, neurotubules, and elements of what we assume to be smooth endoplasmic reticulum. ×18,500.

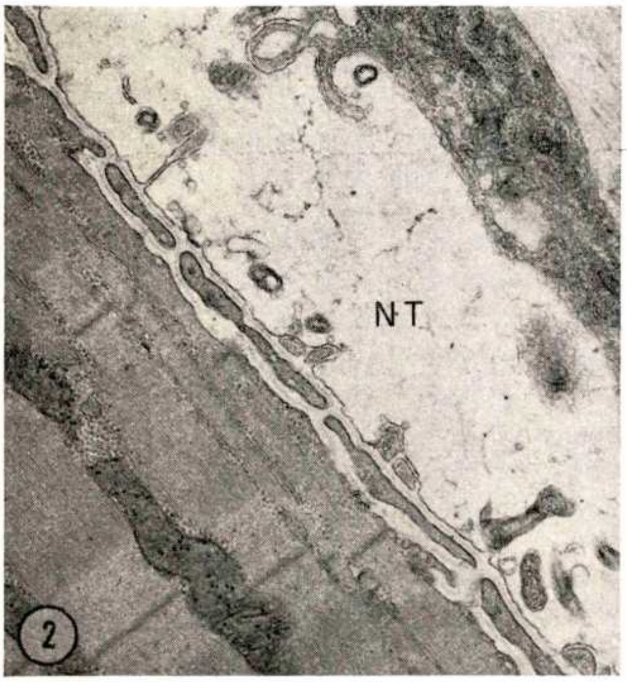


Fig. 2. Electron micrograph showing a portion of an end-plate from the cutaneous pectoris muscle of the frog. Active BWSV was added to this preparation, and it is apparent that very few synaptic vesicles remain in the nerve terminal (NT). ×18,500.

Both cutaneous pectoris muscles with intact nerves were dissected from *Rana pipiens*, and bathed in Ca^{2+}-free Ringer solution. Miniature end-plate potentials (MEPP) were monitored throughout each experiment. Normal MEPP frequency varied from 0·5 to 5 s^{-1}.

Almost immediately after 0·01 ml. of BWSV solution was added to the bath (approximately 2 ml.) directly over the impaling electrode or after beginning the replacement of the bath by hypertonic sucrose solution (360 mM) the MEPP increased greatly and then gradually declined to normal levels over a period of 30–40 min.

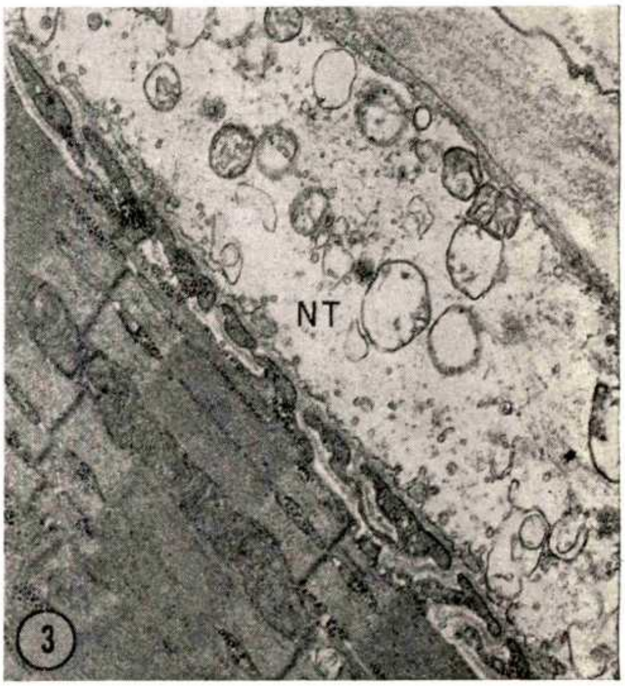


Fig. 3. Electron micrograph showing a portion of an end-plate from the cutaneous pectoris muscle of the frog. Hypertonic sucrose solution was added to this preparation. As in Fig. 2 it is apparent that very few synaptic vesicles remain in the nerve terminal (NT), although the preservation of the other organelles within the terminal is not as good. ×18,500.

One to four hours after the addition of BWSV or hypertonic sucrose, the preparation was rinsed with several changes of fresh Ca^{2+}-free Ringer solution. The bath was then replaced with a solution of 1 per cent formaldehyde, 1 per cent glutaraldehyde, 2 per cent sucrose, and 0·1 M phosphate buffer at *p*H 7·4 (ref. 10). Several changes of fixative were made until all schlieren patterns had disappeared. When the muscle stiffened, it was removed from the 'Lucite' chamber, trimmed and then placed in fresh fixative for a total fixation time of 1 h. The muscle was rinsed for 30 min with several changes of 0·1 M phosphate buffer and 3 per cent sucrose. The muscle was then postfixed for an hour in a solution of 1 per cent OsO$_4$, 2 per cent sucrose, and 0·1 M phosphate buffer. After embedding the muscle in Epon 812 according to standard procedures, it was sectioned with a diamond knife. The sections were picked up in naked copper grids, stained with uranyl acetate and lead citrate, and then viewed on a Hitachi 11B electron microscope.

There were three types of controls. In the first, muscles were pinned out in the same 'Lucite' chamber with the same bath of Ca^{2+}-free Ringer solution, an end-plate region was impaled with a micropipette, and the MEPP frequency was monitored for periods as long as 5 h. The second type of control, specifically for BWSV, was the same as the first except that 0·05 ml. of BWSV solution, inactivated by storage for four weeks at 3° C, was added at the beginning of the experiment. In the first and second type of control, MEPP frequency remained at normal levels throughout the experiment. The third type of control, one of the two muscles from a single animal was soaked in Ca^{2+}-free Ringer solution while the other muscle was being experimented upon.

Control end-plates (Fig. 1) appeared to be comparable with those described by other investigators[5,6,10–12]. In each nerve terminal, there were large numbers of synaptic vesicles that were usually massed along the presynaptic membrane. The terminals were also characterized by mitochondria, neurofilaments, neurotubules and by elements of what seemed to be smooth endoplasmic reticulum.

Experimental end-plates (Figs. 2 and 3) appeared exactly as they did in the controls, with the exception of the nerve terminals. Muscle fibres, Schwann cells, and nearby capillary endothelial cells had a normal appearance. By contrast, the nerve terminals looked relatively "clean" because they contained almost no synaptic vesicles. The other organelles were still present although they seemed to be better preserved in the terminals treated with BWSV. We have not yet answered the question of where the vesicles go when they disappear.

These results correlate closely with those obtained by means of electrophysiology. Although there are alternative interpretations, we believe the most persuasive one supports the idea that the synaptic vesicle and the quantum of chemical transmitter are equivalent.

ALLEN W. CLARK*
ALEXANDER MAURO
HERBERT E. LONGENECKER, jun.†
WILLIAM P. HURLBUT

The Rockefeller University,
New York.

Received August 21, 1969; revised January 19, 1970.

* Present address: Department of Anatomy, University of Wisconsin.

† Present address: Department of Pharmacology, Cornell University School of Medicine.

[1] De Robertis, E., and Bennett, H. S., *J. Biophys. Biochem. Cytol.*, **1**, 47 (1955).
[2] del Castillo, J., and Katz, B., *J. Physiol.*, **128**, 396 (1955).
[3] Palay, S. L., *J. Biophys. Biochem. Cytol.*, **2**, Suppl. 2, 193 (1956).
[4] De Robertis, E., *Exp. Cell Res.*, Suppl. 5, 347 (1958).
[5] Hubbard, J. I., and Kwanbunbumpen, S., *J. Physiol.*, **194**, 407 (1968).
[6] Birks, R., Huxley, H. E., and Katz, B., *J. Physiol.*, **150**, 134 (1960).
[7] Birks, R., Katz, B., and Miledi, R., *J. Physiol.*, **150**, 145 (1960).
[8] Blioch, Z. L., Glagoleba, I. M., Liberman, E. A., and Nenashev, V. A., *J. Physiol.*, **199**, 11 (1968).
[9] Fahrenback, W. H., *Z. Zellforsch.*, **93**, 451 (1969).
[10] Anderson-Cedergren, E., *J. Ultrastruct. Res.*, Suppl. 1, 1 (1959).
[11] Robertson, J. D., *Amer. J. Phys. Med.*, **39**, 1 (1960).
[12] Zacks, S. I., *The Motor Endplate* (Saunders, London, 1964).

Fast Spectrophotometric Measurement of H^+ Changes in *Chromatium* Chromatophores activated by a Liquid Dye Laser

by
B. CHANCE
J. A. McCRAY
J. BUNKENBURG

Johnson Research Foundation,
School of Medicine,
University of Pennsylvania,
Philadelphia,
Pennsylvania

The fast changes in hydrogen ion concentration which follow photoactivation of the bacteriochlorophyll complex of *Chromatium* have been measured by observing absorbance changes of the *p*H indicator bromcresol purple using a ruby-pumped dye laser. This acts as an optical frequency converter with an output pulse corresponding to an absorption peak of the chromatophore. The changes in H^+ binding represent a sort of "membrane Bohr effect".

THE first application of the ruby laser to biological problems was as a pulsed light source for the rapid activation of cytochrome oxidation in the photosynthetically active pale green mutant of *Chlamydomonas reinhardii* in 1962[1,2]. This technique was subsequently applied to *Chromatium*[3]. The experimental results suggested that the normal mode of laser operation provided pulses of sufficient power and brevity to identify a turnover of approximately one cytochrome *b* per 10 chlorophyll *A* per s. A continuation of these studies has involved the measurement of very rapid cytochrome oxidation (half-time, 2 μs) following a saturating 20 ns flash from a Q-switched ruby laser[4].

Another application for laser excitation has arisen in the study of rapid changes in hydrogen ions following light activation of chromatophores of photosynthetic bacteria. In this case, it is necessary to evaluate with accuracy the "one-turnover response" of the photosynthetic system with a light pulse short compared with the 2 μs half-time for the oxidation of cytochromes of type *c*[4]. Because the fast changes of external H^+ concentration[5–7] are measured by following the absorbance changes of the *p*H indicator dye, bromcresol purple, having its absorbance maximum in the region of 580 nm, and because it is also necessary to scan the difference spectrum for other absorbance changes in the wavelength region from 540 to 650 nm, the use of the ruby laser emitting light at 694 nm poses problems in effectively guarding the photodetector from emission at this wavelength. The use of selective laser excitation would make it possible to achieve saturation with a minimum of artefact by choosing a laser wavelength which coincides with a chlorophyll absorption peak for the particular species under study.

It is now possible to use laser-pumped liquid dye lasers[8,9] as optical frequency converters to achieve this objective; in particular, the Q-switched ruby laser may be used to pump cyanine dyes to give 20 ns pulses in the near

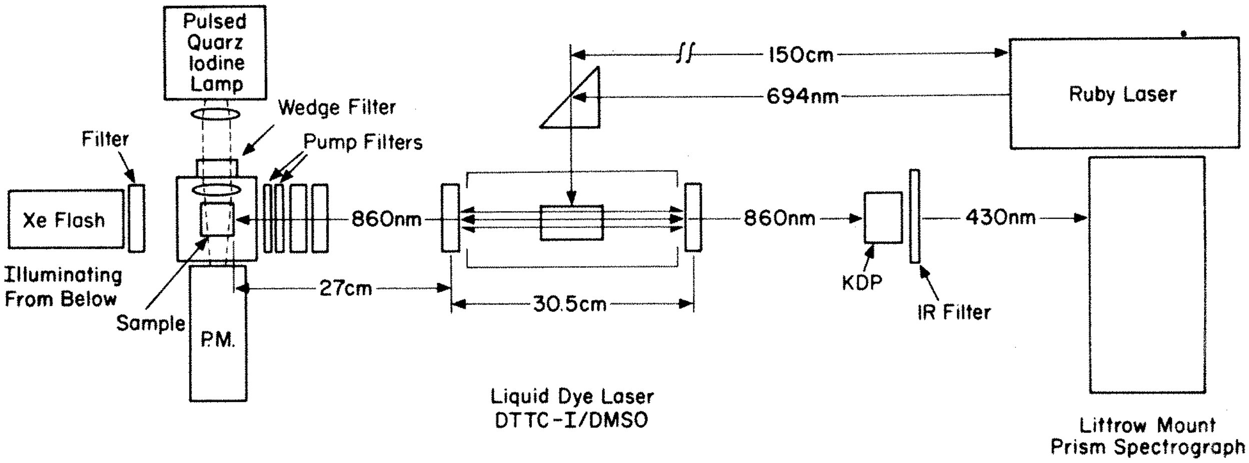


Fig. 1. Schematic diagram of the experimental apparatus (see text for full description).

infrared[10]. An appropriate wavelength may be obtained by selecting the proper dye, solvent, concentration and optical cavity.

We have designed a ruby-pumped liquid dye laser which gives an output laser pulse at 860 nm, which corresponds to an absorption peak of the bacteriochlorophyll complex of *Chromatium*. This laser has been used to obtain the time resolved difference spectrum for the binding of H$^+$ to the chromatophore membrane following a 20 ns pulse over the essential range of measuring light wavelengths from 540 to 650 nm. The experimental results are interpreted to indicate a very rapid light induced change of state of the membrane that is characterized by an increased hydrogen ion binding approximately equal to one H$^+$ per hundred moles of chlorophyll. This reaction has a half-time of 400 µs and is essentially complete in 800 µs.

The Liquid Dye Laser

The laser excitation pulse is obtained by pumping a liquid dye laser with a 20 ns pulse from a Q-switched ruby laser (Fig. 1). The liquid dye laser (LDL) consists of an optical cavity 30·5 cm long, two multiple dielectric mirrors, and a fluorescence cell adjusted transversely to excite the Gaussian mode. The dye solvent system is 3,3′-diethylthiatricarbocyanine iodide in dimethyl sulphoxide, about 10^{-4} M (DTTC–I/DMSO). The wavelength is determined by first frequency-doubling the infrared output of the LDL in an index-matched potassium dihydrogen phosphate crystal and recording the resulting second harmonic wavelength with a Littrow mount prism spectrograph.

Mercury lines were used in combination with the Hartmann formula[11] to calibrate the spectrograph. The resulting spectra (Fig. 2) give a value of 430 ± 2 nm for the second harmonic wavelength, corresponding to an 860 nm infrared laser pulse. The bandwidth is estimated to be about 6 nm.

The mirrors used in the LDL cavity are almost identical, giving two 860 nm output pulses so that wavelength and power measurements can be made without disturbing the apparatus. The conversion efficiency between the ruby pulse and the LDL pulse is about 7 per cent and the energy of one LDL pulse, as measured with a ballistic thermopile, was found to be 109 mJ. The 694 nm ruby pump light was prevented from reaching the photomultiplier by pumping the LDL transversely to the 860 nm beam direction, and by using a filter combination in front of the cuvette which consisted of two Wratten 88A filters, a Corning 4600 glass filter, and an interference filter with a pass band in the infrared. There was no measurable transmission through the filter combination from 340 to 800 nm. Thus light from the xenon flash lamp used to pump the ruby laser was not only attenuated by the distance between the ruby laser and the cuvette (approximately 200 cm) but also adequately eliminated by the filter combination. The transmission of the filter combination, measured at 860 nm, is 6 per cent. Burn patterns were taken at the output of the LDL and, without the filters, just in front of and inside the cuvette, in order to estimate the percentage of the laser beam intercepted by the cuvette; this is found to be 60 per cent. Thus the total energy impinging on the sample from one LDL infrared laser pulse is 4 mJ. The fact that further attenuation by filters or screens caused no measurable diminution of the bromcresol purple response indicates that this energy saturated the fast H$^+$ binding reaction in the chromatophores.

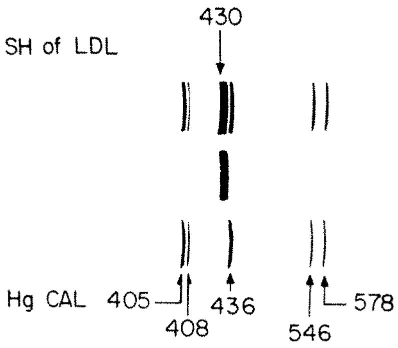


Fig. 2. Optical spectra of the second harmonic of the liquid dye laser output. The first spectrum is a superposition of laser output and mercury calibration. The second and third spectra are, respectively, the laser output and mercury calibration.

Fast Spectrophotometer

The fast spectrophotometer used in these studies was designed to provide maximum acceptable sample illumination with a high degree of stability (about $10^4 : 1$). A tungsten-halogen light source was operated at 6 A. A large aperture optical system, consisting of an $f/2$ lens combined with continuous wedge filters (Schott), 3 mm slit, 7·5 nm spectral interval, was used. The transmitted light was measured in an EMI 9592B photomultiplier equipped with one or two thicknesses of Corning 9782 guard filter. The reaction could be activated by the pulse from the liquid dye laser at 860 nm and by a 200 µs xenon flash as well. Wratten 88A guard filters were used in both cases[12]. The performance of the system is indicated in Fig. 3. It is capable of reading out with a rise time of 40 µs (10 per cent to 90 per cent) an absorbance change of $1 \cdot 6 \times 10^{-4}$ units at a wavelength of 580 nm, through the 8 µM chlorophyll concentration and 67 µM bromcresol purple concentration at a *p*H of 6·5.

Photoactivation of *Chromatium* Chromatophores

The response of the *p*H indicator, bromcresol purple, to 20 ns flashes of 860 nm radiation from the LDL, followed 7 ms later by a 200 µs flash from a xenon lamp over a broad wavelength band from 700 to 900 nm, is illustrated in Fig. 3. In the top trace the LDL flash occurs 6 ms after the recording starts; the laser flash is clearly seen as an upward spindle in the trace, followed by a drop of one scale division (0·2 per cent) with a half-time of 400 µs and a time to plateau of 800 µs. A reaction mechanism is described elsewhere[12]. The apparatus is programmed to provide, 6 ms after the laser flash, a xenon flash with a half-time of 200 µs, which drives the reaction to the maximum of its flash response. The bottom trace merely indicates the absence of electromagnetic artefact; the laser and xenon light is blocked off. The results indicate that photoactivation of *Chromatium* chromatophores causes a fast activation of the *p*H indicator dye, bromcresol purple, with a half-time of 400 µs. Calibrations with HCl show that the laser-induced absorbance change is equivalent to 10^{-7} M H^+ or to 1/100 of the chlorophyll present.

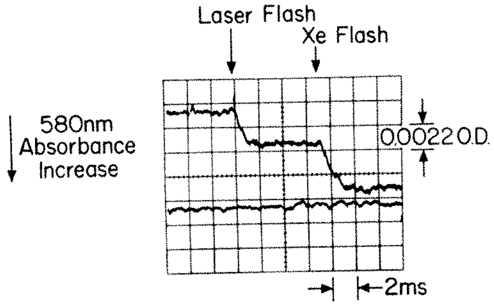


Fig. 3. Response of the *p*H indicator, bromcresol purple, to the 860 nm laser pulse followed by an infrared xenon flash.

Because observations at a single wavelength might be caused by a variety of optical responses of the chromatophores, we have made a scan of the wavelengths from 540 to 650 nm in order to identify bromcresol purple as the absorbing species. This difference spectrum (Fig. 4) is obtained by varying the wavelength of the measuring light incident upon the chromatophore suspension by varying the position of the linear wedge filter. The smaller spectrum (*A*) represents the absorbance changes following the 20 ns laser flash; and the larger (*B*) those arising from the 200 µs xenon flash. Both spectra have peaks at 582 nm, and are sufficiently similar to justify the conclusion that they both correspond to the same chemical species, namely, bromcresol purple.

Hydrogen Ion Binding Reaction

These experiments and others described elsewhere[12] justify the conclusion that the fast bromcresol purple absorbance increase results from the binding of H^+ to the membrane. The extent of the binding (approximately one for every hundred chlorophyll molecules) corresponds roughly to the size of the active centre in these chromatophores[13]. The relative insensitivity of the fast hydrogen ion binding to uncoupling agents and to alterations of membrane cation permeability by antibiotics suggests that the reaction is not intimately associated with an ordinary "pump" mechanism, but rather with primary events of electron transport in the active centre. The time scale of the fast H^+ binding in relation to that of other identifiable electron transport events in *Chromatium* chromatophores[4,12,14-18] is indicated in Fig. 5. The fast H^+ binding reaction, which we have termed "Z"[12], occurs more slowly than the reduction of either of the chlorophyll electron acceptors, X and Y, proposed by Parson[18], or the oxidation of chlorophyll electron donors such as cytochrome c_{555}. This kinetic difference is supported by differences in temperature coefficient and *p*H dependence between the H^+ binding reaction, Z, and acceptor reduction, Y[12,18]. Neither Z nor Y kinetics are affected by reductants of ubiquinone such as ascorbate or succinate[12,18].

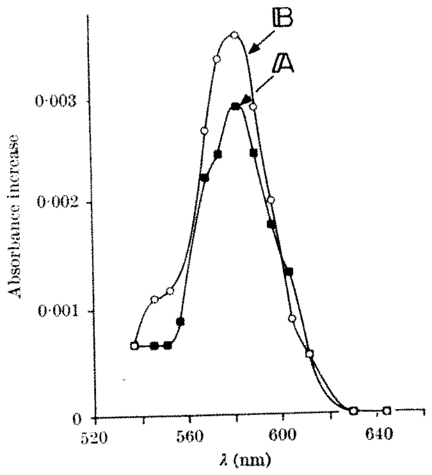


Fig. 4. Laser and xenon flash-induced difference spectra illustrating the specificity of the bromcresol purple absorbance change. *A*, 20 ns laser flash; *B*, 200 µs xenon flash following the laser flash.

It is noteworthy that the H^+ binding reaction does not exhibit reaction kinetics similar to those of the carotenoids of the chromatophore membranes; the more than 10-fold acceleration of the carotenoid absorbance change caused by valinomycin and potassium, observed particularly in *Rhodopseudomonas spheroides*[19] and to a lesser extent in *Chromatium* chromatophores and those prepared from *R. rubrum* (personal communication of M. Baltscheffsky), is not paralleled by a detectable change in the kinetics of the fast H^+ binding—nor, indeed, are the kinetics closely related; the carotenoid absorbance decrease is as short as 100 µs in chromatophores prepared from *Rps. spheroides* and approximately 20 ms in those prepared from *R. rubrum* (unpublished observations of D. DeVault, A. Crofts and M. Baltscheffsky).

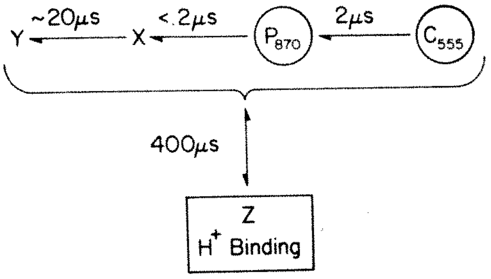


Fig. 5. Simplified scheme for photosynthetic electron transport.

A simple explanation for the light-activated hydrogen ion binding is based on an analogy with haemoglobin, where liganding of the haemoprotein with oxygen leads to structural changes which cause decreased H^+ binding in a phenomenon known as the "Bohr effect"[20]. We would term our finding a "membrane Bohr effect". The half-time of the H^+ binding may be related to a transition between membrane structural states induced by electron transport, in which membrane components—either protein or phospholipid—would exhibit altered charge states.

The alternative viewpoint, that the fast H^+ binding is chemiosmotic in nature[21], requires a correlation of the binding kinetics with the reduction of an electron transport component, presumably other than that indicated in Fig. 5. The correlation of H^+ binding with a reductant of a vectorial membrane-bound electron transport component is, however, not supported by experimental evidence.

The possibility that the light induced increase of H^+ binding causes movements of H^+ across the membrane permeability barrier is of considerable interest, but has not yet been proved by the use of suitable "internal" *p*H indicators.

We thank Dr G. I. Farmer for helpful discussion on liquid dye lasers, Dr M. Nishimura for useful suggestions and Miss R. Fugono for the preparation of chromatophores. The work was supported by a grant from the US Public Health Service.

Received October 27, 1969; revised January 2, 1970.

[1] Schleyer, H., and Chance, B., *Abst. Sixth Annual Meeting Biophys. Soc.*, FC9 (1962).

[2] Chance, B., Schleyer, H., and Legallais, V., in *Microalgae and Photosynthetic Bacteria* (edit. by Japan Soc. Plant Physiol.), 337 (Univ. Tokyo Press, Tokyo, 1963).

[3] Chance, B., and Schoener, B., *Abst. Eighth Annual Meeting Biophys. Soc.*, FD9 (1964).

[4] DeVault, D., and Chance, B., *Biophys. J.*, **6**, 825 (1966).

[5] Chance, B., and Mela, L., *Nature*, **212**, 372 (1966).

[6] Chance, B., Nishimura, M., Avron, M., and Baltscheffsky, M., *Arch. Biochem. Biophys.*, **117**, 158 (1966).

[7] Jackson, J. B., and Crofts, A. R., *Europ. J. Biochem.*, **10**, 226 (1969).

[8] Sorokin, P. P., and Lankard, J. R., *IBM Res. and Dev.*, **10**, 162 (1966).

[9] Sorokin, P. P., Lankard, J. R., Hammond, E. C., and Morruzi, V. L., *IBM Res. and Dev.*, **11**, 130 (1967).

[10] Miyazoe, Y., and Maeda, M., *Appl. Phys. Lett.*, **12**, 206 (1968).

[11] Sawyer, R. A., *Experimental Spectroscopy*, 240 (Dover, New York, 1963).

[12] Chance, B., Crofts, A. R., Nishimura, M., and Price, B., *Europ. J. Biochem.* (in the press).

[13] Clayton, R. K., *Molecular Physics in Photosynthesis* (Blaisdell, New York, 1965).

[14] Parson, W. W., *Biochim. Biophys. Acta*, **153**, 248 (1968).

[15] Ke, B., Vernon, L. P., Garcia, A., and Ngo, E., *Biochemistry*, **7**, 311 (1968).

[16] Ke, B., *Biochim. Biophys. Acta*, **172**, 583 (1969).

[17] Seibert, M., and DeVault, D., *Abst. Third Intern. Cong. Biophys.*, 1969. (in the press).

[18] Parson, W. W., *Biochim. Biophys. Acta* (in the press).

[19] Fleischman, D. E., and Clayton, R. K., *Photochem. Photobiol.*, **8**, 287 (1968).

[20] Bohr, C., Hasselbalch, K. A., and Krogh, A., *Scand. Arch. Physiol.*, **16**, 402 (1904).

[21] Mitchell, P., *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation* (Glynn Research, Bodmin, 1966).

Lack of Eye to Eye Transfer of an Early Response Modification in Birds

by

H. ZEIER*

Department of Psychology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts

A chick reared on glass over the deep side of a "visual cliff" loses its innate aversion to the deep side. If it is reared with one eye occluded, however, the induced loss of preference for the shallow side is not transferable to the other eye.

BIRDS are useful subjects for experiments on interocular transfer because their optic nerves are completely crossed, and their visually controlled behaviour has been well studied. In precocial birds, this behaviour includes certain components that seem to be "innate" (that is to say, independent of early experience); one of the most striking of these behaviour patterns is the avoidance by the newly hatched bird of the seemingly deep side of a "visual cliff"[1,2], an ingenious device for the testing of depth perception (see Fig. 1). Very young precocial birds also show a special form of learning (called "imprinting" by Lorenz[3]), manifested by their tendency to follow the particular stimulus to which they were initially exposed. This following reaction can be established through one eye (with the other eye occluded); once acquired, the reaction transfers immediately to the other eye[4]. Learned pattern discriminations (for example, triangle versus circle) have also been shown to transfer completely in normal birds[5,6], but such transfers are much less effective if the birds have been reared in diffuse light[6].

This article describes an attempt to sample another task for interocular transfer in unoperated birds. It has been shown that the "innate" preference for the shallow-looking side of a visual cliff could be abolished by rearing chicks, from the time of hatching, on the glass over the deep side of the cliff apparatus[7]. After this kind of rearing, the chick will descend indifferently to the deep and shallow side of the cliff; it has lost its innate aversion to the deep side. I replicated this special form of rearing, on the deep side, with the difference that one eye of the chick was occluded. This was to see whether the induced loss of preference for the shallow side would transfer to the other eye when the occluder was shifted from the previously covered to the previously open eye. As will be shown, such transfer definitely fails to occur, in spite of the apparent integrity of intracerebral commissures.

In the experiment here reported, 156 White Rock chicks in ten experimental groups were reared in different conditions either on the deep or shallow side of a visual cliff (Fig. 1). The chicks were taken within 6 h after being hatched in a dark incubator; their visual input was restricted by contact occluders[8], if this was necessary, and they were placed on either side of the cliff for 45 ± 5 h. The experiments were run in series so that there were never more than six chicks sharing the same space within the apparatus. The glass surface was cleaned once, at half-exposure time, for each group. After the chicks had been exposed to the cliff, the contact occluders were removed and the eyes were cleaned with an isotonic solution. The chicks were then given access to food and water for 1 to 2 h in a brooder. Immediately before testing, the vision of each eye was tested independently by eliciting a pecking response to a small rubber ball on a stick held in front of the chick's head. Chicks failing to peck at the ball or those showing any signs of conjunctivitis were discarded from the experiment.

---

* Present address: Swiss Federal Institute of Technology, Department of Applied Physiology, Clausiusstrasse 25, Zürich, Switzerland.

For the test, a centreboard was placed across the glass plate at the borderline between the deep and shallow side. Because electric shocks given to chicks do not influence their choice behaviour on a visual cliff[9], an electrified grid was installed on the centreboard. Each chick was placed twice on the centreboard with its face toward the deep side. If no response occurred within 30 s, a shock of from 1 to 5 mA was administered and gradually increased in strength until the chick jumped from the centreboard. An electrode-paste sensitized the chick's feet to the electric shock. After each response, the glass surface was cleaned and the contact occluders were switched if necessary.

The first two control groups received binocular exposure on the deep or shallow side in order to see if they would show the expected behaviour on two subsequent responses. The results, summarized in Table 1, show that members of the deep-reared group lost their visual-cliff avoidance and descended randomly both times, while those of the shallow-reared group clearly preferred the shallow side as normal chicks would do. There was no significant interaction between first and second response.

Table 1. REACTIONS ON VISUAL CLIFF BY CHICKS REARED UNDER DIFFERENT CONDITIONS

Binocularly tested

			1st response			2nd response		
Group	Rearing conditions	*N*	*D*	*S*	%*S*	*D*	*S*	%*S*
1	Deep side both eyes exposed	12	7	5	42	5	7	58
2	Shallow side both eyes exposed	12	3	9	75	2	10	83
3	Deep side one eye exposed	12	5	7	58	5	7	58
4	Shallow side one eye exposed	12	2	10	83	3	9	75

Monocularly tested

			Exposed eye			Unexposed eye		
Group	Rearing conditions	*N*	*D*	*S*	%*S*	*D*	*S*	%*S*
5	Deep side one eye exposed	36	18	18	50	6	30	83
6	Shallow side one eye exposed	24	5	19	79	4	20	83

			Left eye			Right eye		
Group	Rearing conditions	*N*	*D*	*S*	%*S*	*D*	*S*	%*S*
7	Deep side both eyes exposed	12	5	7	58	5	7	58
8	Shallow side both eyes exposed	12	3	9	75	3	9	75
9	Deep side both eyes covered	12	1	11	92	2	10	83
10	Shallow side both eyes covered	12	1	11	92	3	9	75

N, Number of chicks per group; *D*, number of chicks descending to deep side of cliff; *S*, number of chicks descending to shallow side.

The next two groups received their cliff experience monocularly and performed the test binocularly. The deep-reared chicks (group 3) transferred their loss of visual cliff avoidance from a single eye to both eyes, while the shallow-reared chicks (group 4) showed the expected depth avoidance response. Group 5, however, which had the same exposure history on the deep-cliff side as group 3, but had then been tested monocularly (half of the group with the exposed eye first, the other half with the unexposed eye first), showed the normal visual cliff-avoidance response when using only the unexposed eye, in contrast to randomly distributed responses made when vision was restricted to the exposed eye ($\chi^2 = 7{\cdot}71$, $P < 0{\cdot}01$). Loss of cliff-avoidance response apparently does not transfer from a single eye to a contralateral eye.

Results for group 7 demonstrate that the test performance of binocularly exposed chicks is not affected by occlusion of either the left or right eye, while the shallow-reared control groups 6 and 8 showed the expected cliff-avoidance response regardless of which eye was used. Groups 9 and 10, reared with both eyes occluded, showed the same behaviour, proving that the contact occluders used were effective and that light deprivation during the short (≤ 50 h) experimental rearing period did not distort visual functions in such a way that these animals lose the form of depth perception tested in the visual-cliff situation.

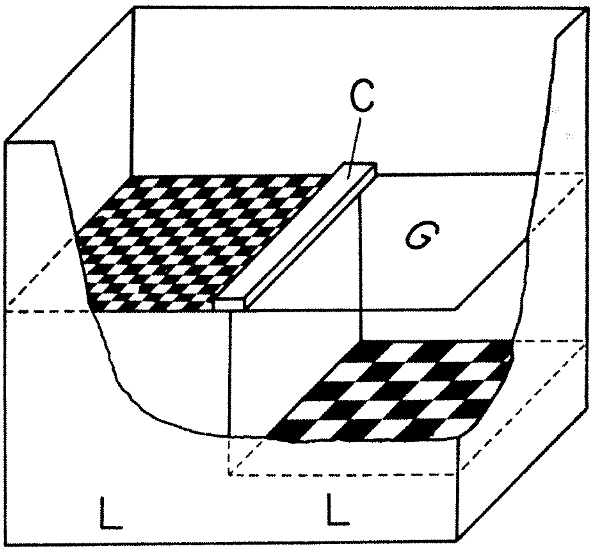


Fig. 1. Visual cliff apparatus with shallow side on the left and deep side on the right. On the shallow side (a square of 35 by 35 cm) textured 6 mm red and white checkered cloth is inserted directly under the glass; on the deep side (35 by 35 cm) an analogous pattern is placed 25 cm below the glass surface, but the squares are 20 mm in length to minimize texture density as a possible cue[2]. *C*, Centreboard (5 cm wide and 2 cm high) for testing procedure; *G*, glass (70 by 35 cm) extending over both sides; *L*, two fluorescent lamps on each side transilluminating the textures.

These findings show that, although the loss of visual-cliff avoidance after exposure on the deep side of a visual cliff transfers from a single eye to both eyes and from both eyes to a single eye, there is no transfer of this behavioural modification from a single eye to the contralateral eye. Such a lack of interocular transfer is particularly significant because chicks "imprinted" with one eye to follow a moving object will readily transfer this following-reaction to the other, untrained eye[4]. It is possible therefore that tasks involving responses for which there is a strong inherent disposition are more easily transferred from eye to eye than are reactions that run counter to an innate response tendency.

The situation in the chick may not be too dissimilar from that in the kitten reared with different exposures to each eye. Hein and Held[10] showed that the visual placing response of the kitten has two components—triggered extension and guided reach. The guided response requires previous sight of the limbs by the controlling eye, and exposure of only one eye to the limb fails to produce guidance of the reach by the other eye. The triggered response requires only exposure of one eye to diffuse light and can then be immediately elicited by stimulation of the other eye[11]. There, too, interocular transfer seems to be less readily accomplished for the response that has a conditioned component[11].

This work was supported in part by a grant from NASA to Professor H.-L. Teuber and by a postdoctoral fellowship to H. Z. from the Swiss National Foundation for Scientific Research and from the University of Zurich. I thank Professors H.-L. Teuber, R. Held, A. Hein and R. D. Walk for their advice on the planning and conduct of this experiment.

Received October 20; revised December 12, 1969.

[1] Gibson, E. J., and Walk, R. D., *Sci. Amer.*, **202**, 64 (1960).
[2] Walk, R. D., and Gibson, E. J., *Psychol. Monogr.*, **75**, No. 15 (1961).
[3] Lorenz, K., *J. Ornithol.*, **83**, 137 (1935).
[4] Moltz, H., and Stettner, L. J., *J. Comp. Physiol. Psychol.*, **55**, 626 (1962).
[5] Levine, J., *J. Genet. Psychol.*, **67**, 131 (1945).
[6] Siegel, A. I., *J. Comp. Physiol. Psychol.*, **46**, 249 (1953).
[7] Tallarico, R. B., and Farrell, W. M., *J. Comp. Physiol. Psychol.*, **57**, 94 (1964).
[8] Chorover, S. L., and Chase, R., *J. Comp. Physiol. Psychol.*, **65**, 238 (1968).
[9] Quinta, T., *Perceptual and Motor Skills*, **22**, 169 (1966).
[10] Hein, A., and Held, R., *Science*, **158**, 390 (1967).
[11] Hein, A., in *Perceptual-Motor Foundations* (edit. by Sloan, M. R.) (Amer. Assoc. for Health, Physical Education, and Recreation, Washington, 1968) (in the press).

LETTERS TO THE EDITOR

PHYSICAL SCIENCES

The Galactic Halo

THE halo of our galaxy is a nearly spherical region containing very old stars which have a smaller content of heavy elements than our Sun. There is no detailed model for its formation. It is usually assumed that somehow a cloud of gas condensed to form our galaxy, and that the halo stars were formed during the collapse process and left with a nearly spherical distribution[1]. In that case, and in view of the success of the theories of nucleosynthesis in stars, it is very puzzling that the halo stars should have any heavy elements at all.

We reconsidered this problem in connexion with our preparations for the symposium on the chemical evolution of the galaxy recently held at the California Institute of Technology in honour of the sixtieth birthday of J. L. Greenstein. This exercise was a new version of a study carried out a few years ago[2] in which the chemical history of the galaxy is followed by numerically integrating the effects of stellar evolution. The general approach was similar to that of the previous study, except that more details of the integrations could be recorded using the storage of the 360/95 computer of the Goddard Institute for Space Studies. The principal difference between the two studies arose in the assumptions which we have now made about the details of stellar evolution. We shall prepare a full report for publication elsewhere; in this report we give only the principal arguments and conclusions which we reached having a bearing on the origin and evolution of the galactic halo.

Our assumptions about stellar evolution have been influenced very heavily by recent studies of supernova explosions by Arnett[3]. For massive stars up to twenty solar masses, we assumed that a complete nuclear explosion would occur, leaving no remnant but injecting into the interstellar medium the main products of nucleosynthesis between carbon and the iron equilibrium peak[4,5]. For more massive stars, we assumed that the implosion would form a Schwarzschild singularity, that is, a black hole or "collapsar". In both cases we assumed that thirty per cent of the initial main sequence mass would be lost in stellar winds during stellar evolution.

The rate of star formation was governed by the assumption that the gas content of the galaxy declined exponentially, reaching five per cent at $1 \cdot 5 \times 10^{10}$ yr (a number chosen to fit cosmochronological considerations). We assumed the same stellar birth-rate function as in the earlier study, where it was taken from the work of Salpeter[6] and Limber[7].

As before, we found that the heavy element content of the galaxy rose very quickly in the first 2×10^9 yr, and that it increased only slowly after that. The present calculations, however, indicated that about ten per cent of all stars of slightly less than the solar mass (which would never have evolved beyond the main sequence) would contain 15 per cent or less of the solar content of heavy elements. Observations show that such stars are extremely rare, however, and that most stars have a heavy element content within a factor three of the solar content. We therefore felt forced to the conclusion which was earlier reached by Schmidt[8]: in the early history of the galaxy almost all the stars formed were more massive than the Sun.

This is consistent with current thinking about star formation. Fragmentation is greatly assisted during the collapse of an interstellar cloud if strong density-dependent cooling is present. Such cooling reduces the velocity of propagation of pressure waves and hence isolates one part of the gas from its neighbouring part. Cooling is very inefficient below about 10^4 K if heavy elements are not present in the gas to form grains and ions with low-lying excited states. It is therefore reasonable to expect that only quite massive stars can have been formed in the galaxy until the heavy element content of the gas rose to a significant fraction of the solar value. For the purposes of our numerical exploration we assumed that only that part of the stellar birth-rate function above ten solar masses was formed by star formation until the content of elements heavier than helium in the interstellar gas reached 0·005 of the mass, about one-third of the solar value.

The galactic evolutionary histories which we computed had a number of strange aspects, but one feature stood out. In the original birth-rate function, about five per cent of the mass ends up in collapsars according to our stellar evolution assumptions. In the revised birth-rate assumptions the collapsar mass fraction becomes about an order of magnitude larger. This feature points the way to the following suggestions about the early history of our galaxy.

Suppose, as is usually done, that a cloud of gas collapses to form our galaxy. Suppose also that about half of the mass of this gas promptly forms massive stars (something like our revised early birth-rate function). These stars contain no heavier elements and hence are likely to have high surface temperatures, of order 10^5 K. The residual gas is likely to be maintained at a similar gas kinetic temperature and to form a roughly spherical body having an approximation to hydrostatic equilibrium and a radius of order 10^5 pc. The rapid evolution of the massive stars will mix substantial amounts of heavy elements into this halo gas. This heavy element contamination greatly increases the cooling efficiency of the halo gas, thus allowing the formation of low mass stars in the halo and simultaneously allowing the halo gas to collapse to form the galactic disk, because the thermal pressure is suddenly reduced.

We have run some models of the galaxy based on this picture. We doubled the mass of the galaxy and put all the additional mass into stars in the first evolutionary time step. This has given us the best fit to the various observed abundance criteria which we wished to fit by our computed galactic histories. While this success is gratifying, we emphasize that a large number of assumptions is involved, so that the only conclusion which can properly be drawn is that our current assumptions about stellar evolution are consistent with our picture of the formation and evolution of the galactic halo.

These calculations indicate that about half the present mass of the galaxy is in the form of collapsars. The many assumptions mean that this number can only be taken as a rough indication of what we should expect. It is not in conflict with observations, however. We must expect the galaxy to be nearly twice as massive as usually assumed, with most of the extra mass consisting of collapsars distributed throughout the halo. This distribution will have very little effect on the dynamical motions of the stars in the galactic disk. Analyses of the motions of the stars in the solar neighbourhood perpendicular to the galactic disk indicate that some thirty per cent of the local mass density is in an unseen form, subject to the assumption that the unseen mass is strongly concentrated to the disk[9]. If the unseen mass has a more nearly spherical distribution, however, its mass can be very much greater than this[10].

For many years it has been a puzzle that giant elliptical galaxies have such a high mass-to-light ratio[11]. The present considerations lead us to suggest that the majority of the mass of such galaxies is in the form of collapsars.

We thank W. D. Arnett and M. Schmidt for discussions. This research was supported in part by grants from the US National Science Foundation and the US National Aeronautics and Space Administration.

J. W. TRURAN
A. G. W. CAMERON

Belfer Graduate School of Science,
Yeshiva University, New York,
and
Goddard Institute for Space Studies,
NASA, New York.

Received February 2, 1970.

[1] Eggen, O. J., Lynden-Bell, D., and Sandage, A. R., *Astrophys. J.*, **136**, 748 (1962).
[2] Truran, J. W., Hansen, C. J., and Cameron, A. G. W., *Canad. J. Phys.*, **43**, 1616 (1965).
[3] Arnett, W. D., *Astrophys. Space Sci.*, **5**, 180 (1969).
[4] Arnett, W. D., *Astrophys. J.*, **157**, 1369 (1969).
[5] Truran, J. W., and Arnett, W. D., *Astrophys. J.* (in the press).
[6] Salpeter, E. E., *Astrophys. J.*, **129**, 608 (1959).
[7] Limber, D. N., *Astrophys. J.*, **131**, 168 (1960).
[8] Schmidt, M., *Astrophys. J.*, **137**, 758 (1963).
[9] Oort, J. H., *Bull. Astron. Inst. Netherlands*, **15**, 45 (1959).
[10] Gould, R. J., Gold, T., and Salpeter, E. E., *Astrophys. J.*, **138**, 408 (1963).
[11] Burbidge, G. R., and Sargent, W. L. W., *Comm. Astrophys. Space Phys.*, **1**, 220 (1969).

An Oscillating State as an Alternative to Gravitational Collapse

THE possibility of an oscillating universe is often disputed on the grounds that oscillations are not possible when there is no equilibrium position[1]. By the same argument, gravitational collapse can be considered as the only alternative when the mass is over critical (there is no configuration of equilibrium for such a big mass). The argument, however, cannot be generalized from the cosmological case to the case of a big mass, as we will see.

The existence of a critical mass of the order of that of the Sun follows from the equation of state obeyed by matter. This can be seen from the following example of a solution representing a mass as big as desired in equilibrium.

$$\mathrm{d}s^2 = \frac{-2}{1-ar^2}\,\mathrm{d}r^2 - r^2\,(\mathrm{d}\theta^2 + \sin^2\theta\,\mathrm{d}\varphi^2) + ar^2\,\mathrm{d}t^2 \qquad (1)$$

from which we obtain

$$8\pi\rho = (1/2r^2) + (3a/2) \qquad (2)$$

$$8\pi p = (1/2r^2) - (3a/2) \qquad (3)$$

The equation of state is

$$\rho = p + k \text{ (with } k = 3a/8\pi) \qquad (4)$$

The element can be joined smoothly to the Schwarzschild exterior solution

$$\mathrm{d}s^2 = -\left(1-\frac{2m}{r}\right)^{-1}\mathrm{d}r^2 - r^2\,\mathrm{d}\,\Omega^2 + \left(1-\frac{2m}{r}\right)\mathrm{d}t^2 \qquad (5)$$

giving for the gravitational mass the value

$$m = (1/3)\,(8\pi k)^{-1/2} \qquad (6)$$

and for the radius

$$R = (8\pi k)^{-1/2} \qquad (7)$$

Pressure and density become infinite at the origin, but there is a theorem[2] applicable in this case which ensures the existence of solutions with the same equation of state, and with a total mass as near as desired to that of our solution provided that the central pressure and density are high enough, though finite.

It is possible therefore to have as large a mass as desired in equilibrium provided we admit an equation of state of the form $\rho = p + k$ small enough. Oppenheimer and Volkoff's proof[3] of the existence of a critical mass of the order of that of the Sun relies on the known equation of state for cold neutron matter.

Misner and Zapolsky[4] have generalized the proof of ref. 3 for the case when matter with over nuclear density obeys an equation of state of the form $p = \rho(\gamma - 1)$ with $1 \leq \gamma \leq 2$ while for under nuclear densities the equation of state is the known equation for cold neutron matter.

Comparing my solution with that of Misner and Zapolsky, both propose the same equation of state for the core (if we take $\gamma = 2$). The essential difference is that the equation $\rho = p$ is considered by Misner and Zapolsky to be valid for over nuclear densities only, while in our case (for k very small) the equation $\rho = p$ remains approximately valid for a considerable range of under nuclear densities. When the equation $\rho = p$ can be extended to a quantity of matter sufficiently larger than that considered by Misner and Zapolsky, there may be equilibrium configurations for masses as great as desired.

The approach of Misner and Zapolsky is sound, so I do not propose my solution as a model for big masses in equilibrium. I accept therefore that there is no configuration of equilibrium for big masses of cold neutron matter. As such big masses collapse, however, most of the matter may become of over nuclear density.

The existence of restoring forces must now be studied with due regard to the equation of state prevailing in the dynamical process during which there exists a much higher ratio of matter with over nuclear density to matter with under nuclear densities than in the static case.

Misner and Zapolsky did not calculate the possibility of equilibrium for such higher ratios of matter obeying the $\rho = p$ equation to matter not obeying it (such higher ratios are unrealistic in the static case). My example proves that in this case there is an equilibrium configuration and therefore there may be restoring forces to this position of equilibrium.

An over critical mass could therefore oscillate in the following way: (1) It starts collapsing because it cannot remain in equilibrium. (2) As it collapses, the ratio of mass with over nuclear to mass with under nuclear density increases and the equation of state of that part of the body, the density of which goes from under nuclear density to over nuclear density, does change. As a result, restoring forces build up. (3) Under the effect of the restoring forces the collapse stops, to be followed by the expansion of the body. (4) While expanding, the ratio of over nuclear densities to under nuclear densities decreases. The body therefore does not cross a position of equilibrium but completes the cycle by reaching the initial state for which no equilibrium position exists.

It would be interesting to establish the precise ratio (as a function of total mass) which separates the cases for which there is or there is not restoring forces. It would be helpful to calculate the limiting mass for which the restoring forces are reversing the collapsing movement before the crossing of the Schwarzschild radius. When the Schwarzschild radius is to be crossed (from the point of view of a comoving observer), the body is to be considered as a collapsing non-oscillating one from the point of view of an external observer who will observe the body as tending asymptotically towards the Schwarzschild radius.

I thank Professor W. Isreal for pertinent remarks.

CLEMENT LEIBOVITZ

Theoretical Physics Institute,
Department of Physics,
University of Alberta,
Edmonton, Alberta,
Canada.

Received November 3, 1969.

---

[1] Hoyle, F., *Galaxies, Nuclei and Quasars*, 24 (Harper and Row, NY, 1965).
[2] Harrison, B., Thorne, K., Wakano, M., and Wheeler, J., *Gravitational Theory and Gravitational Collapse*, 30 (Chicago, 1965).
[3] Oppenheimer, J., and Volkoff, G., *Phys. Rev.*, **55**, 374 (1939).
[4] Misner, C., and Zapolsky, H. S., *Phys. Rev. Lett.*, **12**, 635 (1964).

Long Term Variations of Pulsar Intensities

PULSAR intensity variations may be divided roughly into three classes. There are rapid variations on a time-scale of seconds to minutes which occur simultaneously over a wide range of frequency and must therefore be intrinsic to the source[1]. On a somewhat longer time-scale there are variations typically over a few minutes to a few hours which correlate only over limited bandwidths. There is strong evidence that they are caused by irregular diffraction in the interstellar medium[2,3]. Finally, there are variations on a time-scale of days to months[4] about which little is known because extended regular observations are required. In this report we present some new results obtained during timing observations carried out on a routine basis over 11 months.

The five pulsars CP 0808, CP 0834, CP 0950, CP 1133 and CP 1919 were observed during meridian transit with the 81·5 MHz phased array at Cambridge. A daily measure of the mean intensity was obtained by averaging the ten largest pulses to occur during the 4 min or so that the source was in the beam. Considerable day to day variations must arise from the interstellar fluctuations (extrapolating from the data of Rickett[3]), so the daily values were smoothed by taking a 7 day running mean.

The results are shown in Fig. 1, together with some isolated values at 408 MHz. All five pulsars show similar behaviour in that variations on time-scales extending from a few days to several weeks are clearly evident. CP 0950 is, possibly, the most variable source and has shown the greatest extreme fluctuations. This is not fully apparent in Fig. 1, because the timing records from which the data were taken were saturating for very large pulse intensities. It should be noted, however, that in November 1967, CP 0950 exhibited an increase by a factor of about 200 (ref. 5). The variability of this pulsar is also shown by the data of Downs *et al.* at 12·5 cm (ref. 6).

The points at 408 MHz were included in an attempt to investigate the correlation of long period variations at another frequency. We thank the Nuffield Radio Astronomy Laboratories, Jodrell Bank, for these data, which were obtained from observations with the Mark I telescope usually tracking each source for about an hour. Because the medium period fluctuations are slower at 408 MHz, these points were smoothed by taking a mean over 4 days wherever possible. There is no clear correlation between the strength at the two frequencies for either source, but for CP 0950 the peaks and troughs at 81·5 MHz tend to coincide with those at 408 MHz. The correlation coefficient for the CP 0950 data (unsmoothed at 408 MHz) is 0·37, which is significant at the 5 per cent confidence level. The correlation is almost unchanged by smoothing. There is no significant correlation for the CP 1919 data. This indicates that there may be some correlation of long-term variations over this frequency range, but more extended observations are needed to settle this point.

There seems to be no possibility of accounting for long term variations of intensity by random diffraction in the interstellar medium. Irregularities in the interstellar medium produce a diffraction pattern at the Earth which appears as an intensity variation in time due to the transverse velocities of the irregularities relative to the

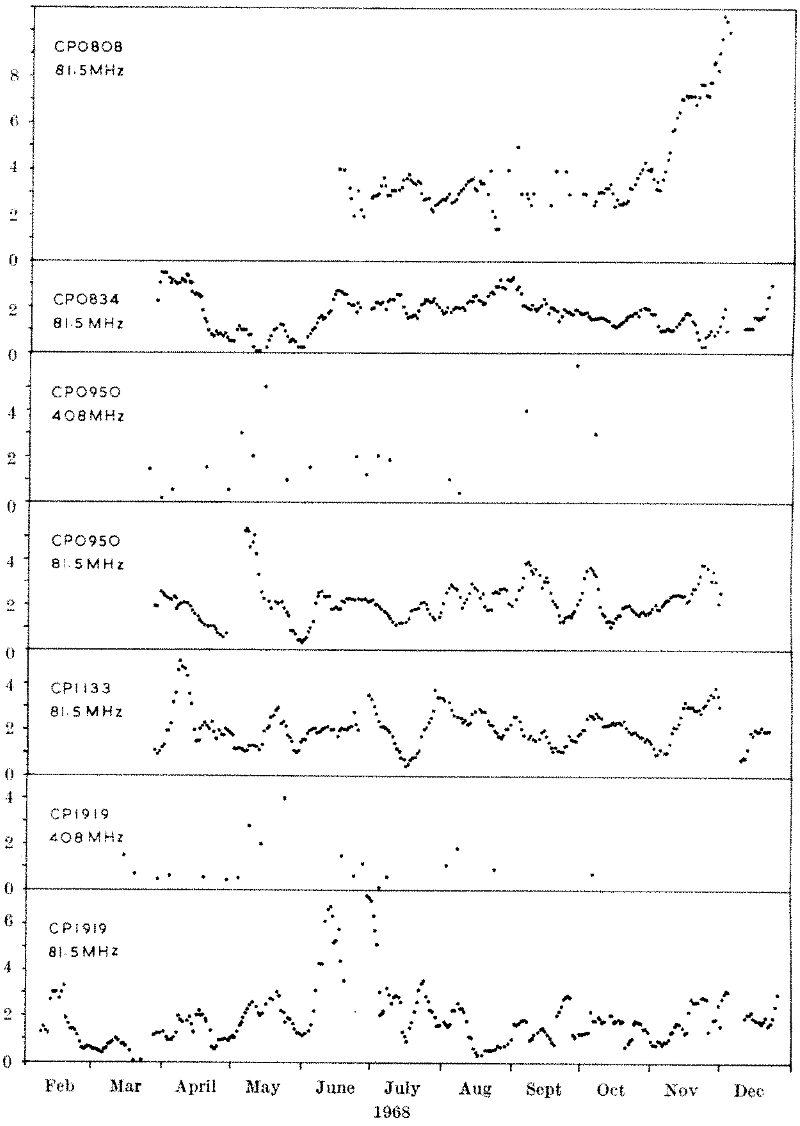


Fig. 1. Plots of intensities (7 day running mean) for five pulsars with an arbitrary linear intensity scale.

Earth. The Earth contributes a transverse velocity component which varies throughout the year, but which is of the order of 30 km s^{-1} for all sources for at least several months in the year. The observed deep amplitude variations have a correlation width of the order of 1 month, so that at 30 km s^{-1} the physical scale, a, of the intensity pattern would have to be greater than 10^{13} cm. The deep modulation implies that the Earth is in the far field of diffraction, however, so the Fresnel distance for the irregularities would have to be less than the distance, d, to the source. Hence $ka^2 < d$, where k is the wave number at 81·5 MHz. For CP 0950, for example, taking $d = 100$ pc, we require $a < 10^{11}$ cm.

Because the scintillation mechanism cannot reconcile these two values for the scale size, and because there is a possible correlation over wide frequency ranges, it may be concluded that the long term variations are intrinsic to the source.

There do not seem to be many processes in a neutron star which will lead to variations on so long a time-scale. Density fluctuations in the escaping plasma might lead to intensity fluctuations for some of the oblique magnetic rotator models, but it is difficult to envisage slow changes in this situation. Variations in the surface magnetic field might also modulate the radio emission, either by altering the beam geometry or by changing the effective dipole moment. Simultaneous observations at different frequencies may provide further clues.

It is also interesting to note that large increases of intensity which persist for a few weeks might give rise to associated variations of period which are large enough to be observable. For example, in the case of CP 0950 radiating 200 times its mean energy for about 3 days, if the radio flux is derived from rotational energy loss at some constant efficiency, the slowdown rate, $\dot{p}$, would rise to about 5×10^{-14}. The change in pulsar phase over the period of $2{\cdot}5 \times 10^5$ s is then $\Delta\varphi = \frac{1}{2}\dot{p}\,t^2 = 1{\cdot}5$ ms. In a plot of pulsar phase, a step of 1·5 ms would be just observable with present observational accuracies; in other cases the effect could be larger. It may be significant, however, that the total radio flux emitted by CP 0950 is only 0·01 per cent of the loss of rotational energy due to the lengthening of the period, whereas pulsars which seem to have less extreme variations in flux have much higher radiative efficiencies. For CP 0808 it is about 1 per cent, and for CP 0328 of the order of 3 per cent. (These values may all be reduced by a factor of up to ten if there is appreciable beaming of the radiation in two dimensions.

Further observations of period changes and of flux variations at several frequencies are needed to investigate these possibilities.

We thank Dr A. Hewish for discussions, and Professor F. G. Smith and G. C. Hunt of Jodrell Bank for the 408 MHz data. T. W. C. and C. G. P. acknowledge maintenance awards from CSIRO and the Science Research Council respectively.

T. W. COLE
H. K. HESSE
C. G. PAGE

Mullard Radio Astronomy Observatory,
Cavendish Laboratory,
University of Cambridge.

Received January 20, 1970.

[1] Lyne, A. G., and Rickett, B. J., *Nature*, **218**, 326 (1968).
[2] Scheuer, P. A. G., *Nature*, **218**, 920 (1968).
[3] Rickett, B. J., thesis, Univ. Manchester.
[4] Hewish, A., Bell, S. J., Pilkington, J. D. H., Scott, P. F., and Collins, R. A., *Nature*, **217**, 709 (1968).
[5] Pilkington, J. D. H., Hewish, A., Bell, S. J., and Cole, T. W., *Nature*, **218**, 126 (1968).
[6] Downs, G. S., Morris, jun., G. A., and Reichley, P. E., *Nature*, **222**, 1257 (1969).

Anisotropic Atomic Hydrogen Distribution in Interplanetary Space

Blum and Farr[1] have suggested modifications of current theories[2,3] concerning the interaction of the solar wind with interstellar hydrogen. One feature of their model involves the motion of the solar system. This motion, coupled with the velocity of the interstellar hydrogen, results in a velocity of 10 to 40 km s^{-1} for interstellar hydrogen relative to the solar system. According to Blum and Fahr the relative velocity gives rise to an anisotropic distribution of neutral hydrogen atoms in interplanetary space. The neutral hydrogen density is enhanced in the direction of approach of the interstellar hydrogen and diminished in the opposite hemisphere. The motion of the interstellar hydrogen is assumed to cause a highly aspherical transition region between the hydrogen and the solar wind.

Our observations[4] with satellite-borne detectors of Lyman α seem to support qualitatively the model of Blum and Fahr. We assume that the radiation detected is primarily solar Lyman α resonantly scattered from neutral hydrogen. The measurements were made with ion chambers filled with NO with LiF windows on two Vela 4 spacecraft launched on April 28, 1967. These Earth-oriented spacecraft are in nearly circular orbits at 110,000 km inclined at 58° to the ecliptic plane and should be above the hydrogen geocorona of the Earth. Each spacecraft carries two collimated ion chambers with 17° half-angle cones of acceptance (0·27 sr). The extensions of the cone axes pass through the centre of the Earth; thus the ion chambers look radially outward from the Earth.

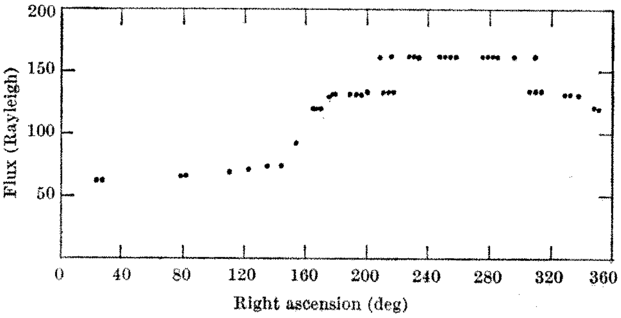


Fig. 1. Scattered Lyman-α flux versus right ascension for June 6 to June 10, 1967. The orbit period is approximately 4·6 days.

The predominant feature of the data is a repetitive and marked asymmetry in the Lyman α flux as a function of right ascension of the cone axes. This feature was evident for a period of 290 days (64 revolutions) after which the sensitivities of the ion chamber were too low for meaningful results. As shown in Fig. 1, the flux typically rises from about 60 Rayleighs to a broad maximum (160 to 190° FWHM) of 160 Rayleighs. The average of many orbits places the centre of the maximum at approximately RA 265°. The motion of the solar system relative to nearby stars is toward RA 270° and δ + 27°. The declination of the cone axes is + 32° at RA 265°.

A distribution of aspherical hydrogen with an enhanced density in the forward hemisphere as suggested by Blum and Fahr could produce the observed asymmetry in scattered Lyman α. A quantitative comparison of the data with the theory is needed and may contribute to a better understanding of the picture of the neutral hydrogen distribution in the vicinity of the solar system, which is still not clear.

This work was carried out under the auspices of the US Atomic Energy Commission.

W. H. CHAMBERS
P. E. FEHLAU
J. C. FULLER
W. E. KUNZ

Los Alamos Scientific Laboratory,
University of California,
Los Alamos,
New Mexico 87544.

Received November 20, 1969; revised January 27, 1970.

[1] Blum, P. F., and Fahr, H. J., *Nature*, **223**, 937 (1969).
[2] Patterson, T. N. L., Johnson, F. S., and Hanson, W. B., *Planet. Space Sci.*, **11**, 767 (1963).
[3] Hundhausen, A. J., *Planet. Space Sci.*, **16**, 783 (1968).
[4] Fuller, J. C., Chambers, W. H., Fehlau, P. E., and Kunz, W. E., *Trans. Amer. Geophys. Un.*, **49**, 254 (1968).

Lunar Rilles and a Possible Terrestrial Analogue

CALCULATIONS by several authors suggest that the Moon, like the Earth, could not have retained a residue of its primordial atmosphere[1-5]. Gilvarry[6-8], however, has considered the possibility of a lunar hydrosphere and of an early lunar atmosphere replenished, like that of the Earth, from within. On this theory the sinuous rilles (Figs. 1 and 2) might be ancient channel-scouring marks. One possibility is the melting of a frozen sub-surface hydrosphere by cometary impacts, allowing the material to flood the surface.

Cameron[9] suggested the scouring of channels by incandescent ash flows, somewhat similar to nuées ardentes. Ash-flows, nuées ardentes, ignimbrites and tuff lavas are types of pyroclastic flow whose origin and behaviour are uncertain, but there seems to be little factual evidence to support the excavation of sinuous rilles by this agency. Urey[10] remarks that the channels are too narrow and such flows could not simply vanish without trace.

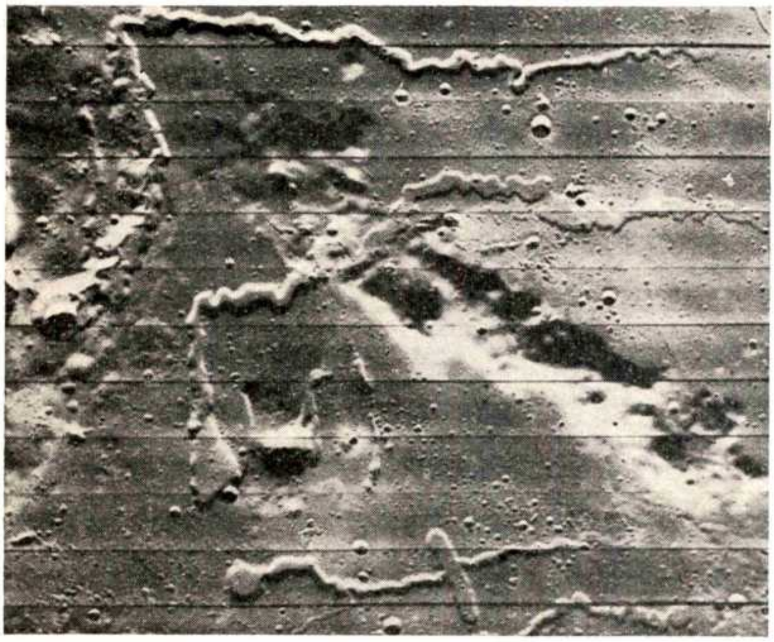


Fig. 1. Harbinger Mountains from Lunar Orbiter V showing Prinz Valleys I and II. The latter starts at a crater on the left, and runs a sinuous course for 40 miles, its width varying from 1,500 to 1,800 feet. A narrower sinuous rille runs within it, 1,000 feet wide. The main rille descends 4,000 feet during its course. Note the control of the rilles by a strong oblique NNW grain and the close genetic relation between a strange sausage-shaped crater and the sinuous rilles.

The question whether the sinuous rilles provide evidence for the movement of surface waters over the lunar surface (or other fluidized scouring agents such as ash-flows) is discussed here from an observational viewpoint.

Apart from sinuosity, there seems to be little real difference in kind between sinuous and straight rilles. Weakly sinuous rilles are a common associate of straight rilles (Figs. 3 and 4). Rilles of all types characteristically appear on dark, smooth areas forming the floors of large craters (Fig. 4), mare floors or floors of obvious trench or graben structures (Fig. 2), of tectonic origin. According to the volcanic–tectonic theory of the lunar surface[11-13], such areas are sites of volcano–tectonic subsidence and thus likely to be traversed by systems of gravity faults. Sinuous and straight rilles grade into one another and intersect, commonly with no variation in smooth floor and shoulder profile (Figs. 1 and 3). In other cases rilles of different depth and profile intersect, but the plateau surface incised by them shows no displacement (Fig. 4). Rilles of all types are commonly interrupted in their course, and even the most sinuous type shown in Fig. 2 reveals, on careful study with a magnifying lens, sectors in which there is no continuous trace but only a weak threadlike continuation, or continuation only in a chain of craterlets of the same width as the rille. These craterlets must be congenetic with the rille[14] and neither water nor a fluidized system such as an ash-flow could flow laterally through such sectors of the rilles. Rilles of all types tend to terminate, haltingly, either in thread-like tails or strings of craterlets (Figs. 1 to 3). There are many examples of undeveloped rilles, trains of craters and incompletely defined groovings (Figs. 3 and 4) and craterlet chains may parallel sinuous rilles (Fig. 3). Echelon arrangements of rilles are not uncommon. Crater floor rilles sometimes show an internal repetition of the shape of the surrounding crater wall, as in the floor of Alphonsus.

Patterns of rilles within rilles are known (Figs. 1 and 2) and may appear to originate in concentric nested craters, through fissures in their walls (Fig. 1). It has been suggested[9] that sinuous rilles always originate from craters in this way and course downhill from them, but this does not seem to be a universal pattern. In Fig. 1 one rille originates in an irregular widening and another in a level floored crater not excavated below the floor of the rille, of very similar character and profile to the widening at the tail of the upper rille in the photograph. The rilles in this photograph do not seem to follow continuous downhill courses. A sinuous rille in an Orbiter IV photo of Mare Orientalis follows the crest of a ridge. The nature of the intersection and cross-over patterns of rilles is not consistent with a scouring origin.

The sinuous deviations tend to coincide with transverse, oblique lineaments (Figs. 1 and 2) and the pattern resembles a progressive linear trace being jogged off laterally to varying degrees. The nature of the surface makes it unlikely that the rille has been displaced after formation (though this explanation cannot be entirely discounted). The more likely explanations are either that a scour channel has deviated to avoid obstructions imposed by crossing fractures or that the sinuosity reflects a mimetic response of a master fracture to intersections with minor transverse fractures, the fracture plane tending to swerve off into them. The topography in Fig. 1 is not consistent with the first explanation and, in any case, it

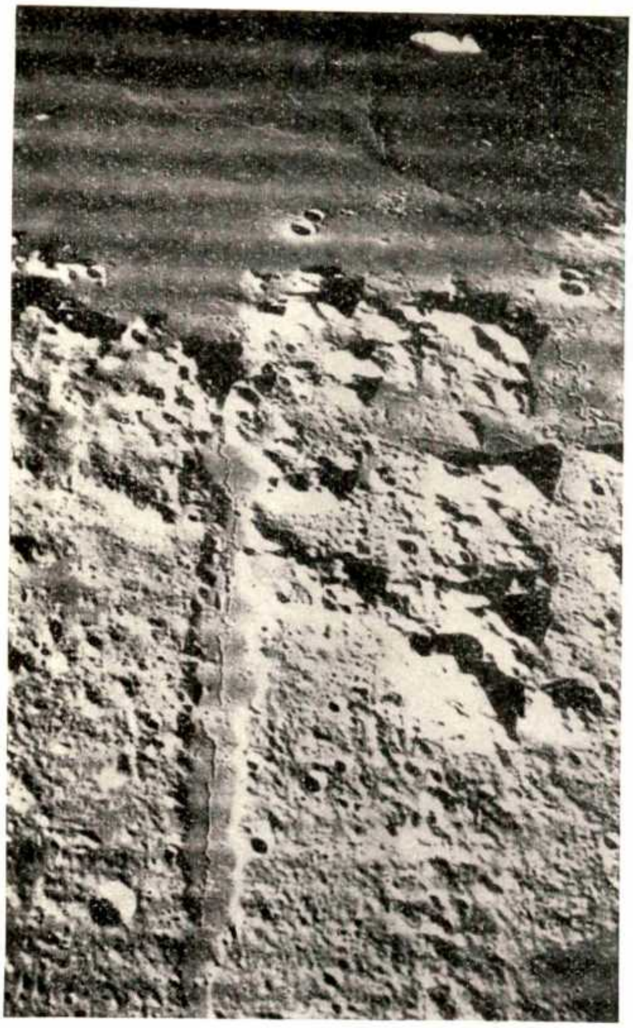


Fig. 2. A narrow sinuous rille following the course of the straight Alpine valley (which is about 25 miles wide). Lunar Orbiter V photograph.

is completely excluded by the fact that the character of the rille within the Alpine Valley in Fig. 2 is interrupted and quite inconsistent with a water scouring process.

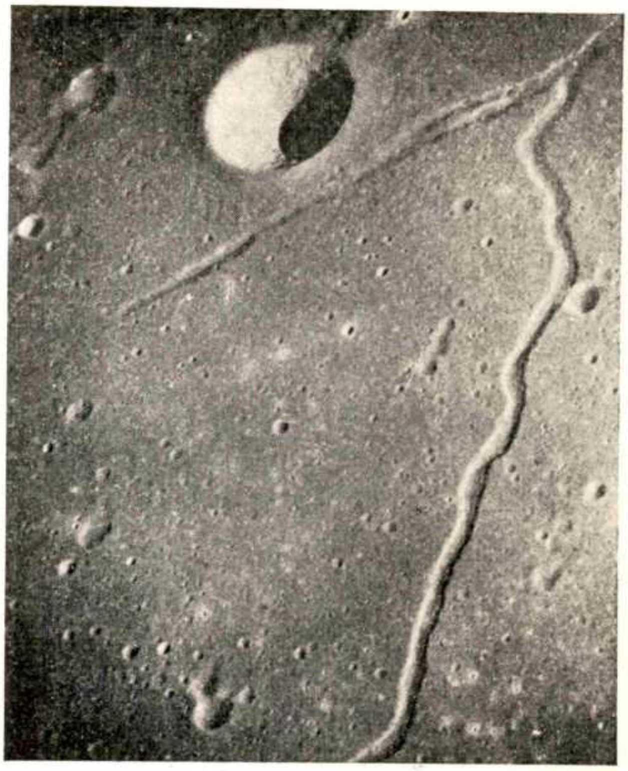


Fig. 3. Diamond-back rille from Apollo 10, a feature close to the Apollo 11 landing site. Note the obvious congenetic relationship of straight and sinuous rilles. No scale given.

My conclusion is that all rilles are reflexions of fractures in the lunar "crust". In some cases the fracture has imperfectly breached the surface (interrupted sections, undeveloped rilles). The craters that punctuate the rilles may show a systematic relationship to rille intersections. Their existence is explained if we interpret the rilles as escape channels for volcanic gases. The gas emission may have been distributed evenly along the fracture line producing a fissure conduit, or a localization of gas emission may have occurred where fractures meet. This pattern, of course, simply repeats the fundamental bimodal control of fissure and central eruption on Earth. The even floor of the rilles in Fig. 1 is, perhaps, surprising, but its character, identical with the surrounding terrain, is not inconsistent with the hypothesis that the present surface has been derived not from the lateral flow of lavas but from a process of "weathering from within" by volcanic gases, bringing up a small amount of solid material with them[13]. The resemblance of the Alpine valley to the Red Sea structure, except for the sinuosity of the internal furrow, may not be fortuitous. This and other rille structures may represent a very feeble process akin to the terrestrial continental dispersion operating long ago in the Moon.

There is some support in a terrestrial analogue for the hypothesis that all rilles are congenetic and reflect volcano-tectonic fractures up which gas has escaped. I have already referred to systems of blowhole craters strung out along closely spaced Quaternary grid faults on the southern slopes of Silali Volcano, Kenya (Fig. 2, ref. 13). On the north-eastern lower slopes of the same volcano there are sinuous fissures, apparently "originating" in large blowhole craters in a similar way to those shown in Fig. 1, and close examination with a magnifying

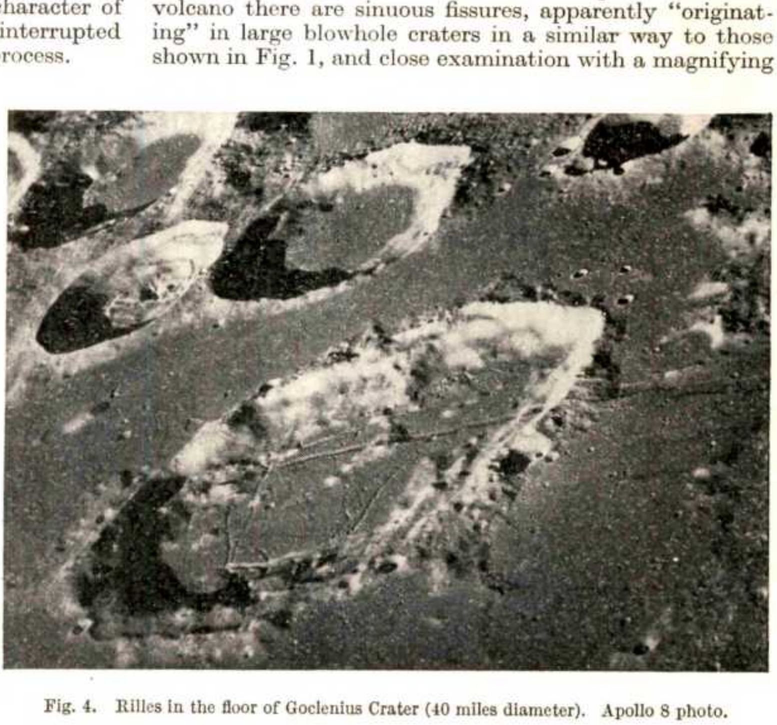


Fig. 4. Rilles in the floor of Goclenius Crater (40 miles diameter). Apollo 8 photo.

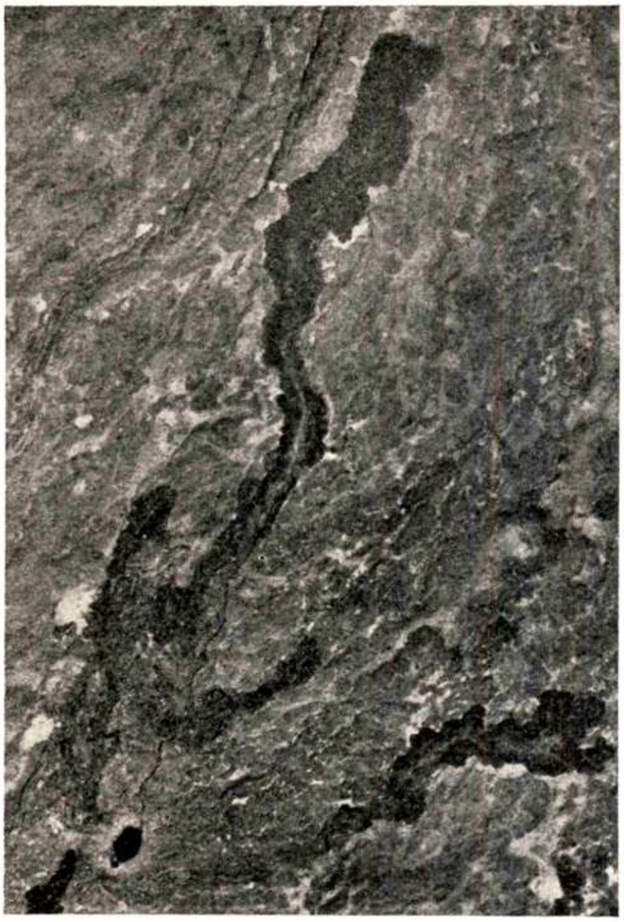


Fig. 5. Blowhole cratering and sinuous fissure association, Silali Volcano, north-east foot, Kenya. The larger blowhole crater (lower left) is slightly more than 100 m in diameter. Sinuous fissures project northwards from it and eastwards from the smaller crater below it. (Photograph, Hunting Aviation, flights for Directorate of Overseas Surveys, Tolworth.)

lens reveals chains of minute blowhole craters along the length of the sinuous fissures (Fig. 5). These fissures cut across the margins of the most recent basalt tongues, and were clearly only channels for the escape of terminal gas. This seems to be a valid analogue of the sinuous rille, and moreover the sinuous fractures are associated with linear fractures in a zone of volcano-tectonic subsidence forming part of an anorogenic zone unquestionably related to the break-up of the continents.

My conclusion is that sinuous rilles are not water scouring patterns, nor are they products of lateral scouring by fluidized systems akin to ash-flows. They are volcano-tectonic fracture traces which are sites of gas emission.

G. J. H. McCall

University of Western Australia.

Received September 30; revised December 8, 1969.

[1] Suess, H. E., *J. Geol.*, **76**, 692 (1949).

[2] Brown, H., in *The Atmosphere of the Earth and Planets*, second ed. (edit. by Kuiper, G. P.), 258 (Univ. Chicago Press, 1952).

[3] Jeans, J. H., *The Dynamical Theory of Gases*, third ed. (Cambridge Univ. Press, 1921).

[4] Baldwin, R. B., *The Face of the Moon* (Univ. Chicago Press, 1949).

[5] Rubey, W. W., in *Crust of the Earth* (edit. by Poldervaart, A. A.), 630 (Geol. Soc. Amer., 1955).

[6] Gilvarry, J. J., *Ann. NY Acad. Sci.*, **123**, 1061 (1965).

[7] Gilvarry, J. J., *Phys. Fluids*, **4**, 2 (1961).

[8] Gilvarry, J. J., *Phys. Fluids*, **4**, 8 (1961).

[9] Cameron, W. S., *J. Geophys. Res.*, **69**, 2423 (1964).

[10] Urey, H. C., *Nature*, **216**, 1094 (1967).

[11] McCall, G. J. H., *Adv. Space Sci.*, **8**, 1 (1966).

[12] McCall, G. J. H., *Ann. NY Acad. Sci.*, **123**, 843 (1965).

[13] McCall, G. J. H., *Nature*, **223**, 275 (1969).

[14] Gilbert, G. K., *Bull. Phil. Soc. Wash.*, **12**, 24 (1893).

Gaseous Species in Equilibrium with the Apollo 11 Holocrystalline Rocks during their Crystallization

The holocrystalline rocks from the Apollo 11 landing site in Mare Tranquillitatis characteristically contain vesicles and vugs[1] which must have been maintained by an internal gas pressure balancing the overburden pressure. Although the landing site may contain rare fragments from distant craters[1], the common rock types of the Apollo 11 sample are probably of local origin. There are no craters closer than about 50 km which could have excavated samples from more than a few hundred metres depth. The groundmass grain size of the holocrystalline rocks, which is from 0·1 to 1·0 mm, and up to 3 mm in vuggy areas[1], also suggests extrusive or hypabyssal crystallization for these rocks. Crystallization probably took place at depths greater than a few centimetres and no more than two kilometres. The total pressure, which was equal to the gas pressure in the vesicles and vugs of the Apollo 11 rocks, was therefore between 0·01 and 100 atmospheres.

The assemblage iron-troilite-ilmenite has been tentatively identified in the lunar holocrystalline rocks[1]. This assemblage places restrictions upon both the p_{O_2} and p_{S_2} of the rock, and the gas in the voids, provided that equilibrium conditions prevail.

Webster and Bright[2] determined $p_{O_2} = 10^{-12 \cdot 4}$ to $10^{-13 \cdot 0}$ as the stability range for the assemblage iron-ilmenite at 1,200° C. Assuming a slope of 1 log unit per 100°, this range becomes $p_{O_2} = 10^{-13 \cdot 1}$ to $10^{-13 \cdot 7}$ at 1,127° C (1,400 K) and $p_{O_2} = 10^{-14 \cdot 1}$ to $10^{-14 \cdot 7}$ at 1,027° C (1,300 K). These temperatures bracket the solidification range of terrestrial basalts and probably that of the lunar rocks also. Although the lunar melts may have reached much higher temperatures, p_{O_2} and p_{S_2} were not controlled until all members of the assemblage iron–troilite–ilmenite had crystallized.

Solid solution of Ni in the Fe would raise the allowable oxygen pressures. Ni is a very minor constituent of the lunar samples, however – 320 p.p.m. or less in all of the published holocrystalline rock analyses[1], and less than 1 p.p.m. in over half of them. The effect of Ni will therefore be neglected.

Table 1. PARTIAL PRESSURES AND PARTIAL PRESSURE RATIOS OF IMPORTANT GASES IN THE PRESENCE OF GRAPHITE

	1,300 K		1,400 K	
Log p_{O_2}	−14·1	−14·7	−13·1	−13·7
Log (p_{H_2O}/p_{H_2})	+0·01	−0·29	−0·20	−0·50
Log $(p_{CH_4}/p_{H_2}{}^2)$	−2·11	−2·11	−2·37	−2·37
Log p_{CO}	2·05	1·75	2·22	1·92
Log p_{COS}	−1·68	−1·98	−1·36	−1·66
Log p_{CO_2}	1·82	1·22	1·69	1·09

Data for the free energy of formation for FeS given by Larimer[3] yield $p_{S_2} = 10^{-5 \cdot 8}$ at 1,400 K and $p_{S_2} = 10^{-6 \cdot 6}$ at 1,300 K for the assemblage iron-troilite.

The gas in the voids is assumed to be composed of the elements H, C, N, O, S and the noble gases because no other volatile elements are likely to have been sufficiently abundant to contribute appreciably to the total gas pressure. The formation equilibrium constants tabulated in the Janaf tables[4] for the gaseous species involving these elements allow pressures of these species to be calculated from the known values of p_{O_2} and p_{S_2} and assumed pressures for the other elements. Only a small number of gaseous species, H_2, H_2O, N_2, CH_4, CO, COS, CO_2 and noble gases, can have pressures exceeding 1 per cent of the pressure of the constituent elements in the interval 1,300–1,400 K and 0·01–100 atmospheres total pressure. The pressures of the various gases, neglecting the noble gases which form no compounds, are given in Table 1, assuming graphite to be present.

In the presence of graphite, the total of p_{CO} and p_{CO_2} is 73–178 atmospheres at 1,300 K and 95–215 atmospheres at 1,400 K. The pressure range at each temperature reflects the range in possible p_{O_2}. These $CO + CO_2$ pressures correspond to a cover of 1·5 to 4·5 km of lunar mare material. It is unlikely that much of the material on the lunar mare surfaces crystallized at such great depths. Graphite is therefore unlikely to have been a stable phase in the lunar samples at these high temperatures. Although graphite could become stable at lower temperatures, the amount of carbon which could be precipitated from the trapped CO and CO_2 is exceedingly small and is probably not detectable. With the restriction that $p_{CO} + p_{CO_2}$ does not exceed the total pressure, COS cannot exceed 1 per cent of the total pressure. In the absence of graphite, the tabulated data may be simplified as shown in Table 2.

Table 2. PARTIAL PRESSURE RATIOS OF IMPORTANT GASES IN THE ABSENCE OF GRAPHITE

	1,300 K		1,400 K	
Log p_{O_2}	−14·1	−14·7	−13·1	−13·7
Log (p_{H_2O}/p_{H_2})	+0·01	−0·29	−0·20	−0·50
Log $(p_{CH_4}/p_{H_2}^2 p_{CO})$	−4·16	−3·86	−4·59	−4·29
Log (p_{CO_2}/p_{CO})	−0·23	−0·53	−0·53	−0·83
Log p_{CO} ≤	2·05	1·75	2·22	1·92

The pressures of N_2 and the noble gases are not restricted by any important gaseous reactions. Several conclusions may be drawn from these data: (1) Graphite is stable only at total pressures above about 70 atmospheres and is therefore probably absent from the Apollo 11 samples. (2) Only H_2, H_2O, N_2, CH_4, CO, CO_2, and noble gases are likely to contribute significantly to the vesicle gas pressure. (3) H_2 is more abundant than H_2O and CO is more abundant than CO_2, as would be expected in a highly reducing environment. (4) CH_4 can only be a significant constituent at the higher pressures. (5) N_2 and the noble gases are not significantly restricted by gaseous reactions.

I acknowledge support by the National Research Council—NASA resident research associateship programme at the Manned Spacecraft Center, Houston, Texas.

T. R. WELLMAN

Geochemistry Branch,
NASA Manned Spacecraft Center,
Houston, Texas.

Received December 15, 1969.

[1] Lunar Science Preliminary Examination Team, *Science*, **165**, 1211 (1969).
[2] Webster, A. H., and Bright, N. F. H., *J. Amer. Ceram. Soc.*, **44**, 110 (1961).
[3] Larimer, J. W., *Geochim. Cosmochim. Acta*, **31**, 1215 (1967).
[4] Janaf, *Thermochemical Tables* (Dow Chemical Company, Midland, Michigan, 1964).

Meteoritic Spherules in the Soil surrounding Terrestrial Impact Craters

ANALYSES of lunar material returned by the Apollo missions[1] will raise many questions regarding the formation of splatter particles distributed about the lunar surface by meteoritic impacts, and we report here some results of our searches for and studies of microscopic (10–300 μm) meteoritic particles in the soil surrounding five terrestrial meteorite craters: Sikhote-Alin, Canyon Diablo, Odessa, Boxhole and Henbury. Our aim was to search for particles that had their origin in the cratering process and to investigate their chemical and physical properties. Because of the difficulties of identifying small particles in soil samples, we have limited our attention so far to the magnetic component. Thus our results bear only on the problem of the formation of impact particles from iron rather than from stony meteorites and that contain at least some material from the parent meteorite. We do not report here any information relevant to the problem of the nonmeteoritic impact products or those produced by impacts of chondritic meteorites. It should be noted that virtually all recognized impact craters on the Earth are produced by iron meteorites, a fact that apparently can be ascribed to the greater erosion rate for chondrites, both in interplanetary space and in the terrestrial atmosphere. The lunar surface is expected to show a greater percentage of chondritic craters than is found on Earth.

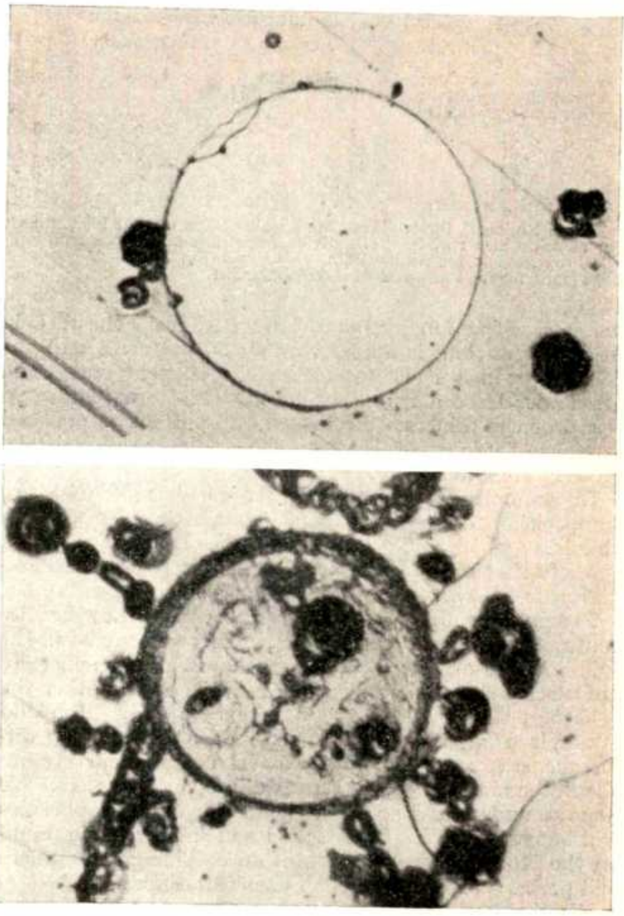


Fig. 1. Photomicrographs of polished sections of two meteoritic impact spherules from the Henbury craters. Top: a typical particle (170 μm diameter) of composition (a) (Table 1); bottom: a particle (150 μm diameter) of composition (b). The black areas (bottom) are cavities in the particle and spots made by the probe beam in the epoxy of the mount.

Table 1 summarizes the results of chemical analyses of meteoritic spherules from the soil surrounding the Henbury and Sikhote-Alin craters. For the Odessa craters we have not found any spherules, and those found in our Boxhole samples have not been completely analysed. (A preliminary report was given by P. W. H. and F. W. R. at the thirtieth annual meeting of the American Meteoritical Society, 1967.) Chemical analyses are by electron microprobes either at the Smithsonian Astrophysical Observatory or at the Advanced Metals Research Corporation. For comparison, similar particles from the Canyon Diablo craters are also described in Table 1; for them we have quoted chemical compositions reported from gross chemical analyses by Nininger[2] and by Mead, Littler and Chao[3]. For the Sikhote-Alin craters, we give data taken from our previous reports[4,5] on particles supplied to the Smithsonian Astrophysical Observatory by Krinov.

The spherical particles found in the soil near the Sikhote-Alin and Canyon Diablo craters are very different from those from the soil of the craters at Henbury. The former are almost purely metallic and/or iron oxide, while the latter are a mixture of iron and nickel from the meteorite and other elements from the soil. In the case of the Henbury samples, the spherules are divided primarily into two chemical families—one in which the

Table 1. PROPERTIES OF METEORITE CRATER SPHERULES

Crater(s):	Henbury		Sikhote-Alin	Canyon Diablo*
Location:	Australia		USSR	Arizona
Chemical composition: (per cent by weight)	(a)†	(b)†		
Al	7	4	0	0
Si	25	13	0	?
Fe	17	34	~55	56
Ni	0·75	1·4	2·5	9
No. of particles analysed	7	16	4	676

* From Nininger[2], for total sample normalized to a total of 100 per cent. For more detailed chemistry of individual spherules and separate mineralogical constituents, see Mead *et al.*[3].

† Figures for the two principal chemical families.

meteoritic fraction by mass, estimated from the nickel–iron composition, is approximately 20 per cent, and another in which it is approximately 10 per cent. The remainder have a composition that can be explained in terms of the minerals in the soil. Fig. 1 shows polished sections of two spherules from the Henbury samples, one of each chemical type.

In comparing the analyses of our spherules with the composition of the Henbury meteoritic glass[6], large chunks of which make up a layer at the Henbury craters[7], we note that our spherules are significantly richer in iron and nickel than is the meteoritic glass.

In summary, we find for three crater areas that spherules containing iron and nickel from the meteorite exist in the soil. At two of these crater sites, however, the spherules are mostly metallic or iron oxide, while at the other the spherules show a mixture of meteoritic nickel–iron and minerals from the soil. The reason for this striking difference is not known, and we feel that similar studies should be made of other terrestrial meteorite craters. We hope an understanding of the production of these spherules will be useful for study of particles of similar origin found on the Moon, where the lack of an oxidizing atmosphere will perhaps provide the chief chemical differences.

We thank F. L. Whipple for support, J. A. Wood and Janice F. Bower for help with the electron-probe analysis, and the National Aeronautics and Space Administration for partial support through a grant to the University of Washington.

PAUL W. HODGE*
FRANCES W. WRIGHT

Smithsonian Institution
Astrophysical Observatory,
Cambridge, Massachusetts 02138.

Received January 23, 1970.

* Also at: Astronomy Department, University of Washington, Seattle, Washington 98105.

[1] Lunar Sample Preliminary Examination Team, *Science*, **165**, 1211 (1969).

[2] Nininger, H. H., *Arizona's Meteorite Crater*, 88 (American Meteorite Laboratory, Denver, 1956).

[3] Mead, C. W., Littler, J., and Chao, E. C. T., *Amer. Mineralogist*, **50**, 667 (1965).

[4] Wright, F. W., Hodge, P. W., and Langway, jun., C. C., *J. Geophys. Res.*, **68**, 5575 (1963).

[5] Hodge, P. W., Wright, F. W., and Langway, jun., C. C., *J. Geophys. Res.*, **69**, 2919 (1964).

[6] Frondel, C., *The System of Mineralogy of James Dwight Dana and Edward Salisbury Dana, Yale University 1837–1892*, **3** (John Wiley, New York, 1962).

[7] Milton, D. J., *Geological Survey Professional Paper 599-C* (US Geol. Survey, Washington DC, 1968).

Retro-reflexion

THE article by J. A. Howard[1], "Increased Luminance in the Direction of Reflex Reflexion—a recently observed Natural Phenomenon", is no doubt interesting. It may, however, be useful to point out that this "recently observed phenomenon" is identical with the *heiligenschein*, extensively discussed in the classical book of Pernter–Exner, *Meteorologische Optik*[2], with several references to earlier reports. The first observation goes back to Winterfeld[3] (1804), which can hardly be called "recent". A brief description is also found in my book[4].

The aureole around one's shadow is especially bright on a bedewed lawn, but it can also be observed on most other backgrounds. It has been noticed around the shadow of a balloon and lately Professor F. Whipple and others have seen it also from an aircraft[5]. Really recent is the recognition of the *heiligenschein* on the Apollo films and photographs. Two reports of Pohn and Wildey have already appeared[6,7] where the effect is quantitatively measured.

It is, after all, only the opposition effect, studied many times on objects of planetary astrophysics: Saturn's rings[8], the zodiacal light[9], the planetoids[10] and the Moon[11].

It would seem useful to compare and to combine all these various manifestations of the same fundamental and well understandable phenomenon.

M. MINNAERT

Sterrekundig Instituut te Utrecht,
Utrecht.

Received January 9, 1970.

[1] Howard, J. A., *Nature*, **224**, 1102 (1969).

[2] Pernter–Exner, *Meteorologische Optik*, 464 (Wien–Leipzig, 1922).

[3] Winterfeld, *Gilbert's Annalen*, 57 (1804).

[4] Minnaert, M., *Light and Colour in the Open Air*, 230 (London, 1940).

[5] Butler, *J. Opt. Soc. Amer.*, **45**, 328 (1955).

[6] Pohn, H. A., Radin, H. W., and Wildey, R. L., *Astrophys. Lett.*, **157**, L 193 (1969).

[7] Wildey, R. L., and Pohn, H. A., *Astrophys. Lett.*, **158**, L 129 (1969).

[8] Seeliger, H., in *Handbuch d. Astrophysik*, **4**, 390 (1929).

[9] Seeliger, H., in *Handbuch d. Astrophysik*, **4**, 423 (1929).

[10] Gehrels, T., *Astrophys. J.*, **123**, 331 (1956).

[11] Van Diggelen, J., *Planet. Space Sci.*, **13**, 271 (1965).

Wall Precursor Effects in Gaseous Detonation

THE response of electric probes in the region ahead of ionizing shock waves has been known for some time[1]. The distances over which precursor signals have been obtained are striking; they may vary from a few millimetres to more than one metre ahead of the shock[2]. Various explanations of electrical precursors have been proposed: (1) diffusion of electrons ahead of the shock front from the ionized region behind the front; (2) photoemission from the probe or tube wall; (3) photoionization of the upstream gas[1,3,4]. Although these explanations may well apply in certain circumstances, results of the work described here suggest that at least in some cases so-called precursor effects may be due to electrostatic charging of the shock tube walls by lateral diffusion of electrons from the shock-generated plasma.

Cylindrical brass probes, 1 mm diameter and 5 mm long, were used in an investigation of electrical phenomena in stoichiometric oxyhydrogen detonations produced in a 4 m long stainless steel tube of hexagonal cross-section. The distance between parallel faces was 25·4 mm. To facilitate removal for cleaning and renewal, the probes were sealed into 'Perspex' inserts which in turn were set

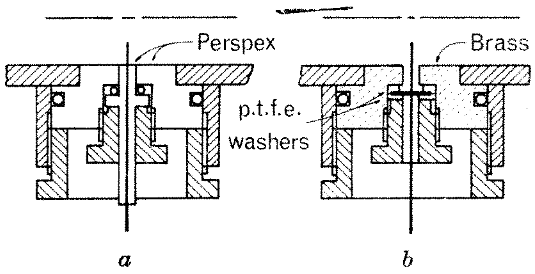


Fig. 1. *a*, Original probe holder. *b*, Modified probe holder.

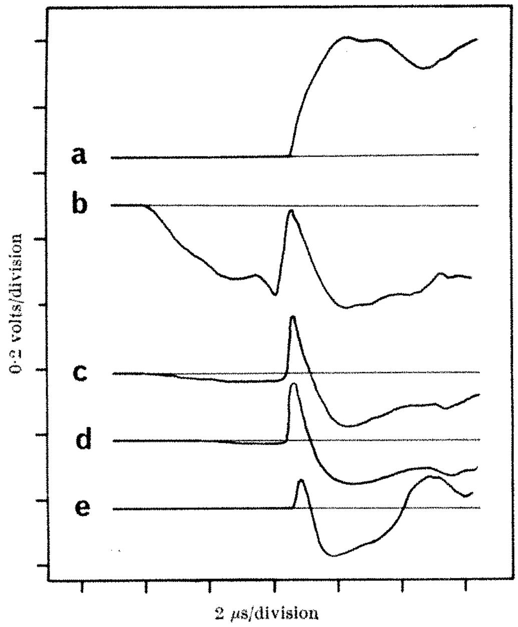


Fig. 2. Probe responses: *a*, double probe; *b–d*, single probe (cleaned before *b* only); *e*, modified probe.

into 'Perspex' windows. The windows replaced one face of the hexagonal tube for a distance of 25·4 mm as shown in Fig. 1*a*. The signal developed across a 10 kΩ load resistor connected between the probe and the earthed tube wall, which acted as a reference electrode, was observed by means of a Tektronix 551 oscilloscope. Two additional probes, separated by 1 mm, were positioned diametrically opposite the single probe and a potential of 1·5 V in series with a 10 k resistor applied between them. This double probe, which was insulated from the tube wall, recorded the time of arrival of the detonation plasma at the plane of observation. Only when both the probes and insulating surfaces were cleaned were reproducible results obtained.

Fig. 2*a* records the response of the double probe indicating the arrival of the detonation plasma at the probe position. Curves *b–d* record typical signals obtained from the single probe immediately after cleaning, and from succeeding identical detonations without cleaning in between. It may be seen that the cleaned probe responds some 4 μs before the arrival of the plasma, exhibiting a pronounced negative precursor signal. In subsequent detonations this precursor progressively disappears. The duration of the precursor signal was found to correspond to the transit time of the detonation wave (velocity 2·9 km s^{-1}) over the surface of the insulator. The influence of the insulator surface on the precursor signal was confirmed by using the modified probe holder shown in Fig. 1*b*. Here the plasma only makes contact with the insulator several microseconds after reaching the probe. With this type of probe, the response of which is shown in Fig. 2*e*, no "precursor" was ever observed in oxyhydrogen detonations.

A net negative charge is always established on insulating surfaces in contact with a plasma so as to ensure zero ambipolar current[5]. As the detonation plasma moves across the surface of the insulating section of the tube wall the insulator becomes progressively charged. While the advancing charge is being established the probe experiences an increasingly negative electrostatic field. It is thus driven to progressively greater negative potentials. The positive excursion on the arrival of the detonation at the probe has been investigated in detail and will be described elsewhere. Provided the surface charge is not disturbed, subsequent detonations are able to add little additional charge and so the precursor effect is diminished. Cleaning the insulator serves to remove this surface charge, allowing the maximum charge to be established by the succeeding detonation. The accumulated charge may also be removed by placing the exposed surface in contact with a bath of mercury. After such treatment the maximum precursor signal is obtained from the subsequent detonation regardless of the number of detonations that had preceded the immersion of the insulator. A similar procedure was used to show that possible charging of the insulator surface during cleaning had no significant effect in the production of the precursor.

Although many precursor signals can no doubt be adequately explained by the theories quoted here, the present findings show that an insulating surface can give rise to similar effects. This could be of particular importance to shock studies carried out in glass-walled shock tubes; in the case of metal shock tubes spurious precursor signals of the type discussed in this paper can be completely suppressed by a suitable design of probe holder.

M. C. CAVENOR

Department of Chemical Engineering
and Chemical Technology,
Imperial College,
London SW7.

Received November 27, 1969; revised January 2, 1970.

[1] Petschek, H., and Byron, S., *Ann. Phys.*, **1**, 270 (1957).
[2] Weymann, H. D., and Holmes, L. B., *Bull. Amer. Phys. Soc.*, **10**, 1143 (1965).
[3] Weymann, H. D., *Phys. Fluids*, **3**, 545 (1960).
[4] Appleton, J. P., *Phys. Fluids*, **9**, 336 (1966).
[5] von Engel, A. H., *Ionized Gases* (Clarendon Press, Oxford, 1965).

Self-induced Transparency and Dispersion Delays in Potassium Vapour

SELF-INDUCED transparency[1] first demonstrated in ruby[2] was subsequently reported[3] observed in gaseous SF$_6$. We wish to report the observation of this nonlinear transparency effect in the propagation through potassium vapour of coherent pulses of an organic dye laser, tuned to resonance with the λ7699 Å (ground state $4S_{1/2}$–$4P_{1/2}$) transition. Because linear and nonlinear dispersion delays may be confused with delays arising from self-induced transparency[4,5] we have also investigated dispersion effects at frequencies away from resonance. The dye laser frequency can be readily tuned[6] to resonance with transitions between single pairs of energy levels coupled to the laser field by dipole matrix elements, so complexities arising from degenerate transitions[5] are avoided. Dye lasers covering the spectral range 3000 Å to 11,000 Å are now available and nonlinear coherent pulse propagation effects can thus be studied in many absorbing atomic vapours with our experimental arrangement which does not depend on the chance coincidence of a gas or solid-state laser frequency with a suitable electric-dipole transition in the material[2,3].

The apparatus is shown in Fig. 1. A mode-locked ruby laser, producing a train of 1 ns 100 MW peak power

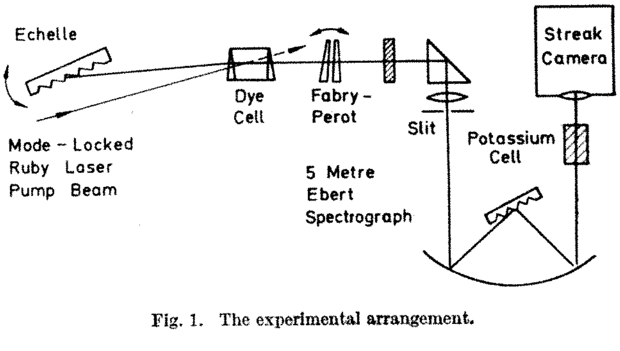


Fig. 1. The experimental arrangement.

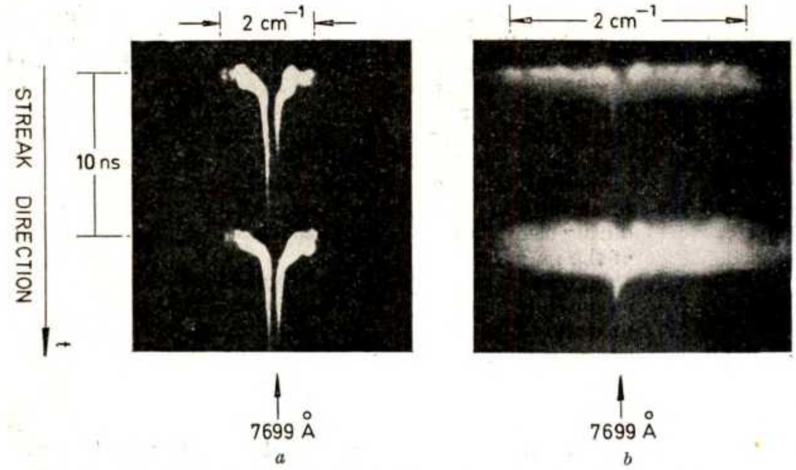


Fig. 2. *a*, Streaked dye laser spectra showing linear dispersion delays. Separation between pulses is 10 ns. Potassium cell temperature of 150° C corresponds to $N \sim 2 \times 10^{13}$ cm^{-3}. Laser power at cell ~200 W per pulse. *b*, Self-induced transparency delays for $N \sim 5 \times 10^{11}$ cm^{-3}. Laser power at cell ~3 kW per pulse.

pulses, pumped, in the near longitudinal configuration[6], a matched dye laser cavity[7]. A rotatable echelle diffraction grating reflector provided both narrowing and tuning of the dye laser output spectrum. Using a 10^{-4} M solution of 1,1′ diethyl-γ-cyano 2,2′ dicarbocyanine tetrafluoroborate in pyridine in a 3 cm wedged-window cell, a train of 1 ns 5 MW pulses at intervals of 10 ns was obtained.

Inserting in the dye laser cavity a tilted Fabry–Perot etalon of plate reflectivity 65 per cent and free spectral range 1 cm^{-1} results in a narrowing of the 2 cm^{-1} wide spectrum of these pulses into two spectral components separated by the interferometer free spectral range and each of width $\sim 5 \times 10^{-2}$ cm^{-1} (ref. 8). With the interferometer tilted at $\sim 10^{-2}$ radians to the output mirror, accurate frequency tunability and stability to $\pm 2{\cdot}5 \times 10^{-2}$ cm^{-1} were obtained. The dye laser spectrum was focused on a 30 μm slit of a 5 m Ebert echelle spectrograph (dispersion 3 mm per Å and limiting resolution $1{\cdot}5 \times 10^{-2}$ cm^{-1}). A 6 cm path length potassium vapour cell was placed inside the spectrograph 50 cm in front of the focal plane. This position improved both the laser beam uniformity at the cell and the overall optical efficiency. Time resolution was achieved by streaking the spectrum with an image tube camera recording on 3,000 ASA polaroid film.

With a potassium cell temperature of 150° C (inhomogeneous line width 3×10^{-2} cm^{-1}) and the 2 cm^{-1} spectral width output of the dye laser (peak pulse power at the potassium cell ~200 W) we obtained streaked spectra as shown in Fig. 2*a*. It can be seen that the pulse delays increase rapidly as the frequency approaches resonance with the λ7699 Å transition, becoming > 10 ns at $7{\cdot}5 \times 10^{-2}$ cm^{-1} from exact resonance. At this point the streaked pulse intensity falls below the polaroid threshold. These delays are just those to be expected from linear dispersion theory[9]. It can be easily shown that far from resonance the small signal pulse delay is

$$\tau_D = \frac{\pi N e^2 f l}{mc}\,\frac{l}{(\omega_0 - \omega)^2} = S(\omega_0 - \omega)^{-2} \qquad (1)$$

where l is the path length, N is the particle density, f is the oscillator strength and ω_0 is the resonance frequency of the potassium transition. For potassium vapour at 150° C, $N \simeq 2 \times 10^{13}$ cm^{-3}, $f = 0{\cdot}33$ and $l = 6$ cm gives $S \simeq 10^{12}$ s Hz^{-2}. An experimental plot of pulse delay τ_D as a function of $(\omega_0 - \omega)^{-2}$ gives a slope of $2{\cdot}5 \times 10^{12}$ s Hz^{-2}, in reasonable agreement with the calculated value of S. (The value of N, which is affected by any uncertainty in the cell temperature measurement, is not known to better than this accuracy.) When the laser beam power at the potassium cell was increased by a factor of 25 (to 5 kW) the pulse delays decrease to ~3 ns at $\sim 4 \times 10^{-2}$ cm^{-1} from resonance, with saturation of the absorption.

Reducing the cell temperature to 100° C, corresponding to $N \simeq 5 \times 10^{11}$ cm^{-3}, greatly reduced the dispersive delays as expected from (1), but delays of 2 ns were observed exactly on resonance as shown in Fig. 2*b*. The results obtained with the interferometer frequency narrowed laser beam are shown in Fig. 3. The set of higher frequency pulses on the left were 1 cm^{-1} off resonance and provided time reference points on the streaked spectrum. The spectra of the first and last of the resonance frequency pulses show absorption, while the other pulses show narrow delay tails exactly on resonance. A maximum delay of 4 ns occurred on the second pulse, which was the most intense with ~100 W total power at the absorption cell. The observation of absorption without delay, and delays which increased with pulse intensity, rules out the presence of any simple dispersion effects[9]. The individual pulse duration, τ_p, of ~1 ns satisfies the condition $\tau_p \ll T_2'$ (ref. 2). (T_2', the dephasing relaxation time, is determined mainly by the spontaneous lifetime of 28 ns of the transition.) Calculating the threshold for self-induced transparency from the condition[2,3]

$$2p/\hbar \int_{-\infty}^{+\infty} \varepsilon(t)\,\mathrm{d}t = n(2\pi) \qquad (2)$$

and putting $n = 1$, $p = 7{\cdot}2 \times 10^{-18}$ e.s.u. gives a threshold power of ≈30 W for $\tau_p = 1$ ns for linearly polarized light in a single laser mode. Using the threshold pulse delay

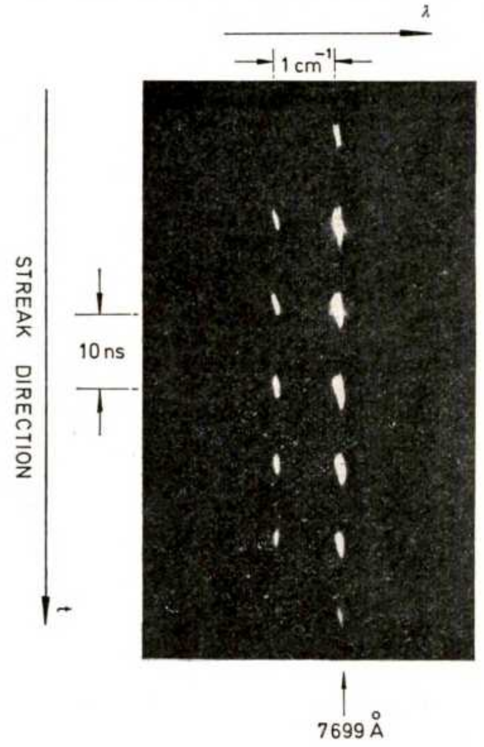


Fig. 3. Self-induced transparency delays with narrow band (5×10^{-2} cm^{-1}) dye laser tuned to resonance with potassium $4S_{1/2}$–$4P_{1/2}$ transition. The second, higher frequency, set of pulses provide time reference points on the streaked spectrum. Pulses are separated by 10 ns. Laser power ~100 W per pulse.

result of McCall and Hahn[2],

$$\tau_D = \alpha l \tau/2 \qquad (3)$$

with α, the small signal absorption coefficient $= 4{\cdot}5$ cm^{-1} ($N = 5\times10^{11}$ cm^{-3}), $l = 6$ cm, and τ, the pulse width, $= 1$ ns, gives $\tau_D = 13{\cdot}5$ ns. Relation (3) was derived assuming plane wave conditions. Any intensity variations across the laser beam, phase modulation or lack of coherence would affect both the output pulse shape and the delay.

These results show that frequency tunable dye lasers have many advantages for experiments involving optical propagation effects including linear and nonlinear dispersion, self-induced transparency and photon echoes[10].

We thank the Science Research Council for the provision of equipment and technical support. Two of us (P. D. S. and G. M. G.) were supported by postgraduate studentships from SRC and the Ministry of Education for Northern Ireland respectively. The special laser mirrors were manufactured by Mr D. Blanc. This work was reported at the Belfast Conference on Nonlinear Optics, September 1969.

D. J. Bradley
G. M. Gale
P. D. Smith

Department of Pure and Applied Physics,
The Queen's University of Belfast,
Belfast BT7 1NN, Northern Ireland.

Received December 8, 1969.

[1] McCall, S. L., and Hahn, E. L., *Bull. Amer. Phys. Soc.*, **10**, 1189 (1965).
[2] McCall, S. L., and Hahn, E. L., *Phys. Rev. Lett.*, **18**, 908 (1967).
[3] Patel, C. K. N., and Slusher, R. E., *Phys. Rev. Lett.*, **19**, 1019 (1967).
[4] Courtens, E., and Szöke, A., *Phys. Lett.*, **28A**, 296 (1968).
[5] Rhodes, C. K., Szöke, A., and Javan, A., *Phys. Rev. Lett.*, **21**, 1151 (1968).
[6] Bradley, D. J., Gale, G. M., Moore, M., and Smith, P. D., *Phys. Lett.*, **26A**, 378 (1968).
[7] Bradley, D. J., and Durrant, A. J. F., *Phys. Lett.*, **26A**, 73 (1968).
[8] Bradley, D. J., Durrant, A. J. F., Gale, G. M., Moore, M., and Smith, P. D., *J. Quant. Electron*,. *Q.E.-4*, 707 (1968).
[9] See, for example, Brillouin, L., *Wave Propagation and Group Velocity*, 121 (Academic Press, New York and London, 1960).
[10] Kurnit, N. A., Abella, I. D., and Hartmann, S. R., *Phys. Rev. Lett.*, 13, 567 (1964).

Electronic Properties of Well Oriented Graphite

It has recently become apparent that conventional interpretations[1,2] of Hall effect and magnetoresistive phenomena in graphite are consistent neither with one another nor with current ideas on the locations of electrons and holes within the Brillouin zone. Our Shubnikov–de Haas measurements on irradiated pyrolytic and single-crystalline graphite support the reassignment of the positions of the electron and hole Fermi surfaces made by Schroeder *et al.*[3]. We have also obtained evidence that apparent contradictions between the low field Hall coefficients of pyrolytic and single-crystalline graphites[1,2] represent genuine extremes of behaviour. Studies in this laboratory of the anisotropy of the pressure dependence of conduction[4,5] have suggested that electron-hole scattering of majority carriers is more important than carrier–phonon interactions[6–8] in the basal planes, while at the same time the drag effect of majority on minority carriers[9] is unimportant in explaining the low field Hall effect. A potentially confusing situation has thus arisen.

Elegant magneto-optical experiments[3] have shown that the curves of E against $\boldsymbol{k}$ (refs. 1 and 2) as conventionally plotted along the Brillouin zone edge HKH should in effect be inverted so that the Fermi surface centred on K (de Haas–van Alphen period[10,11] about $1{\cdot}6\times10^{-5}$ G^{-1}) encloses electron states, and the two outer Fermi surfaces around H (de Haas–van Alphen period about $2{\cdot}1\times10^{-5}$ G^{-1}) now represent hole states. The assignments of cyclotron masses are also interchanged such that the electron mass is about $0{\cdot}057\ m_0$ and the hole masses range from high values through $0{\cdot}039\ m_0$ at the extremal orbit to about $10^{-3}\ m_0$ at H. The carriers thus comprise heavy majority electrons and holes together with light minority holes.

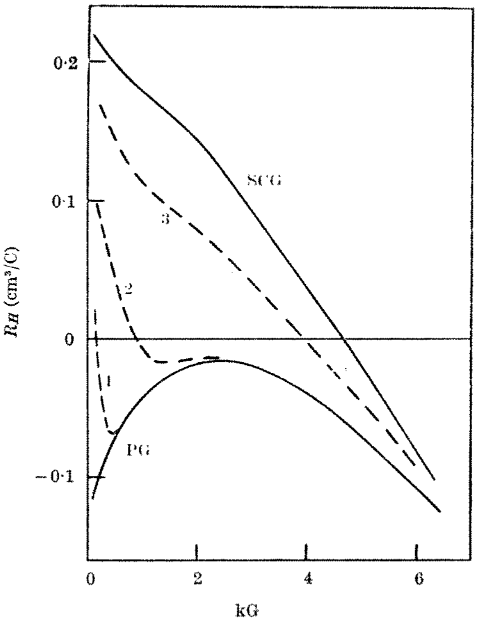


Fig. 1. The low-field Hall coefficient measured in the basal plane at 77 K for single-crystalline graphite (SCG) by Soule[2] and for well oriented pyrolytic graphite (PG) by Spain[1]. The dashed curves 1, 2 and 3 represent three pyrolytic graphites of increasing crystallite size.

The association of majority electrons and holes with the short and long de Haas–van Alphen periods respectively is confirmed by our observations of shifts in these periods induced by light pre-irradiation with fast neutrons (< 10^{17} neutrons cm^{-2} at 30° C). Pre-irradiation produces acceptors which become negatively charged, thus lowering the Fermi energy. According to the conventional (before 1968) band model, the de Haas–van Alphen periods should diverge with increasing neutron dose. In fact, they converge, supporting the new locations of electrons and holes. Furthermore the Dingle temperature of the "new holes" increased more than that for the "new electrons" in agreement with the expectation that negatively charged centres scatter holes more strongly than electrons[12].

These findings necessitate a complete reassessment of the field-dependence of the Hall coefficient. It had been thought[1] that either the low-field characteristics of single crystalline and pyrolytic graphite were fundamentally different, or else one set of data was invalid. Our observations, however, show that if the mean free path in pyrolytic graphite is systematically increased by recrystallization, then the extremes of behaviour can be bridged, as illustrated schematically in Fig. 1 for 77 K.

We conclude that both sets of results are valid, and that the positive (SCG) zero-field coefficient represents long mean free paths, whereas the negative coefficient (PG) results from crystallite boundary scattering.

Using the original (before 1968) version of the effective mass distribution[1,2], the PG result could be easily explained in terms of independent heavy majority carriers and light minority electrons, whereas the SCG result appeared anomalous. Nevertheless, Sugihara[13] managed to account for the positive zero-field coefficient in SCG by introducing a drag effect on the extremely mobile minority electrons by majority holes, thus reversing the sign of their mobility. He found majority electron-hole scattering to be unimportant, at least at the lowest temperatures where the carriers are degenerate. We agree with Sugihara on this last point.

With the new effective mass distribution it is the SCG result which receives a natural explanation on an independent particle model. It is also clear that a reduction of the mean free path will eliminate the strong positive trend at the lowest fields by reducing the mobility of light holes, but an explanation of the strong negative trend now causes difficulty, for here the mosaic boundary scattering which is characteristic of PG precludes the introduction of drag terms. In any event it is unlikely that light carriers which are so strongly influenced by heavy carriers as to have their effective sign reversed will retain a high mobility. It may well be, however, that the general shape of the Hall curves for PG can be interpreted using the majority electron-hole scattering terms which have helped to explain behaviour under pressure of specimens containing charged point defects and mosaic boundaries[4,5] in the nondegenerate region, the essential point being that an attractive elastic scattering event via a screened coulombic field is stronger than a repulsive one[12].

J. D. Cooper
J. Woore
D. A. Young

Department of Chemical Engineering
and Chemical Technology,
Imperial College,
London SW7.

Received December 4, 1969.

[1] Spain, I. L., Ubbelohde, A. R., and Young, D. A., *Phil. Trans. Roy. Soc.*, A, **262**, 345 (1967).

[2] Soule, D. E., *Phys. Rev.*, **112**, 698 (1958).

[3] Schroeder, P. R., Dresselhaus, M. S., and Javan, A., *Phys. Rev. Lett.*, **20**, 1292 (1968).

[4] Yeoman, M. L., and Young, D. A., *J. Phys. C (Solid St. Phys.)*, **2**, 1742 (1969).

[5] Howell, J. A., Yeoman, M. L., and Young, D. A., *Third Conf. Ind. Carbon and Graphite, Soc. Chem. Ind. London* (April 1970).

[6] Sugihara, K., and Sato, H., *J. Phys. Soc. Japan*, **18**, 332 (1963).

[7] McClure, J. W., and Smith, L. B., *Proc. Fifth Carbon Conf.*, **2**, 3 (1963).

[8] Young, D. A., *Carbon*, **6**, 135 (1968).

[9] McLean, T. P., and Paige, E. G. S., *J. Phys. Chem. Solids*, **18**, 139 (1961).

[10] Soule, D. E., McClure, J. W., and Smith, L. B., *Phys. Rev.*, **134**, A453 (1964).

[11] Williamson, S. J., *et al.*, *Solid State Comm.*, **4**, 37 (1966).

[12] Blatt, F. J., *J. Phys. Chem. Solids*, **1**, 262 (1956).

[13] Sugihara, K., *J. Phys. Soc. Japan*, **21** (Suppl.), 324 (1966).

Interaction of Atomic Hydrogen and Nitrogen with Graphite Surfaces

Recent optical studies of the topographical changes occurring at graphite surfaces during oxidation indicate that the cores of non-basal dislocations are often the preferred sites for attack by O_2 molecules[1]. The orientation of the hexagonal etch pits on the graphite basal plane reveal that below 800° C the oxidation rate is faster in the $\langle 10\bar{1}0\rangle$ than in the $\langle 11\bar{2}0\rangle$ directions, whereas the reverse is true above 1,000° C (ref. 2).

Atomic oxygen reacts with graphite to cause pitting uncharacterized by any particular symmetry[3]. In direct contrast to this behaviour, we have observed the formation of regular hexagonal pits on the cleavage surfaces of graphite crystals on exposure of such surfaces to either atomic hydrogen or atomic nitrogen.

Our experiments were carried out with carefully cleaved crystals of Ticonderoga graphite, which were trimmed to approximately 15×4 mm^2 before mounting in the vacuum system. The samples were first degassed in vacuum at 800° C by resistive heating between platinum clips. Purified hydrogen or nitrogen was then passed over the sample at a system pressure of 2 mm, and the temperature of the graphite was measured with an optical pyrometer. The flowing gases were dissociated with a microwave discharge (2,450 MHz) maintained by a cavity[4] located 28 cm upstream from the sample. The cleavage plane of the sample was normal to the flowing gas. During the experiment the gas flow rate, sample temperature and power input to the discharge tube were maintained constant. After termination of the run, the graphite sample was cooled in nitrogen gas and then examined optically with bright field, reflected illumination. No quantitative measurement of the atom concentration in the gas streams was attempted, but our experience suggests that degrees of dissociation of 20–30 per cent may be expected.

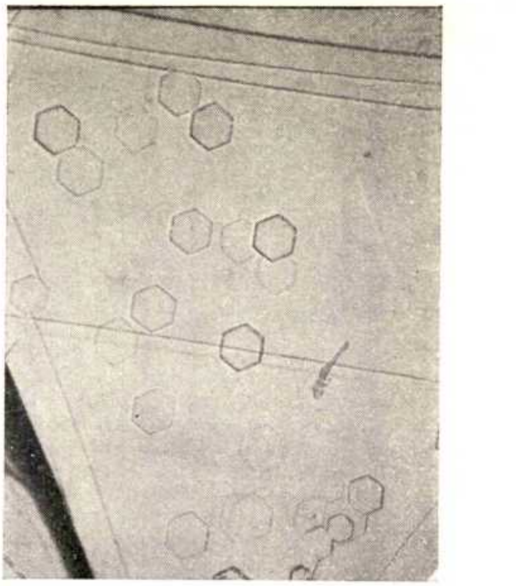


Fig. 1. Hexagonal etch pits of perpendicular orientation produced by atomic hydrogen on graphite crystal at 720° C. × *c*. 130.

Exposure of a sample at 720° C to flowing molecular hydrogen (no discharge) for 8 h produced no observable changes in the surface structure of the graphite crystals. In the presence of the discharge, however, well defined features appeared rapidly on the graphite basal plane exposed to the dissociated gas. Fig. 1 shows hexagonal etch pits formed on the graphite surface at 720° C after exposure to atomic hydrogen for 1 h. The pits were all perpendicular to the $\{11\bar{2}0\}$ twin bands[2]. That is, the sides of the pits consist of $\{10\bar{1}l\}$ prismatic faces. As with molecular oxygen above 1,000° C, atomic hydrogen reacts preferentially at the cores of the non-basal dislocations that thread the graphite crystals, the greatest rate of reaction in the basal plane being in the $\langle 1\bar{1}20\rangle$ directions. Other topographical features elicited by etching with atomic hydrogen include terraced pits, pits within pits, and pits on twist boundaries comprised of *c*-axis screw dislocations. All of these hexagonal pits are of perpendicular configuration.

In the absence of the discharge, an 8 h exposure to flowing nitrogen produced no detectable topographical changes on a graphite surface maintained at 1,100° C. With the discharge switched on, however, hexagonal etch pits were formed in many regions of the graphite basal plane at this temperature. In these conditions the pits developed the parallel orientation, as shown in Fig. 2. The preferred directions of attack in the *a*-plane are along the $\langle 10\bar{1}0\rangle$ directions. The etching behaviour of atomic nitrogen observed here is identical to that observed for diatomic oxygen below 800° C (ref. 5).

The development of macroscopic pits implies that there are significant inequalities in the chemical reactivity of the real, microscopically imperfect graphite surface. With the information now at hand, the nature and relative contributions of all the mechanistic steps that comprise these

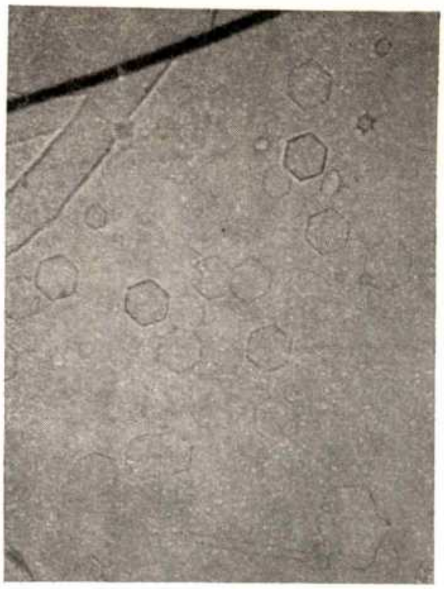


Fig. 2. Hexagonal etch pits of parallel orientation produced by atomic nitrogen on graphite crystal at 1,100° C. × *c*. 130.

reactions are not obvious. The observed differences in the effects produced by atomic oxygen and atomic hydrogen may, however, be tentatively related to the differences in the surface mobilities of these absorbed species. Adsorbed oxygen atoms are believed immobile[3] and adsorbed hydrogen atoms exhibit high mobility on a carbon surface[6]. The behaviour observed here for nitrogen atoms strongly suggests that a mobile layer is involved during the attack on the graphite crystal surface.

BRUCE MCCARROLL
D. W. MCKEE

Research and Development Center,
General Electric Company,
Schenectady,
New York 12301.

Received December 4, 1969.

[1] Thomas, J. M., *Carbon*, **7**, 359 (1969).

[2] Thomas, J. M., *Chemistry and Physics of Carbon* (edit. by Walker, jun., P. L.), **1**, 121 (Marcel Dekker, New York, 1965).

[3] Marsh, H., O'Hair, T. E., and Reed, R., *Trans. Faraday Soc.*, **58**, 285 (1965).

[4] McCarroll, B., *Rev. Sci. Inst.* (in the press).

[5] Hughes, E. E. G., Williams, B. R., and Thomas, J. M., *Rev. Sci. Instrum.*, **58**, 2011 (1962).

[6] Robell, A. J., Ballou, E. V., and Boudart, M. J., *J. Phys. Chem.*, **68**, 9 (1964).

Vapour Pressure of $H_2{}^{18}O$ Ice (I) (−17° C to 0° C) and $H_2{}^{18}O$ Water (0° C to 16° C)

WE have measured the vapour pressure isotope effect (VPIE) between $H_2{}^{18}O$ and $H_2{}^{16}O$ ice from −17° C to 0° C and have obtained results in disagreement with those reported by Matsuo and Matsubaya[1]. In order to check our measurements we have also determined the VPIE in the liquid phase and obtained results in good agreement with Szapiro and Steckel[2]. Also we have calculated from the present data an ice–liquid fractionation factor

$$\alpha_{s/l} = (H_2{}^{16}O/H_2{}^{18}O)_{\text{liq}}/(H_2{}^{16}O/H_2{}^{18}O)_{\text{solid}}$$
$$= 1{\cdot}0026 \pm 0{\cdot}0003$$

in good agreement with O'Neil's directly measured value of 1·0030 ± 0·0002 (ref. 3).

Our differential vapour pressure apparatus will be described elsewhere. The samples are contained in a common copper block whose temperature is controlled and measured to 0·001° C. We estimate that the temperature difference between samples is significantly less than 0·0002° C. The pressure differences are measured with a calibrated differential capacitance manometer. The vapour pressure of $H_2{}^{16}O$ water at each temperature can be obtained to sufficient accuracy for the present purpose from standard tables[4], but we have chosen to use a new theoretical relation derived by us which is substantiated by new measurements of the vapour pressure of $H_2{}^{16}O$ ice (results to be published shortly).

The 0·75 cm³ sample of $H_2{}^{18}O$ (H normalized) was kindly supplied to us by Dr G. Jakli of the Central Research Institute for Physics, Hungarian Academy of Sciences, Budapest. Their mass spectrometric analysis gave for oxygen, $^{18}O = 69{\cdot}0 \pm 0{\cdot}5$ per cent, $^{17}O = 1{\cdot}4 \pm 0{\cdot}2$ per cent, ^{16}O by difference. The deuterium concentration was only $1{\cdot}03 \pm 0{\cdot}01$ times normal abundance. The pressure differences were corrected to 100 per cent $H_2{}^{18}O$ assuming Raoult's law and the law of the geometric mean for $H_2{}^{16}O$, $H_2{}^{17}O$, $H_2{}^{18}O$.

In the experiments 71 points were measured in the ice range, 0° C to −17° C, and 40 points in the liquid, 0° C to 16° C, over a ten day period. The two parameter least square fits are

$$\ln(R_{\text{solid}}) = \ln\left(\frac{P_{H_2{}^{16}O}}{P_{H_2{}^{18}O}}\right)_{\text{solid}} = \frac{2{\cdot}110}{T} + 0{\cdot}00656 \qquad (1)$$
$$256 < T < 273$$

$$\ln(R_{\text{liquid}}) = \ln\left(\frac{P_{H_2{}^{16}O}}{P_{H_2{}^{18}O}}\right)_{\text{liquid}} = \frac{13{\cdot}764}{T} - 0{\cdot}03872 \qquad (2)$$
$$273 \leq T \leq 289$$

The calculated logarithms are good to ± 3 to 4×10^{-4} between 0 and −17° C, and to $\pm 2 \times 10^{-4}$ between 0 and 16° C. Our results for ice are thus consistently about 0·002–0·003 ln (R) units above those of reference 1, the only other reported measurements. In the liquid we calculate $\ln(R) = 0{\cdot}0090 \pm 0{\cdot}0002$ at 15° C in agreement with the value $\ln(R) = 0{\cdot}0097 \pm 0{\cdot}0003$ reported by Szapiro and Steckel[2] at the low temperature end of their measurements. Simultaneous solution of (1) and (2) at the melting point gives $\ln(\alpha_{s,l}) \approx \ln(R_{\text{solid}}) - \ln(R_{\text{liq}}) = 0{\cdot}0026 \pm 0{\cdot}0003$ in agreement with O'Neil's value of $0{\cdot}0030 \pm 0{\cdot}0002$. We are thus forced to conclude that Matsuo and Matsubaya[1] are some 20 per cent low in their value for $\ln(R_{\text{solid}})$ $(\alpha_{s,v})$. This is probably due to experimental difficulties arising from their extremely small (10 mg) samples.

A more complete report, including theoretical interpretation, will be submitted for publication in the near future. We acknowledge the financial support of the United States Department of Interior, Office of Water Resources Research, and the Petroleum Research Fund, American Chemical Society.

GABOR JANCSO*
JOVAN PUPEZIN†
W. ALEXANDER VAN HOOK

Chemistry Department,
University of Tennessee,
Knoxville, Tennessee 37916.

Received December 23, 1969.

---

* Permanent address: Central Research Institute for Physics, Hungarian Academy of Sciences, Budapest.

† Permanent address: Boris Kidrich Institute of Nuclear Sciences, Belgrade, Yugoslavia.

[1] Matsuo, S., and Matsubaya, O., *Nature*, **221**, 463 (1969).

[2] Szapiro, S., and Steckel, F., *Trans. Faraday Soc.*, **63**, 883 (1967).

[3] O'Neil, R., *J. Phys. Chem.*, **72**, 3683 (1968).

[4] *Handbook of Chem. and Phys.*, 46th ed., D-93 (Chemical Rubber Co., Cleveland, 1966).

Pulsatile Flow Instrument for detecting Viscoelasticity in Dilute Polymer Solutions

In very dilute solutions of high molecular weight polymers, turbulent friction may be much smaller than in corresponding flows of the solvent alone[1]. It is widely believed that this drag reduction must be associated with viscoelastic properties of the liquid, but such properties are difficult to detect in very dilute solutions. I describe here an instrument that reveals departures from Newtonian behaviour even with very low concentrations.

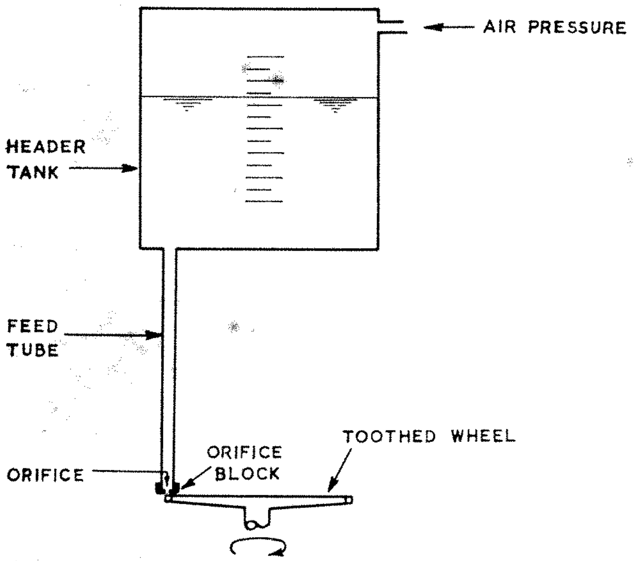


Fig. 1. Diagram of apparatus.

The instrument is sketched in Fig. 1. The liquid flows through an orifice of diameter 0·4 mm, the exit of which is obstructed periodically by a toothed wheel. The wheel has a diameter of 60 mm and 120 square cut teeth with gaps equal to the widths of the teeth. The clearance between the orifice block and the teeth is approximately 0·06 mm. The rate of flow of liquid can be found as a function of wheel speed and applied driving air pressure by timing the passage of the liquid surface in the header tank past graduated marks. The marks that showed the beginning and end of the flow in these experiments were 220 mm and 190 mm above the orifice, representing a flow quantity of $8\cdot7 \times 10^4$ mm^3.

Typical results are shown in Figs. 2–5. Fig. 2 shows the flow times as functions of wheel speed with an applied air pressure of 250 mm of water in addition to the mean gravity head of 205 mm. The results for zero speed correspond to a gap opposite the orifice. With increasing rotational speed the flow times for water at first increase and then decrease slightly. The kink in the curve is associated with liquid leaking between the orifice and the wheel. A relief of pressure occurs, with liquid spurting slightly on to the downstream edge of the orifice block. At zero wheel speed the point for Polyox *WSR* 301 solution is slightly above that for water. With increasing wheel speed the flow times at first increase more than those for water, but decrease markedly between about 1,100 and 1,500 r.p.m. This is associated with spurting, much more marked than for water, of the liquid emerging at the downstream edge of the orifice block. Liquid drops are thrown upwards and backwards from the rotating wheel so that they collect on the top of the orifice block. At higher speeds a narrow jet emerges tangentially from the downstream edge of the orifice block, and spurting ceases. It seems that elastic stresses in the fluid are released as the liquid emerges from under the block.

This behaviour is not critically dependent on mean flow rate. Fig. 3 shows similar behaviour when no air pressure was applied, the flow taking place under the gravity head alone. For more dilute concentrations of Polyox the decrease of flow time occurs at higher wheel speeds. Aged solution has a smaller decrease of flow time, postponed to a higher speed, than fresh solution. Turbulent flow rate measurements in a pipe of bore 1·7 mm, with a flow speed of about 3·3 m/s, showed the aged solution to be considerably less effective for turbulent drag reduction than the fresh solution of the same concentration.

Fig. 4 shows flow curves for other liquids at a pressure of 250 mm of water, as in Fig. 2. The curves for water are a little different in the two figures, possibly because of a slight difference in the clearance between orifice and wheel. Nevertheless, the polyacrylamide *PA* 905, which for a given concentration is a little less effective in reducing turbulent friction than Polyox *WSR* 301, shows a similar, though much less sharp, decrease in flow time at similar wheel speeds. Spurting was also observed at these speeds, though less marked than for Polyox. The whole flow curve for *PA* 905 in Fig. 4, however, is shifted bodily upwards,

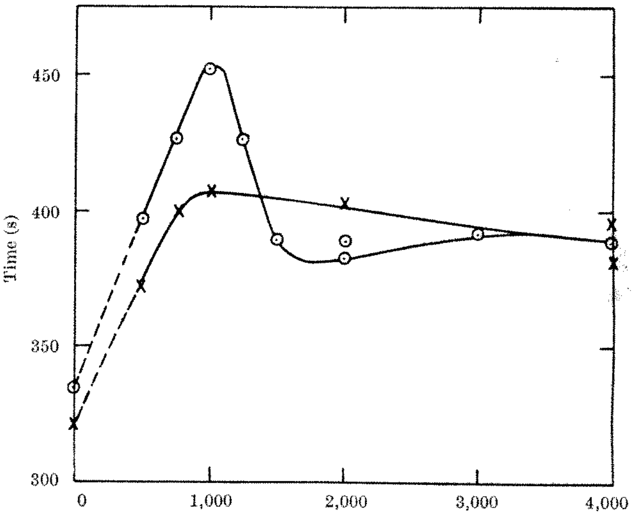


Fig. 2. Results for air pressure of 250 mm water. ×, Water; ○, 46 p.p.m. Polyox *WSR* 301.

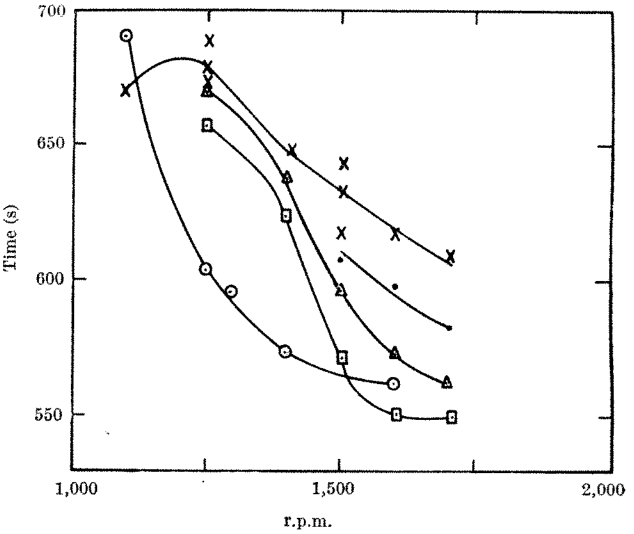


Fig. 3. Results for zero air pressure with Polyox *WSR* 301 solutions. ×, Water; ●, 2·3 p.p.m.; △, aged 23 p.p.m.; □, 23 p.p.m.; ○, 46 p.p.m.

and at zero rotational speed the polyacrylamide produces a far greater resistance to flow through the orifice than Polyox. It has been suggested[2] that such a resistance to extensional flow might be the cause of turbulent drag reduction, but this idea seems to be inconsistent with the relatively small effect of Polyox at zero wheel speed.

The micellar solution of cetrimide and α-naphthol, though effective at reducing turbulent friction at low wall shear rates[3], has a different type of flow curve, with no marked spurting. The shear rates are possibly too high for viscoelastic effects to be evident.

The guar gum solution in Fig. 4 shows a decrease in flow time at higher wheel speeds than for Polyox, as confirmed in more detail in Fig. 5. The flow curve is similar to the curves for Polyox in Fig. 3, though shifted upwards. This behaviour is associated with spurting which begins at about 1,800 r.p.m. A minimum flow time occurs at about 3,500 r.p.m., when there is a pronounced tangential jet flow. The corresponding minimum time for 46 p.p.m. Polyox takes place at about 1,500 r.p.m. The ratio of the two speeds, 7/3, is smaller than the ratio of the threshold wall shear stresses τ_{WC} for the onset of drag reduction[1] in Polyox and guar gum solutions. Lowe's measurements[4] for Polyox *WSR* 301 thus show τ_{WC} to be about 6·5 dyne/cm^2 for 2 p.p.m., decreasing to 1·5 dyne/cm^2 for 35 p.p.m. For guar gum, White[5] finds a value of about 24 dyne/cm^2. Evidently, therefore the wheel speeds for spurting flow in the present experiments do not correspond directly to dominant turbulent frequencies (proportional to wall shear stress) in the wall layer region at the onset of drag reduction. The viscoelastic effects in my experiments may, however, be associated, not with the frequency of passage of teeth past the orifice but rather with the impact of individual teeth on the emerging jet, like the effects that Metzner[6] noted. The maximum impact pressures might be expected to vary roughly as the cube of the speed, as in the experiments of ref. 7, because of the cushioning effect of the entrapped air carried around by the teeth. If turbulence dynamic pressures at drag onset, proportional to τ_{WC}, are compared with the cube of wheel speeds for spurting flow, the correlation is fair. In any case, these speeds are in the same order as the threshold shear stresses for turbulent drag reduction onset, so that my findings suggest that drag reduction occurs when turbulent frequencies close to the wall become high enough for viscoelasticity to affect the turbulence.

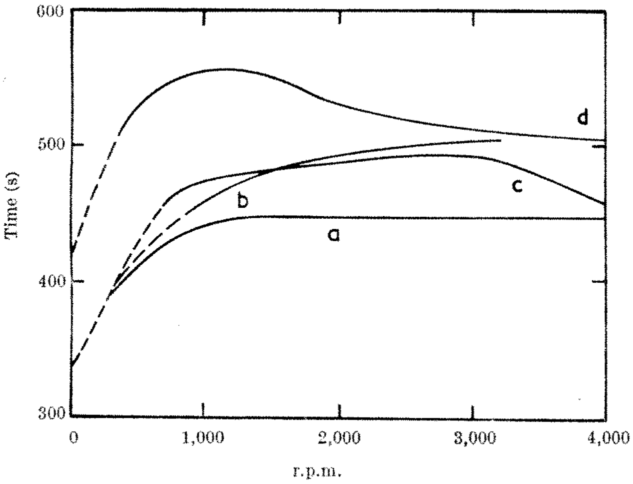


Fig. 4. Results for air pressure of 250 mm water. *a*, Water; *b*, 364 p.p.m. cetrimide, 144 p.p.m. α-naphthol; *c*, 117 p.p.m. guar gum; *d*, 35 p.p.m. *PA* 905.

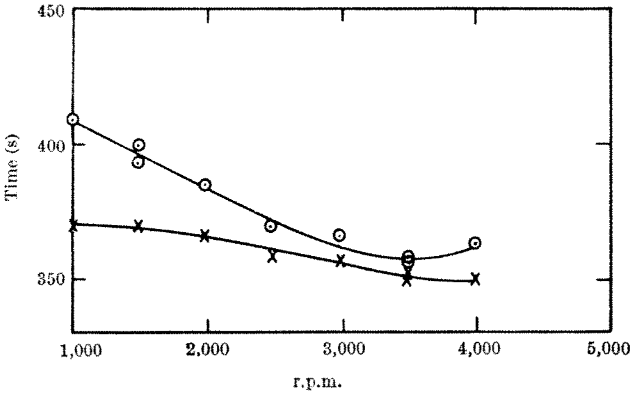


Fig. 5. Results for air pressure of 250 mm water. ×, Water; ○, 234 p.p.m. guar gum.

G. E. Gadd

National Physical Laboratory,
Ship Division,
Foggs Road,
Feltham,
Middlesex.

Received December 18, 1969; revised January 8, 1970.

[1] Gadd, G. E., *Nature*, **212**, 874 (1966).
[2] Seyer, F. A., and Metzner, A. B., *Canad. J. Chem. Eng.*, **45**, 121 (1967).
[3] White, A., *Nature*, **214**, 585 (1967).
[4] Lowe, R. J., thesis, Liverpool Univ. (1969).
[5] White, A., *J. Mech. Eng. Sci.*, **8**, 452 (1966).
[6] Metzner, A. B., *Trans. Soc. Rheol.*, **12**, 57 (1968).
[7] Gerlach, C. R., *Southwest Research Institute Technical Report*, 1 (1967).

Visualization of Spiral Motion in Curved Open Channels of Large Width

The difficulty of recording spiral motion in curved open (river) channels and of visualizing the secondary currents at a cross-section of a bend, particularly when the stream is very wide, has led to differing, and sometimes contradictory, conclusions on the behaviour of flow around bends. Indeed, some observations on sand movements around river bends[1] seem to reject the very existence of spiral motion.

This report describes a method of observing motions in two laboratory open channels of 180° bends, each of different curvature and width. The secondary currents (which are mainly responsible for forming the spiral motion) were made visible and recorded by a photographic technique[2]. The narrow channel was 25·5 cm wide (denoted by b) and 202 cm central radius of curvature (r_c); the wide channel was of $b = 61$ cm and $r_c = 183$ cm. Fig. 1 represents a general layout of the apparatus.

Photographs of the secondary currents were taken using exposures at two cross-sections, and at two depths of flow ($h = 7·6$ cm and 3·75 cm). The patterns of secondary flow at these cross-sections were recorded by photographing in a direction perpendicular to the cross-section concerned, illuminated tracer particles having been fed in at the channel entry, Fig. 2. Two tracer agents were used: in laminar flow, a plastic powder, trade name 'Telco' (specific gravity, 0·96; particle size range, 0·1–0·2 mm); in turbulent flow, another tracer agent, consisting of droplets of a mixture of nitrobenzene, olive oil and water (specific gravity = 1). The latter tracer was adopted because its relatively larger particle size reflected more light on to the film. The cross-sections were illuminated by a 1,500 W Phillips 'Lino-lite' lamp through a 2·5 cm slit. The projection of the pathlines of the illuminated particles on the plane of the cross-section gives the pattern of secondary flow. To define the

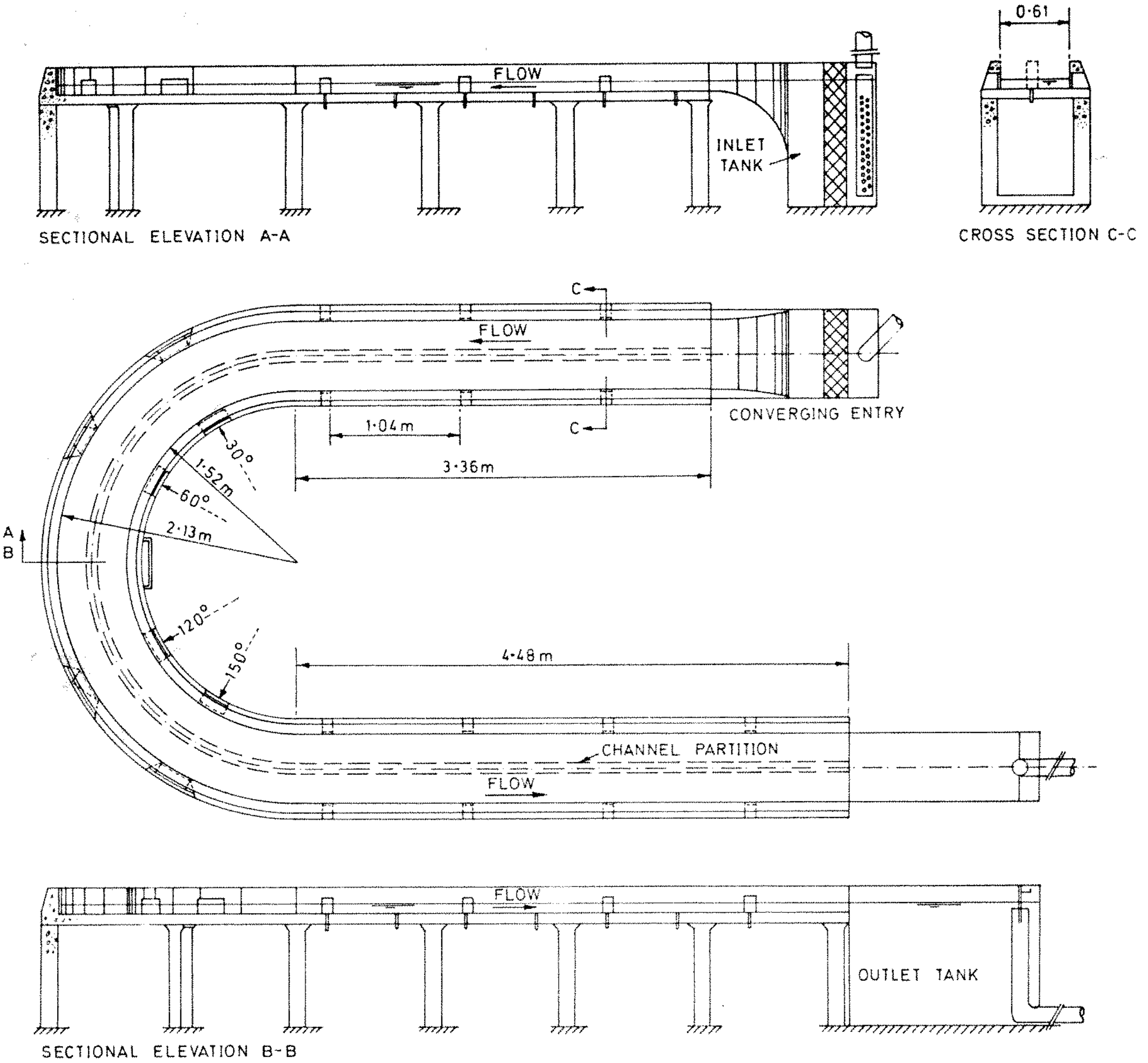


Fig. 1. Plan and elevation of concrete channel.

direction of the secondary flow at every point, the rear (upstream) half of the slit width was covered by a strip of unexposed developed negative film, so that the brighter half of a particle pathline image on the picture showed the secondary flow direction at that point. By changing the velocity only, the two depths of flow were studied for different values of Reynolds' number (defined as $\frac{\text{velocity} \times \text{mean hydraulic radius}}{\text{kinematic viscosity}}$). At each Reynolds' number, photographs were made at angles $\theta = 30°$ and $150°$ from the beginning of the circular bend.

In Figs. 3–8 some of the flow patterns at the two cross-sections $\theta = 30°$ and $150°$ for different Reynolds' number are represented.

When the flow is laminar ($R_e = 246$), and at the early region of the bend ($\theta = 30°$) (Fig. 3), there exist a number of vortices (three or four) in the cross-section. At the developed region of the bend ($\theta = 150°$) (Fig. 4), these vortices adjust themselves to show a predominant cell near the middle of the channel width. There is no sign of the single large cell usually assumed to occupy the whole cross-section.

For higher Reynolds' number ($R_e = 2{,}000$), the two vortices existing at $\theta = 30°$ in the narrow channel (there are fewer vortices when the channel width is narrower) (Fig. 5) are also adjusted to show a main vortex at $\theta = 150°$, as represented in Fig. 6.

When the Reynolds' number attains a higher value ($R_e = 6{,}160$), as shown in Fig. 7, the turbulent velocity fluctuations destroy the structure of the spiral motion; in this last case, the radial velocity very near the channel

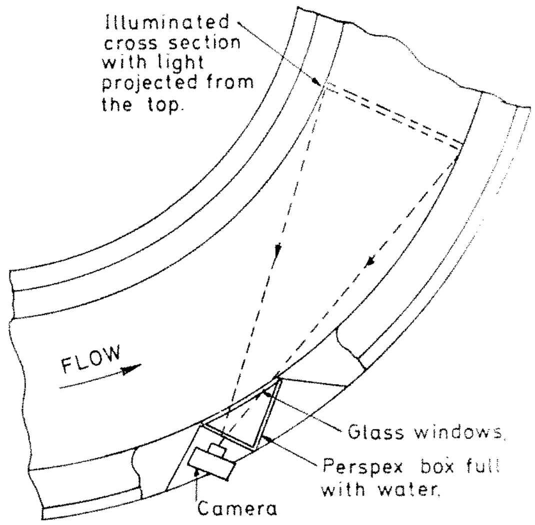

Illuminated cross section with light projected from the top.
FLOW
Glass windows.
Perspex box full with water.
Camera

Fig. 2. Plan of curved channel showing arrangements for photography of secondary currents.

bed and water surface is relatively strong and the effect of turbulence fluctuations in these regions is weak. In the remainder of the flow cross-section, Fig. 7 shows the rather random direction of the secondary flow due to turbulence effects.

For a yet higher value of R_e ($=7{,}910$) and wider channels ($b/h \gg 1$) (Fig. 8), the entire flow cross-section can be considered as a turbulent flow near a flat solid surface, and in this case the turbulent fluctuations have the predominant effect everywhere in the cross-section and no very clear spiral motion exists.

Despite several studies on turbulent flow in straight channels[3], the combined effect of secondary currents due to turbulent fluctuations and those due to channel curvature has not yet been analysed. At this stage, however, the present study (and further investigation which will be published later) concludes that when the aspect ratio of the flow (b/h) increases, the strength of secondary currents, as represented by the flow deviation from the circumferential direction, decreases. In this case the flow pattern in the cross-section tends to break into a large number of spirals and eventually, when $b/h \gg 1$, the

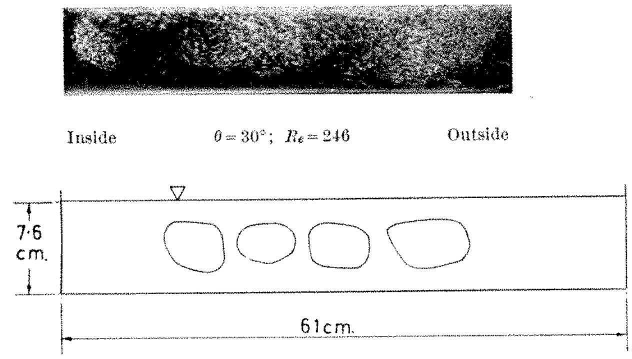


Fig. 3.

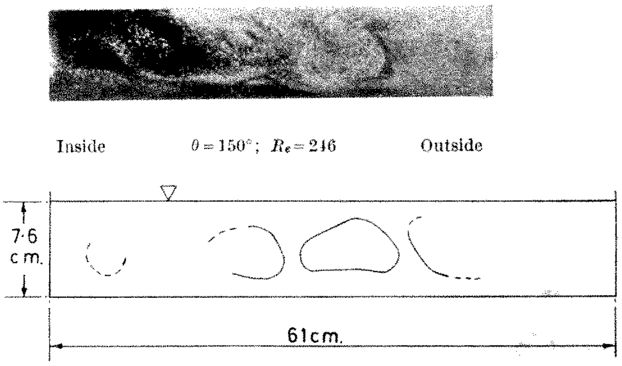


Fig. 4.

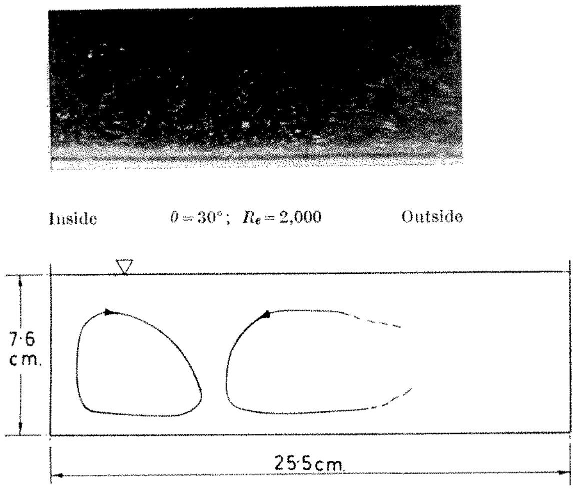


Fig. 5.

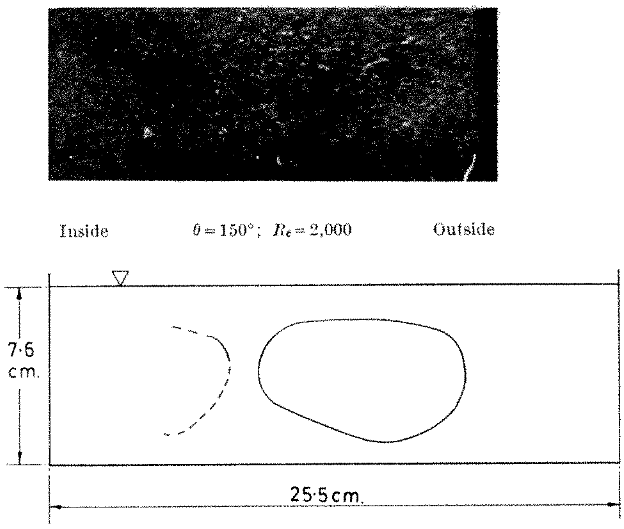


Fig. 6.

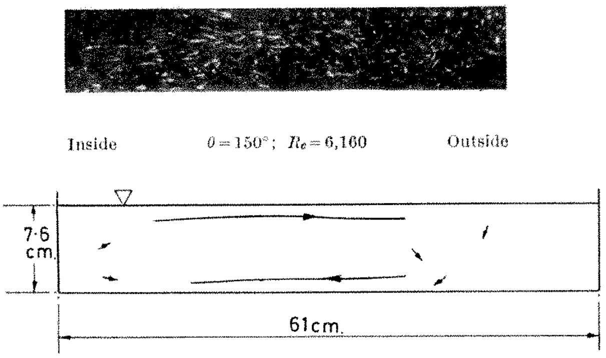


Fig. 7.

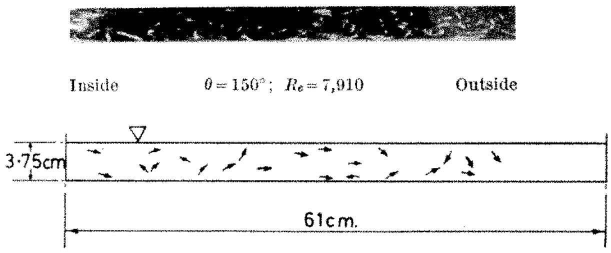


Fig. 8.

Figs. 3–8. Photographs and inferred secondary current patterns in curved channel. All diagrams have the inside wall of the channel to the left.

effect of turbulence fluctuations gives the flow pattern a random structure and no spiral motion appears.

J. R. D. Francis
A. F. Asfari

Imperial College,
London SW7.

Received December 16, 1969.

[1] Vogel, H. D., and Thompson, P. W., *Civil Engineering (ASCE)*, **5**, 3 (1933).

[2] Asfari, A. F., thesis, London Univ. (1968).

[3] Tracy, H. J., *ASCE Proc.*, **5**, 91, No. HY6 (1965).

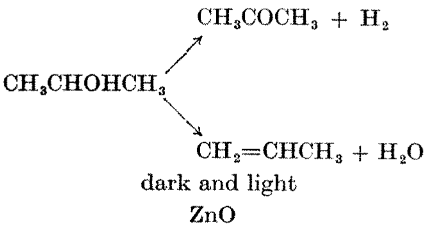
Decomposition of Isopropyl Alcohol photosensitized by Zinc Oxide

In previous investigations the activation energy of a catalytic reaction at the surface of a semiconducting oxide has been shown to depend on the strength of the metal oxygen bond in the surface of the semiconductor[1-8]. In a study of the oxidation of carbon monoxide on NiO, ZnO and Co_3O_4, the density of the bond electrons and the distribution of the electrons in the quantum states of the semiconductor surface bonds had been varied by supporting small grains of the oxides (1000 Å in diameter) on metals of different work functions (Ag, Au, Pd, Pt) and by excitation of the bond electrons by ultraviolet irradiation. The rate determining step of the reaction was found to be the transition of an oxygen atom from the potential valley of a metal oxygen bond (Ni—O, Zn—O, Co—O) in the semiconductor surface into the potential valley of the oxygen carbon bond of the newly formed CO_2 molecule. Because a semiconductor surface bond is destroyed during the rate controlling oxygen transition, the dependence of the activation energy on the strength of the metal oxygen bond is easy to understand.

In order to find out whether this dependence is valid also for a reaction in which no oxygen transition occurs we have investigated the decomposition of isopropyl alcohol vapour on a ZnO catalyst in dark and light conditions.

A dynamic reaction system with an all-quartz reaction vessel was used, containing a ZnO catalyst powder (ZnO colloidal extra, Zinkweiss-Forschungsgesellschaft, Oberhausen, grain diameter 1000 Å) supported on a quartz frit of 50 mm diameter. The catalyst could be illuminated by a high pressure mercury arc (220 V a.c., 300 W, Deutsche Quarzlampengesellschaft Hanau) mounted 50 mm above the catalyst bed. A constant flow of helium (99·995 per cent, Linde) at atmospheric pressure saturated with isopropyl alcohol vapour at 16° C (partial pressure of isopropyl alcohol 26·7 torr) passed continuously through the catalyst powder and was then analysed by a Perkin–Elmer F6/3T gas chromatograph with a Siemens recorder (2·5 mV) and a three column valve (molecular sieve 5 Å, silicagel, carbowax).

In the temperature range from 250° C to 340° C isopropyl alcohol is decomposed in two parallel reactions



$$CH_3CHOHCH_3 \begin{array}{l} \nearrow CH_3COCH_3 + H_2 \\ \searrow CH_2{=}CHCH_3 + H_2O \end{array}$$

dark and light
ZnO

The reactions are observable in both dark and light environments. Both reaction rates are increased on illumination, the dehydrogenation to a smaller extent than the dehydration. No wall reaction is detectable in this temperature range.

By varying the flow rate in the reaction vessel, we have measured the reaction isotherms of both reactions over a wide range of conversion of isopropyl alcohol (up to 80 per cent) in order to establish the kinetics of the reaction. The dehydration was found to be a zero order reaction, the dehydrogenation to be a first order reaction with a strong inhibition by water. Illumination was found to produce no change in the kinetics.

Table 1

	Activation energy, kcalories/mol		Pre-exponential factor, log *A*, torr/s	
	Dehydration	Dehydrogenation	Dehydration	Dehydrogenation
Dark	39	80	15	30·5
Light	31	65	13	25·7

As is seen from Table 1, the activation energy and pre-exponential factor are affected in the same way. The activation energy of both reactions shows a marked decrease due to illumination. The activation energies of the dehydration, a zero order reaction, are "true" activation energies, whereas the activation energies of the dehydrogenation, a reaction with a strong inhibition, are "apparent" activation energies. Because the heat of adsorption of water on ZnO (25–30 kcalories/mol)[9,10] is incorporated into these values, the "true" activation energies of the dehydrogenation may be smaller.

The main conclusion from the experimental results is that although no transition of oxygen atoms of the ZnO surface bond occurs during the decomposition reactions, the excitation of the surface bonds by light decreases the activation energy of both reactions. The activation energy is again found to depend on the strength of the ZnO surface bond. This may be understood by considering the mechanism. The isopropyl alcohol molecule forms a transition state at the surface with two hydrogen bridges to adjacent oxygen and zinc atoms. The products are then formed by a cyclic electron shift of the transition state (Fig. 1). The formation of the H bridges and the subsequent electron transition depend on the electron distribution in the ZnO surface bond. The surface bonds may be described by a resonance model

$$\mathrm{Zn}^{2+}|\overline{\underline{\mathrm{O}}}|^{2-}\leftrightarrow\mathrm{Zn}^{+}-\overset{\cdot}{\overline{\underline{\mathrm{O}}}}|\leftrightarrow\mathrm{Zn}^{\circ}|\overline{\underline{\mathrm{O}}}|^{\circ}$$

and the bond strength results as a linear combination of the resonance forms[8]

$$\psi_{\mathrm{ZnO}} = a\psi_{\mathrm{Zn}^{2+}\,\mathrm{O}^{2-}} + b\psi_{\mathrm{Zn}^{+}\,-\,\mathrm{O}^{-}} + c\psi_{\mathrm{Zn}|\overline{\underline{\mathrm{O}}}|}$$

Increasing the bond strength (decreasing c) must cause an increase in the activation energy; decreasing the bond strength (increasing c), as, for example, excitation by light,

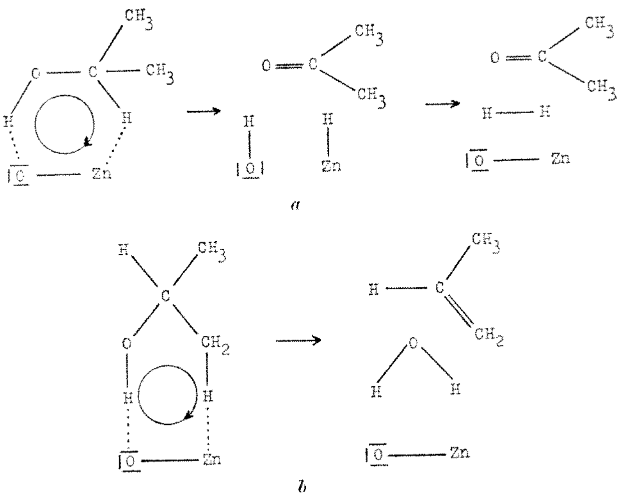


Fig. 1. *a*, Dehydrogenation; *b*, dehydration.

must cause a decrease in the activation energy. The last equation also shows that oxygen desorption from the ZnO surface decreases the bond strength (that is, increases *c*). Because the catalyst is constantly in contact with inert or reducing gases during the reaction, a continuous oxygen desorption from the ZnO surface is observed. This should cause a decrease of the activation energy which in fact is observed. We are now studying this in more detail.

We thank the Deutsche Forschungsgemeinschaft for sponsoring this investigation.

HEINRICH DIETER MÜLLER
FRIEDRICH STEINBACH

Physikalisch-Chemisches Institut,
The University,
Sophienstrasse 11,
8 Munich 2.

Received December 16, 1969.

[1] Steinbach, F., *Nature*, **215**, 152 (1967).
[2] Steinbach, F., *Angew. Chem.*, **79**, 1019 (1967).
[3] Steinbach, F., *Angew. Chem. Int. Ed.*, **6**, 999 (1967).
[4] Steinbach, F., *Z. Phys. Chem. N.F.*, **61**, 235 (1968).
[5] *Photocatalytic Effect of Semiconductors*, in *Electronic Phenomena in Chemisorption and Catalysis* (edit. by K. Hauffe) (Walter de Gruyter, Berlin) (in the press).
[6] Steinbach, F., *Nature*, **221**, 657 (1969).
[7] Steinbach, F., and Krieger, K. A., *Z. Phys. Chem. N.F.*, **58**, 290 (1968).
[8] Steinbach, F., and Barth, R., *Ber. Bunsenges. Phys. Chem.* (in the press).
[9] Taylor, H. S., and Sickman, D. V., *J. Amer. Chem. Soc.*, **54**, 602 (1932).
[10] Wicke, E., *Z. Elektrochem.*, **53**, 279 (1949).

Hückel-level Calculations on the Band Structure of Poly-AT

THE linear combination of molecular orbitals (LCMO) approximation (ref. 1 and unpublished results of R. L. F.) has been suggested as a feasible method for studying the electronic properties of random biopolymers[2]. In the course of some preliminary Hückel-level calculations on random polymers of adenine and thymine we found some interesting behaviour in the energy bands of the polymer, which we report here.

The method[1,2] approximates the wave function of the polymer as a linear combination of the molecular orbital functions of the components. A further simplification is achieved by including only a few of the molecular orbitals from each component in the basis set for the polymer. The Hückel-level calculations reported here were based on the Hückel-level LCAO band theory calculations on regular, single-helix polymers by Hoffmann and Ladik[3]. Our method gives essentially the same results as the direct band theory calculations for the bands considered in homopolymers. The key point is that, in the homopolymers, the energy bands have a small spread about the values for the corresponding levels in the isolated components. The energy spread for the highest occupied band of an infinite polymer of either adenine or thymine is approximately 0·10 Hückel β units. The spread for the lowest vacant band is 0·06 β for adenine and 0·02 β for thymine. The result is similar if a heteropolymer is constructed with a regular alternation of units. The band spreads in this case are even smaller. The two highest occupied bands in an infinite polymer of alternating poly-AT have a spread of 0·15 β while the two lowest vacant bands have a spread of only 0·0002 β. This is also confirmed by the band-theory type calculations of Avery *et al.*[4].

When the order of the units in the polymer is random, no such grouping of the energy levels occurs. The energy bands quickly spread so that there is overlapping between the occupied bands. In the present case, the spread in the unoccupied bands is more gradual as the polymer is built up, because of the very small off-diagonal elements between these; there is every indication, however, that these too could overlap for an infinite polymer. Figs. 1 and 2 show this effect. In Fig. 1 a thirty-two unit random polymer is built up. In Fig. 2, four different eight-unit subpolymers are combined in various ways to give larger random polymers of up to thirty-two units. Fig. 3 presents an integrated energy density plot for the bands arising from the highest occupied orbital of adenine and of thymine for an alternating A–T–A–T- - - - - structure and for several different random polymers containing equal numbers of A and T. In such a plot, a band of closely spaced energy levels yields a nearly vertical region on the graph while an energy gap is represented by a horizontal line. This is the behaviour observed for the alternating structure. Perfect crystals also yield this type of plot for energy bands and gaps. A straight sloping line would indicate a uniform energy distribution over the range considered. The points for the random polymers approximate to such a straight line over much of the band. The behaviour found for the random polymers is very similar to that for the energy bands of a one-dimensional, one-component liquid[5] or for those of a one-dimensional, random binary alloy[6].

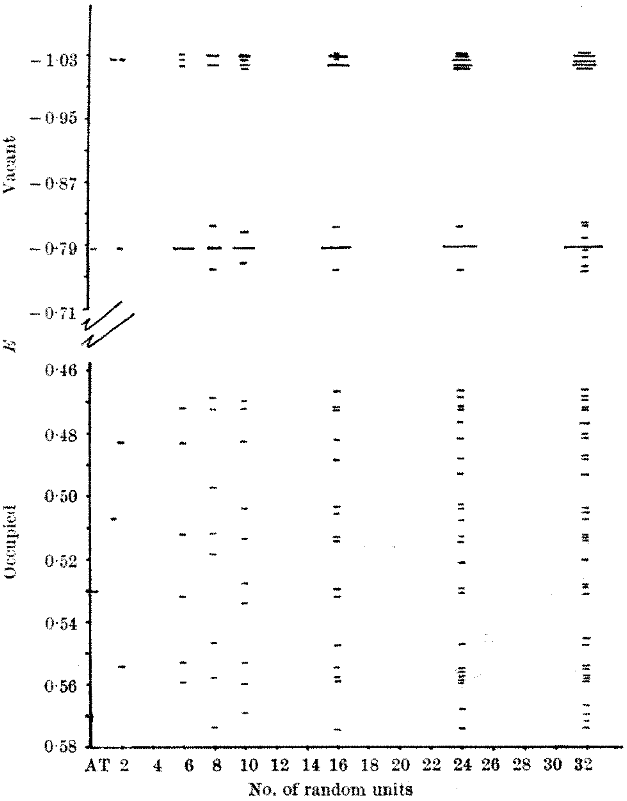


Fig. 1. Energy levels as a function of number of units in a random polymer. Energy unit, standard Hückel β_0. Lengthened lines indicate degeneracies. Order of units (starting at the bottom): ATTAAATTT-ATTATTATTTAATAATTATAAAA.

It has often been suggested that there should be extensive electron delocalization along the chain in biological polymers[7]. This suggestion has previously been justified theoretically by the calculations of Ladik *et al.*[3,4] and Yomosa *et al.*[8]. Such delocalization should have important effects on the electrical and magnetic properties, the optical properties, energy transfer and many other properties of the system. Qualitative discussions of these phenomena can be based on any model that allows delocalization along the chain, and, in many cases, on a perturbation theory treatment of the individual components. The available computational techniques are probably not refined enough to warrant any serious attempt at quantitative predictions of the properties of large polymeric systems. The band shapes we have obtained, however, suggest some ideas that should be considered even in qualitative discussions.

Calculations of optical properties are usually based on perturbation theory or exciton theory. Perturbation theory calculations involve summations of terms that have differences in energy or in powers of energy in the denominator.

$$\text{Property} \propto \sum_{j \neq i} \frac{1}{E_i^n - E_j^n}$$

The terms with energies near the specific energy under consideration are in general most important. Calculations for polymers show that a given electronic energy level occurs within a band of closely spaced energy levels. In a periodic polymer, these bands are narrow and near the energy levels for the isolated components, so that a perturbation theory calculation based on the isolated components should be a fair approximation of such a calculation on the polymer. For a random polymer, on the other hand, the bands are much more diffuse; consequently, the calculated properties should be significantly different from either the isolated component or the periodic polymer. For an infinite polymer, the summation should be replaced by an integration over the energy bands. Because the energy under consideration lies within the energy bands, such integrations would require the use of Dirac delta functions in order to eliminate the singularities as E_j approaches E_i.

Electrical conduction is theoretically described by band theory. Systems having partially filled energy bands are intrinsic conductors. Those with only totally filled and totally vacant bands are insulators or semiconductors. Polymers of the type under consideration fall into the latter category. They can, at most, be semiconductors. If a few electrons are placed in a normally vacant energy band, they can be moved through the system by an external field to achieve conduction. The charge carriers are the electrons. This is said to be n-type conduction. If, however, a few electrons are removed from a filled band, conduction can again occur. The net effect in this case is that the vacancy is the charge carrier. This is said to be p-type (or hole) conduction. In band theory calculations, the electrical conductivity of a system, for a given number of charge carriers, is proportional to $d^2E/d\mathbf{k}^2$ (via the effective mass of the carrier) where $\mathbf{k}$ is the propagation vector in the system. A plot of $E(k)$ against $\mathbf{k}$ (the energy dispersion curve) has the same form as Fig. 3 but with the axes interchanged. The quantity $d^2E/d\mathbf{k}^2$ is equal to the curvature of the dispersion curve. An estimate of the curvature near the band edges of the occupied and vacant bands should thus give a rough indication of whether the system should behave as a better n-type or p-type conductor, assuming an equivalent population or depopulation of the bands could be achieved. From a consideration of our data, it seems that random poly-AT should be a better n-type conductor than p-type.

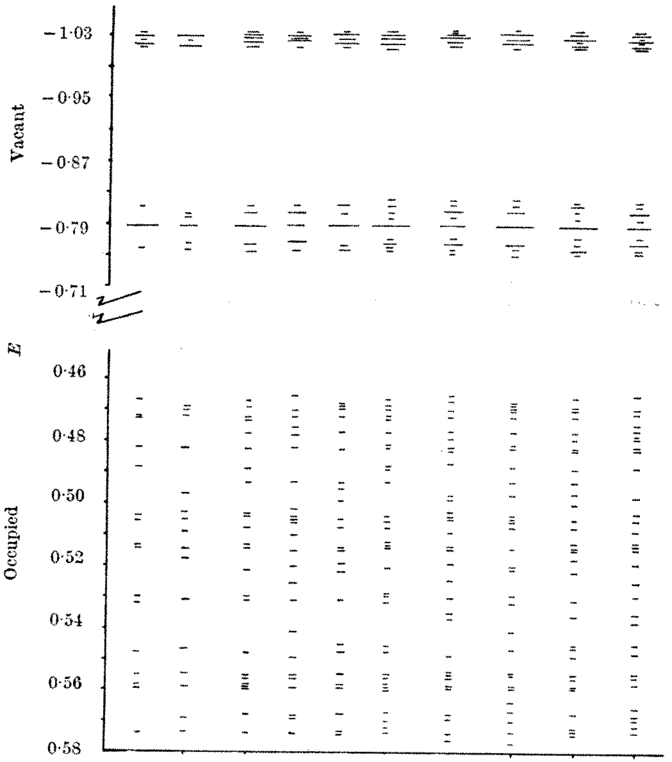


Fig. 2. Energy levels of several different 16, 24 and 32-unit random polymers made from different combinations of basic random 8-unit subpolymers. Energy unit, standard Hückel β_0. Basic subpolymers: (1) ATTAAATT; (2) TATTATTA; (3) TTTAATAA; (4) TTATAAAA.

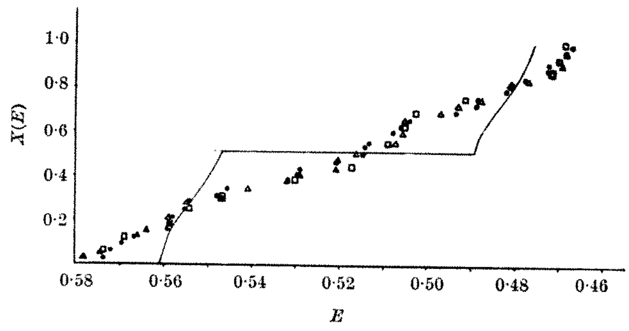


Fig. 3. Integrated energy density for an alternating AT polymer and several random polymers. $X(E)$ is the fraction of levels having an energy less than or equal to E. Energy unit, standard Hückel β_0. Solid curve, alternating AT (32 units); closed circles (1234); triangles (2341); squares (21).

Similar arguments to these can be applied to the theories of energy transfer, magnetic properties, and so on. Work is under way in our laboratory to obtain better wave functions for random polymers so that such discussions can be put on a more sound basis.

One further point. The calculations reported here are for a random ordering of the polymer units, but assume a fixed, rigid geometrical arrangement. Similar results would be obtained for ordered polymer units, but a randomized geometrical arrangement[5]. The forces holding biopolymers in their helical conformations are so weak that it would be expected that, at any instant, there would be a great deal of randomization of the positions of the constituent molecules. The considerations we have presented should thus be valid even for polymers with regular recurrence monomer units.

We thank the Petroleum Research Fund of the American Chemical Society for financial support. We also acknowledge the assistance of the LSU New Orleans and LSU Baton Rouge computer research centres.

R. L. Flurry, jun.
Donna Breen

Department of Chemistry,
Louisiana State University in New Orleans,
New Orleans,
Louisiana 70122.

Received October 13, 1969; revised January 7, 1970.

[1] Flurry, jun., R. L., *J. Phys. Chem.*, **69**, 1927 (1965); **73**, 2111 (1969).
[2] Flurry, jun., R. L., *Israel Academy of Science and Humanities* (in the press).
[3] Hoffmann, T. A., and Ladik, J., in *The Structure and Properties of Biomolecules and Biological Systems* (edit. by Duchesne, J.), 84 (Interscience, New York, 1964).
[4] Avery, J., Packer, J., Ladik, J., and Biczó, G., *J. Mol. Spectros.*, **29**, 194 (1969).
[5] Gubanov, A. I., *Quantum Theory of Amorphous Conductors*, ch. IV (Consultants Bureau, New York, 1965).
[6] Agacy, R. L., and Borland, R. E., *Proc. Phys. Soc.*, **84**, 1017 (1964).
[7] Szent-Györgyi, A., *Nature*, **148**, 157 (1941).
[8] Yomosa, S., Miyata, T., and Suzuki, H., *J. Phys. Soc. Jap.*, **24**, 878 (1968).

BIOLOGICAL SCIENCES

Induction of Immunity and Tolerance *in vitro* in the Absence of Phagocytic Cells

In previous reports on the induction of immunological tolerance *in vitro*[1,2] we presented evidence suggesting that specific unresponsiveness is mediated by the direct encounter of antigen molecules with immunocompetent cells. The kinetics of the antigen-dose response *in vitro*[1,2] to H-antigens of *Salmonella adelaide* (polymerized flagellin, POL)[3] suggested that in this antigen system not only tolerance, but also immunity, may result from such direct interaction. This assumption, however, contradicts certain current concepts which regard the macrophage as an essential participant in the induction of humoral immunity to all antigens. In this report, we will describe experiments which help to elucidate some aspects of this situation.

Peritoneal macrophages treated with antigen are thought to participate in immune induction[4–7]. In certain conditions, however, such cells can act as inhibitors of the immune response *in vivo*[1–8] and of antigen induced DNA synthesis *in vitro*[1–9]. This has been substantiated in the following way.

CBA male mice aged 80–120 days were used for all experiments. 10×10^6 peritoneal exudate cells (PEC) containing on the average 60 per cent macrophages as tested by carbon uptake were cultured together with 20×10^6 syngeneic spleen cells using the *in vitro* system described previously[10]. The cells were stimulated with immunogenic levels of POL (20 ng POL/ml.) and sheep erythrocytes (SRC) (2×10^6). Control cultures contained $25–30 \times 10^6$ spleen cells only and both antigens. Cultures were harvested after 4 days and assayed for numbers of antibody forming cells (AFC) to POL and SRC. Inhibition of the *in vitro* immune response by PEC was obtained with both POL and SRC antigens (Table 1).

Table 1. EFFECT OF ADDING PERITONEAL EXUDATE CELLS (PEC) TO CULTURES OF MOUSE SPLEEN CELLS ON *in vitro* AND *in vivo** IMMUNE RESPONSES

	In vitro (4 days of culture) Antibody forming cells per culture (mean ± *S.E.M.*)		*In vivo* (1 day *in vitro*, 7 days *in vivo*) 5×10^6 viable lymphocytes injected Antigen reactive cell foci (mean ± *S.E.M.*)	
Antigen *in vitro*	SRC	POL	None	POL
30×10^6 spleen cells	2,745 ± 245	3,490 ± 787	3·0 ± 0·5	3·3 ± 0·4
20×10^6 spleen cells 10×10^6 PEC	132 ± 37	135 ± 37	1·2 ± 0·3	3·9 ± 0·4

* Adoptive immune responses of mouse spleen cells cultured *in vitro* for 24 h in the presence or absence of peritoneal exudate cells were measured at the level of the antigen reactive cell (ARC). An antigen reactive cell focus produced in the host's spleen is taken as an indication that an ARC has produced a clone of cells secreting specific antibody to polymer of *S. adelaide* flagellin[11].

To test the effect of this inhibitory mechanism on antigen reactive cells (ARC), similar cultures were harvested after 24 h and cells injected intravenously together with 25 μg POL into lethally irradiated syngeneic recipients. Seven days later, recipient spleens were assayed for the number of ARC foci as described elsewhere[11]. Control groups received spleen cells cultured for 24 h in the absence of PEC. The results showed that the presence of PEC during a 24 h period of *in vitro* culture of spleen cells did not irreversibly diminish their content of immunocompetent cells to POL (Table 1).

The inhibition of the immune response *in vitro* could be due to (*a*) competition of macrophages and immunocompetent cells for antigen, (*b*) the secretion of inhibitory substances by PEC, or (*c*) a mechanism requiring direct contact between PEC and spleen cells. Antigenic competition and the secretion of inhibitory substances were excluded by the fact that a normal immune response could be induced by spleen cells cultured with the supernatant from cultures containing spleen cells, PEC and antigen. By culturing PEC and spleen cells in compartments physically separated from each other by a 'Nucleopore' membrane it was further established that PEC did not secrete inhibitory substances but required direct contact with spleen cells to inhibit the immune response.

Because of basic differences in experimental design, our findings on the inhibition of antibody formation *in vitro* by added PEC cannot be regarded as contradictory to other results which showed that these cells[6,7] or their cytoplasmic content[4,5] mediate immunity after exposure to antigen *in vivo* or *in vitro*. They do agree well, however, with the following experiments on the capacity of spleen cells to be immunized or rendered tolerant *in vitro* after removal of phagocytic cells.

Table 2. EFFECT OF REMOVAL OF PHAGOCYTIC ELEMENTS FROM MOUSE SPLEEN CELL SUSPENSIONS ON THE INDUCTION OF IMMUNITY AND TOLERANCE *in vitro* TO POLYMER OF *S. adelaide* FLAGELLIN

	Level of phagocytic cells per cent nucleated cells ± *S.D.*		Immune response in culture (AFC/culture ± *S.D.*)	
	Morphological assessment (macrophages + polymorphs)	Functional assessment (^{125}I-POL labelled cells)	Immunogenic dose (20 ng POL per culture)	Tolerogenic dose (3 μg POL per culture)
Original suspension	5·7 ± 1·9	1·41 ± 0·07	1,835 ± 605	25 ± 9
Filtrate fraction	0·05 ± 0·05	0·05 ± 0·04	1,390 ± 118	20 ± 9
Adherent fraction	13·3 ± 3·3	1·55 ± 0·12	905 ± 110	75 ± 75

Cell suspensions were assayed for the number of antibody forming cells (AFC) after 4 days of culture.

Phagocytic cells were separated from spleen cell suspensions by a modification of the active adherence column of Rabinowitz as described elsewhere (ref. 12, and K. S., in preparation). The separation procedure was monitored by differential cell counts which showed that 99 per cent of macrophages and polymorphonuclear cells had been removed by the separation, and by functional tests for phagocytosis based on the uptake of ^{125}I-labelled POL *in vitro* (Table 2). The small proportion of radiolabelled cells present in the filtrate were lymphocytes and polymorphonuclear cells, but never macrophages. The adherent fraction eluted as described elsewhere[13] comprised polymorphonuclear cells, macrophages and lymphocytes. Cultures containing 12×10^6 viable cells were incubated for 4 days in the presence of either 20 ng or 3 μg POL/ml. culture fluid. An antigen dose of 20 ng is optimal for the induction of immunity *in vitro*, while a concentration of more than 1 μg POL/ml. induces immunological tolerance[1,2]. The cell fractions cultured comprised: (*a*) the filtrate, depleted of phagocytes, (*b*) the adherent fraction, consisting of phagocytes and lymphoid cells, and (*c*) the original unseparated spleen cells as a control. Results in Table 2 gave clear evidence that both normal immunity and immunological tolerance to POL occurred in the absence of phagocytic cells.

These findings provide strong evidence in favour of the hypothesis that both immune induction and immune tolerance to POL are initiated by the direct recognition of antigen molecules by antigen-reactive cells. This recognition is presumably mediated by specific cell surface bound recognition sites and does not require the participation of phagocytic cells. The only parameter which decides whether immunity or tolerance to POL is induced *in vitro* is a quantitative one, the antigen dose. This does not conflict with the possibility that, *in vivo*, the binding of antigen on reticular cells in germinal centres may counteract immunity by tolerance induction, as was suggested by Ada and Parish[13]. Rather it suggests that macrophages may act as a "buffering system" to reduce the concentration of free antigen within the organ to levels too low to induce tolerance in order to favour immunity rather than tolerance.

Our results on immune tolerance contrast with those of Fishman's group[5] and others[6,7,15] who regard the phagocytosis of antigen by macrophages as an essential step in immune induction, and with those of Mosier[16], who demonstrated that the removal from mouse spleen cell suspensions of a macrophage-rich cell population resulted in the loss of immunocompetence on subsequent culture. As we have demonstrated in another article, however[13], and as recently pointed out by others[17], these apparent discrepancies are in fact a reflexion of different cellular mechanisms of immune induction depending on the type of antigen used.

This work was supported by the National Health and Medical Research Council, Canberra (Australia), the US Atomic Energy Commission, the US National Institute of Allergy and Infectious Diseases and the Anna Fuller Fund. We thank Mr J. Pye for supplying the antigen. The technical assistance of Misses J. Burkitt, M. Crawford and H. Jackson is acknowledged.

ERWIN DIENER
KEN SHORTMAN
PAMELA RUSSELL

Walter and Eliza Hall
Institute of Medical Research,
Melbourne, Australia.

Received August 15; revised October 23, 1969.

[1] Diener, E., and Armstrong, W. D., *Lancet*, ii, 128 (1967).
[2] Diener, E., and Armstrong, W. D., *J. Exp. Med.*, **129**, 591 (1969).
[3] Ada, G. L., Nossal, G. J. V., Pye, J., and Abbot, A., *Austral. J. Exp. Biol. Med. Sci.*, **42**, 267 (1964).
[4] Byrt, P. N., and Ada, G. L., *Immunology*, **17**, 501 (1969).
[5] Adler, F. L., Fishman, M., and Dray, S., *J. Immunol.*, **97**, 554 (1966).
[6] Askonas, B. A., and Rhodes, J. M., *Nature*, **205**, 470 (1965).
[7] Ford, W. L., Gowans, J. L., and McCullagh, P. J., in *The Thymus, Experimental and Clinical Studies* (edit. by Wolstenholme, G. E. W., and Porter, R.), 73 (1966).
[8] Perkins, E. H., and Makinodan, T., *J. Immunol.*, **94**, 765 (1965).
[9] Parkhouse, R. M. E., and Dutton, R. W., *J. Immunol.*, **97**, 663 (1966).
[10] Diener, E. J., *Immunology*, **100**, 1063 (1968).
[11] Armstrong, W. D., and Diener, E., *J. Exp. Med.*, **129**, 371 (1969).
[12] Shortman, K., *Austral. J. Exp. Biol. Med. Sci.*, **44**, 271 (1966).
[13] Shortman, K., Diener, E., Russell, P., and Armstrong, W. D., *J. Exp. Med.*, **131**, 461 (1970).
[14] Ada, G. L., and Parish, C. R., *Proc. US Nat. Acad. Sci.*, **61**, 556 (1968).
[15] Gallily, R., and Feldman, M., *Immunology*, **12**, 197 (1967).
[16] Mosier, D. E., *Science*, **158**, 1573 (1967).
[17] Unanue, E. R., and Askonas, B. A., *Immunology*, **15**, 287 (1968).

Properties of Protective Malarial Antibody

PROTECTIVE malarial antibody has been demonstrated by passive transfer tests in monkey[1] and human[2] infections and, to a lesser degree, in rodent malaria[3-8]. Such tests have not, however, provided a suitable basis for detailed studies on the mechanism of malarial immunity. It has been shown recently that serum from monkeys immune to *Plasmodium knowlesi* specifically inhibits the cyclical proliferation of this simian parasite maintained *in vitro*[9]. This technique has now been used to obtain information about levels of protective antibody in immune sera, the distribution of such antibody among the major immunoglobulin classes and its mode of action on the parasite. Cultures of *P. knowlesi* were initiated with trophozoites and taken through a 24 h cycle of maturation, division, red cell reinvasion and renewed growth[9]. The average multiplication rate was six-fold in flasks containing normal serum. The course of parasite growth was followed as before by measurement of ^{3}H-leucine incorporation into parasite protein[9].

In the presence of normal rhesus sera, trophozoites and schizonts incorporate ^{3}H-leucine at an approximately linear rate for about 4 h. During parasite division (schizogony) the incorporation diminishes, becomes stationary for about 2 h after red cell reinvasion, and is renewed during the succeeding cycle of parasite development (Fig. 1). Immune serum has little effect on initial intracellular growth, but inhibits the second cycle of development which follows schizogony (Fig. 1). Normal and immune preparations were assayed in flasks containing 0·25 ml. of washed red blood cells with an initial parasitaemia of 0·5–1·0 per cent. The dose-dependent nature of parasite inhibition for one pool of antiserum from monkeys immunized for 17–20 months (Table 1, pool 1) is shown in Fig. 1. In flasks containing less than 0·3 ml. of antiserum increasing proportions of parasites enter a second cycle of development (Fig. 1).

Table 1. DATA CONCERNING IMMUNE SERUM POOLS ANALYSED

Pool No.	No. of donors	Duration of immunization	No. of challenges	Final challenge
1 (27.5.69)	4	17–20 months	11–12	10^8 parasites
2 (17·9.69)	4	17–23 months	10–14	10^8 parasites

For comparison of different immune preparations, dose-response data relating to inhibition of the second cycle of *in vitro* growth were derived from radioactivity measurements at 24 h. The standard inhibitory dose of immune serum was taken as 0·4 ml. of pool 1 (Table 1) because this consistently produced complete suppression of the second cycle of development. The normal increment was the difference between 24 h radioactivity values in flasks containing 0·4 ml. of the immune serum and those containing normal serum (Fig. 1). Radioactivity increments in other flasks expressed as a percentage of the normal showed an approximately linear relationship to dose on a logarithmic plot (Fig. 2). The mean data from four experiments for immune pool 1 (Table 1) and one experiment for pool 2 (Table 1) indicate that both these sera contain titres of protective antibody near the threshold required for inhibition of progressive parasite growth when red cells (0·25 ml., parasitaemia 0·5–1 per cent) and immune serum (0·3 ml.) are mixed in physiological proportions.

Malarial immunity in many species, including the rhesus monkey, develops slowly and is associated with persistence of the infecting organism as shown by periodic low-grade parasitaemia in clinically immune subjects, and the induction of malarial relapse by splenectomy in immune animals. This form of relative immunity, described as "premunition" or "tolerance"[10], may be a consequence of the fact that the intracellular plasmodium has limited access to immunologically competent cells so that protective antibody levels may remain inadequate for

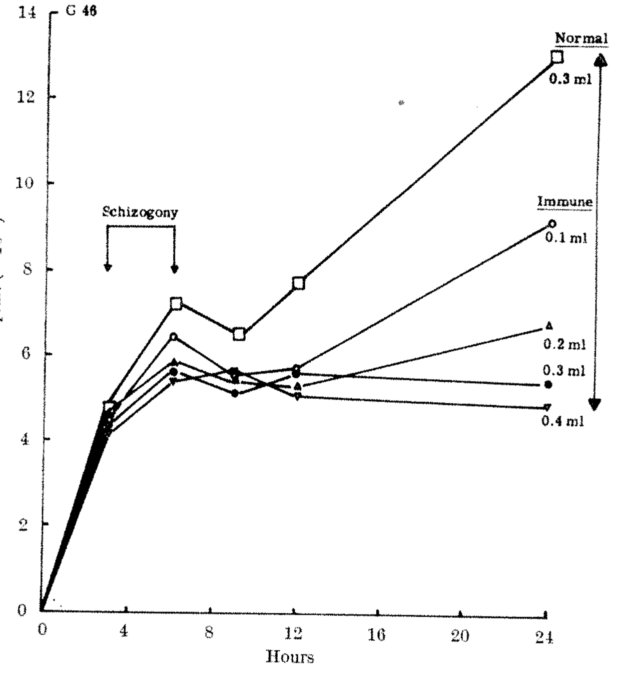


Fig. 1. Incorporation of ^{3}H-leucine by cultures of *P. knowlesi* in the presence of normal serum and various doses of immune serum (pool 1, Table 1). The vertical arrow on the right shows the normal growth increment (see text). The initial parasitaemia was 1·0 per cent and the multiplication rate in flasks containing normal serum was × 6·5. Schizogony denotes the period of parasite division.

Table 2. IgG AND IgM LEVELS IN VARIOUS RHESUS MONKEY PREPARATIONS TESTED FOR ANTIMALARIAL ACTIVITY (SEE FIGS. 2 AND 3)

Preparation	Fractionation method	IgG (mg/ml.)	IgM (mg/ml.)
Normal serum	—	13	0·7
Immune serum pool 1	—	13	0·8
Immune serum pool 2	—	13·5	0·3
Whole Ig	0·4 Saturated $(NH_4)_2SO_4$	13	0·4
$IgG_{(2)}$	DEAE-cellulose—0·015 M phosphate (*p*H 8·0)	13	< 0·1
$IgG_{(1)}$	DEAE-cellulose—0·02 M phosphate (*p*H 8·0)	13	< 0·1
IgM	'Sephadex *G*-200', first peak	< 0·1	0·8
Normal IgG	DEAE-cellulose—0·04 M phosphate (*p*H 6·0)	11	< 0·1

Ig fractions were isolated from pool 1 immune serum.

complete suppression of parasite multiplication even after long infection. This concept is supported by evidence of relatively low titres of protective antibody in serum from monkeys repeatedly immunized for about 2 years. Protective antibody levels are probably also low in human adults exposed to lifelong malarial infection, for passive immunization of African infants required doses of immune adult γ-globulin equivalent to about half the total circulating immunoglobulin pool of the recipient[2].

Various immunoglobulin fractions were prepared from immune rhesus serum by standard methods (Table 2). The IgG and IgM content of these was adjusted to that of the original immune pool (Table 2). Whole Ig (0·4 saturated $(NH_4)_2SO_4$ fraction) simulated the inhibitory effect of the original immune serum (Fig. 3), whereas IgG fractions of fast or slow electrophoretic mobility and IgM had significantly less inhibitory action (Fig. 3). The antimalarial effect of whole immune serum or salt precipitated Ig can be accounted for by the additive effects of IgG and IgM. In accordance with this finding, the inactivation of either IgA or IgE by addition of an excess of specific anti-IgA or anti-IgE sheep sera (the latter serum is known to react with monkey IgE[11]) had little effect on the inhibitory action of the immune serum. Similarly, attempts to inactivate IgE by heating at 56° C for 2 to 4 h produced little change in the activity of the immune serum.

The association of protective antibody with IgM in chronically immunized animals could be attributed to antigenic variability of the parasite. Variants of *P. knowlesi*, distinguishable by a schizont agglutination test[12,13], appear during the course of chronic infections and each might induce a primary immune response associated with IgM antibody[13]. The *in vitro* response to both IgG and IgM was, however, linear over the range investigated and at the highest doses tested IgM produced 40 per cent and IgG 80 per cent inhibition of normal parasite growth. These results suggest that, in regard to the parasite population analysed, IgG and IgM contained protective antibodies of comparable specificities. Persistence of IgM antibody after prolonged immunization has been observed with other particulate antigens[14]. The failure to demonstrate antimalarial activity in monkey IgE is of interest because IgE levels are high in human[15] and monkey[16] parasitic infections and this had suggested a possible protective role for the class of antibody known to be associated with immediate hypersensitivity reactions.

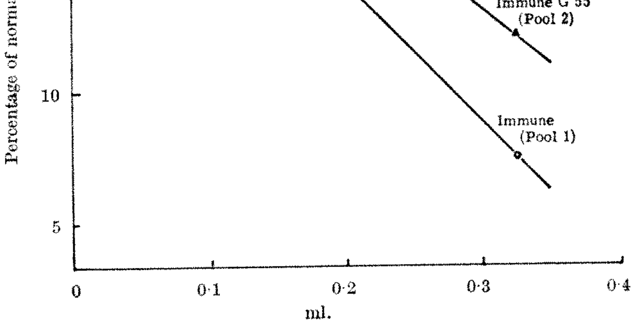


Fig. 2. Dose-response data (see text) for immune sera pools 1 and 2 (Table 1).

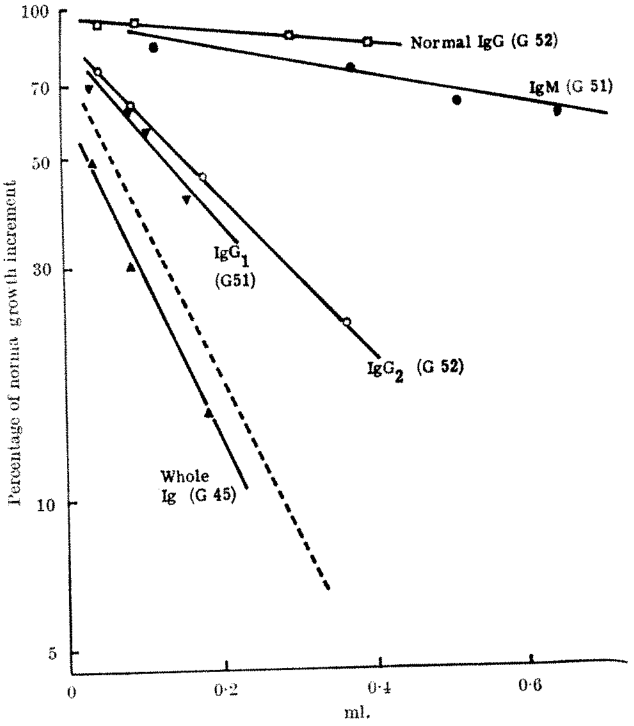


Fig. 3. Dose-response data for immune preparations of whole Ig, IgG and IgM and for normal IgG. Fractions were tested at concentrations corresponding to those of the original immune serum (see Table 2). The broken line shows the response for immune serum—pool 1 (Fig. 2).

Studies on the enzymatic splitting of γ-globulin have provided a means for investigating the molecular basis of various antibody reactions[17]. The role of bivalence and the importance of the Fc fragment in malarial antibody were studied by comparing papain fragments (univalent Fab together with Fc) and peptic fragments (bivalent $F(ab')_2$)[18] of IgG. The immune papain digest had no demonstrable effect whereas divalent $F(ab_1)_2$ fragments simulated the antimalarial action shown by equivalent concentrations of the original IgG (Fig. 4).

It is concluded that protective malarial antibody has many features in common with viral neutralizing antibody[19]. Both seem to inhibit cell reinvasion, act independently of complement, require at least two combining sites to exert their protective action and do not affect the behaviour of organisms already within cells. In the bacteriophage neutralization system, the effectiveness of bivalent as compared with monovalent antibodies[20-22] is not attributable to differences in size[21,22], but apparently reflects the considerably greater affinity of molecules carrying two combining sites[23]. In malaria the agglutinating capacity of bivalent antibody may also be significant for microscopical observation has shown that merozoites liberated into normal serum during schizogony remain discrete before reinvading red cells, but in the presence of immune serum the free parasites tend to agglutinate and fail to penetrate erythrocytes (unpublished data of G. A. B.).

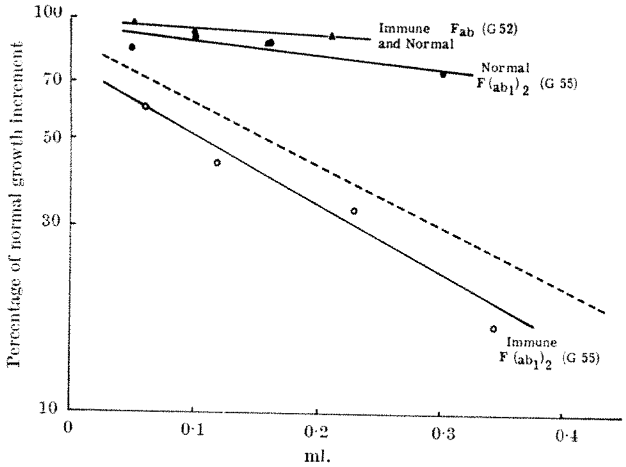


Fig. 4. Dose-response data for papain (Fab and Fc) and pepsin F(ab′)$_2$ fragments of normal and immune IgG. Concentrations of fractions tested were Fab + Fc 13 mg/ml., F(ab$_1$)$_2$ 8·7 mg/ml. Broken line shows the response for the original whole IgG (13 mg/ml.).

We thank Mr E. D. Dennis and Miss S. Wanstall for technical assistance, Dr M. T. Cwynarski for immunoglobulin estimations and Dr S. G. O. Johansson for anti-IgE serum. This work was supported by grants from the Medical Research Council, the World Health Organization and the Wellcome Trust.

S. Cohen
G. A. Butcher

Department of Chemical Pathology,
Guy's Hospital Medical School,
London SE1.

Received November 19; revised December 8, 1969.

[1] Coggeshall, L. T., and Kumm, H. W. J., *J. Exp. Med.*, **66**, 177 (1937).
[2] Cohen, S., McGregor, I. A., and Carrington, S., *Nature*, **192**, 733 (1961).
[3] Fabiani, G., and Fulchiron, G., *CR Soc. Biol.*, **147**, 99 (1952).
[4] Bruce-Chwatt, L. J., and Gibson, F. D., *Trans. Roy. Soc. Trop. Med. Hyg.*, **50**, 47 (1955).
[5] Isfan, T., *Arch. Roum. Path. Exp. Microbiol.*, **25**, 65 (1966).
[6] Briggs, N. T., Wellde, B. T., and Sadun, E. H., *Milit. Med.*, **131** (Suppl.), 1243 (1966).
[7] Diggs, C. L., and Osler, A. G., *J. Immunol.*, **102**, 298 (1969).
[8] Stechschulte, D. J., Briggs, N. T., and Wellde, B. T., *Milit. Med.*, **134**, 1140 (1969).
[9] Cohen, S., Butcher, G. A., and Crandall, R. B., *Nature*, **223**, 368 (1969).
[10] Sergent, E., and Sergent, E., *Indian J. Malar.*, **10**, 53 (1956).
[11] Ishizaka, K., and Ishizaka, T., *J. Allergy*, **41**, 96 (1968).
[12] Eaton, M. D., *J. Exp. Med.*, **67**, 857 (1938).
[13] Brown, K. N., and Brown, I. N., *Nature*, **208**, 1286 (1965).
[14] Torrigiani, C., and Roitt, I. M., *J. Exp. Med.*, **122**, 181 (1965).
[15] Johansson, S. G. O., Mellbin, T., and Vahlquist, B., *Lancet*, i, 1118 (1968).
[16] Ishizaka, K., Ishizaka, T., and Tada, T., *J. Immunol.*, **103**, 445 (1969).
[17] Porter, R. R., *Biochem. J.*, **73**, 119 (1959).
[18] Nisonoff, A., Wissler, F. C., Lipman, L. N., and Woernley, D. L., *Archs. Biochem. Biophys.*, **89**, 230 (1960).
[19] Burnet, F. M., in *Principles of Animal Virology*, second ed., 265 (Academic Press, New York, 1960).
[20] Stemke, G. W., and Lennox, E. S., *J. Immunol.*, **98**, 94 (1967).
[21] Klinman, N. R., Long, C. A., and Karush, F., *J. Immunol.*, **99**, 1128 (1967).
[22] Stemke, G. W., *J. Immunol.*, **103**, 596 (1969).
[23] Greenbury, C. L., Moore, D. H., and Nunn, L. A. C., *Immunology*, **8**, 420 (1965).

Evidence against the Presence of H2 Histocompatibility Antigens in Ehrlich Ascites Tumour Cells

The use of a hybrid between Ehrlich ascites tumour and hamster cell lines to immunize mice against the tumour has been reported[1]. We report here the use for the same purpose of a hybrid line between Ehrlich ascites tumour cells and cells derived from mouse embryo cells obtained from an inbred strain of mice (CBA). This hybrid is less immunologically foreign than the hamster × Ehrlich hybrid for the randomly bred Swiss mice used in the immunization experiments; it differs antigenically only in histocompatibility antigens, in particular in its H2 antigen, which, for CBA mice, is the H2^k antigen. Such a hybrid gave the opportunity of investigating whether the immunity arising after injection of Ehrlich × hamster hybrids[1] was the result of strong histocompatibility antigens (of H2 type) in the Ehrlich cells.

A primary culture of mouse embryo cells was obtained by trypsinization of minced, two-week-old embryos of a CBA mouse. The disaggregated cells were maintained in Dulbecco's modified Eagle's medium for one week before fusion. They were fused with Ehrlich ascites tumour cells as described previously[1], and the fusion mixture was incubated in a plastic bottle (area 25 mm^2, Falcon Plastics) for 24 h, when non-adherent cells were removed by pouring off the medium and repeatedly washing with Earle's balanced saline. Fresh medium (5 ml.) was then added, and incubation continued. One week later the bottle was shaken to suspend cells which were in mitosis, and the medium, with the suspended cells, was placed in a fresh plastic bottle. The medium was changed after 24 h incubation at 37° C, and incubation continued for another week. After four repetitions of this process the cell population obtained consisted of rapidly growing cells resembling morphologically the hamster × Ehrlich hybrid cells previously described[1]. Chromosome counts on this population showed a range of 95 to 114 chromosomes, with a model value of 110 chromosomes. All the chromosomes were telocentric or acrocentric with the exception of one large metacentric chromosome characteristic of the Ehrlich ascites tumour line[2]. The expected modal number of chromosomes in a hybrid line between mouse diploid cells and Ehrlich cells would be about 117. The cell population seemed therefore to consist of hybrids formed by the fusion of one mouse embryo cell and one Ehrlich cell, with reduction in the number of chromosomes. The karyotype of the population remained the same over several months cultivation *in vitro*, which was carried out by transfer of 10^5 to 10^6 cells at each passage. The CBA cells had no marker chromosomes of use in this cross, but the behaviour of the cells *in vivo* and their growth *in vitro* support the idea that they were, in fact, hybrids.

10^6 hybrid cells were injected intraperitoneally or subcutaneously in 0·2 ml. of Earle's saline into groups of five Swiss (randomly bred) or CBA (inbred) mice, with the following results. (1) Swiss mice, intraperitoneal injection : The mice developed an ascites tumour indistinguishable in time of first appearance, time of death, and cell concentration (determined at daily intervals) from that developing after injection of Ehrlich ascites tumour cells. The karyotype of the population ten days after injection differed, however, from the karyotype of the *in vitro* population. In one experiment the range of chromosome numbers at ten days was 73 to 83, with a mode at about 80 chromosomes, and in another experiment the range was 85 to 107, with a mode at about 95 chromosomes. (2) Swiss mice, subcutaneous injection: About one week after injection a small tumour developed at the injection site in all the mice. This increased slowly in size to a maximum never greater than 5 mm in diameter. From about 15 days after injection the tumours began to regress, and there was no trace of a tumour in any mouse three weeks after injection. Ehrlich cells, on the other hand, produced progressive tumours which reached 2 to 3 cm in diameter, often became ulcerated (the mice were then killed), and finally caused death with no sign of regression. (3) CBA mice, intraperitoneal injection: The mice developed a moderate degree of ascites. The total volume of exudate—which was in all cases heavily bloodstained—was never more than a few ml. and the cell concentration was always low, never more than 10^5 cells per ml. Multiple solid tumours were present on the mesentery, intestines and at the site of injection. The mice died between 10 and 12 days after injection. Ehrlich cells in these mice, on the other hand, produced a more marked ascites, also bloodstained, with cell concentrations reaching more than 10^7 cells per ml. A few small solid tumours were present in the peritoneal cavity. The mice died between 10 and 13 days after injection. The hybrid cells therefore seem to have been as malignant as Ehrlich cells in CBA mice. (4) CBA mice, subcutaneous injection: Hybrid cells by this route behaved in the same way as Ehrlich cells. Tumours were first detected six days after injection, and progressively increased to a size of 2–3 cm, often with ulceration. The mice died between 60 and 70 days after injection.

Immunization experiments were all carried out in randomly bred Swiss mice. The mice were immunized by subcutaneous injection of 10^6 cells into the flank, and challenged by intraperitoneal injection three to four weeks later. This time interval was adopted in order to allow the small tumours arising from subcutaneous injection of hybrid cells to regress completely. In several experiments the mice which survived the first challenge were rechallenged with 10^5 cells thirty days later. The results are summarized in Table 1. All the mice which died developed a massive ascites.

Table 1. IMMUNIZATION OF SWISS MICE AGAINST EHRLICH ASCITES TUMOUR (EAT)

Immunizing cells (10^6 subcutaneously)	Challenge cells (10^6) intra-peritoneally	Challenge dose	Mice surviving 30 days (no ascites)	Mice surviving rechallenge at 30 days with 10^6 cells
None	CBA × EAT	10^5	1/4	—
None	CBA × EAT	10^6	0/5	—
None	EAT	10^4	0/5	—
	EAT	10^5	0/5	—
	EAT	10^6	0/5	—
CBA × EAT	CBA × EAT	10^5	5/5	5/5
CBA × EAT	CBA × EAT	10^6	4/4	4/4
CBA × EAT	EAT	10^4	11/11	9/11
CBA × EAT	EAT	10^5	3/4	3/3
CBA × EAT	EAT	10^6	0/5	—
CBA mouse embryo fibroblasts	CBA × EAT	10^5	5/5	5/5
CBA mouse embryo fibroblasts	EAT	10^5	1/5	0/1

The results provide evidence against the presence of H2 antigens in Ehrlich cells, for the following reasons: Suppose Ehrlich cells possess a strong histocompatibility antigen of type "$H2^a$". If the strong antigens are co-dominant, CBA × Ehrlich cells should have antigens $H2^k$ and "$H2^a$". The rejection of the hybrid after subcutaneous injection into Swiss mice can be attributed to its $H2^k$ antigen, and possibly also to its "$H2^a$" antigen. The failure of CBA mice to reject the hybrid after subcutaneous injection, however, indicated that the "$H2^a$" antigen is either not expressed in the hybrid or is identical with $H2^k$. The fact that CBA × Ehrlich immunized against Ehrlich indicates that it is probably expressed. "$H2^a$", on this hypothesis, must therefore be identical with $H2^k$. Fibroblasts from CBA embryos immunized against CBA × Ehrlich, as would be expected, because both have $H2^k$. They failed, however, to immunize against Ehrlich ascites tumour cells which could mean that the Ehrlich $H2^k$ is in a "masked" form, insusceptible to immunological attack. Because CBA × Ehrlich was effective in immunizing against Ehrlich, the antigen cannot exist in such a "masked" form. It is therefore impossible to explain these results on the hypothesis that an antigen comparable in strength with H2 antigens is present in Ehrlich cells. Immunity after injection of Ehrlich × hamster (1) or CBA × Ehrlich hybrids must therefore have been directed against "weak" antigens of unknown nature.

One of us (L. C.) was in receipt of a Lord Marks fellowship from the National Research Council of Israel.

L. CHEN
J. F. WATKINS

Sir William Dunn School of Pathology,
Oxford.

Received December 23, 1969.

[1] Watkins, J. F., and Chen, L., *Nature*, **223**, 1018 (1969).
[2] Harris, H., Watkins, J. F., Ford, C. E., and Schoefl, G. I., *J. Cell Sci.*, **1**, 1 (1966).

Aberrant Karyotypes and Spontaneous Abortion in a Japanese Family

CHROMOSOME aberrations involving trisomy, monosomy and translocations may be aetiologically related to spontaneous abortion in humans[1-3]. We describe here a family in which translocations seem to be associated with repeated spontaneous abortions.

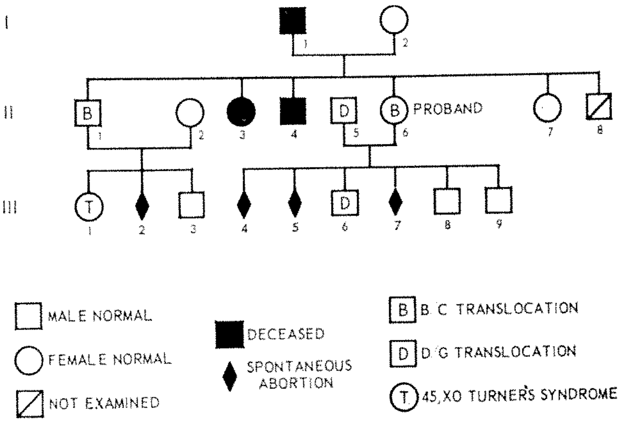


Fig. 1. Pedigree of family with *B/C* and *D/G* translocations, *XO* (Turner's syndrome) and multiple spontaneous abortions.

The proband was a female, aged 43. Her husband was 45 years old and all the family (Fig. 1) are physically and mentally normal with the exception of one with Turner's syndrome (III–1). In twenty-one years of married life, the proband has had three live births (III–6, 8, 9) and three spontaneous abortions (III–4, 5, 7) each at three months gestation. An elder brother of the proband (II–1) has two children: a normal son and a daughter with Turner's syndrome. His wife had one miscarriage at three months gestation.

Chromosome studies were carried out on leucocyte cultures according to a modification of the method of Moorhead *et al.*[4]. For each individual, the chromosomes in sixty to one hundred cells were examined, and at least ten metaphases were karyotyped. In cases with chromosome abnormalities, twenty to thirty cells were karyotyped.

The proband (II–6) and her elder brother (II–1) had chromosome counts of 46. Two chromosomes were missing, one each from group *B* and group *C*. Further, two unusual extra chromosomes were present: a large submetacentric element corresponding in length to the *A2* autosomes, and a small metacentric resembling *E16* (Fig. 2). It is likely that a distal part of the long arm of a *C* chromosome had been translocated to a distal portion of the short arm of a *B* chromosome. The abnormal chromosomes are tentatively regarded as heteromorphic partners of the *B5* and *C11* chromosomes in Fig. 2.

The husband of the proband (II–5) and one son (III–6) consistently had 45 chromosomes. Two were absent, one from group *D* and the other from group *G*, and in their place was one medium-sized submetacentric, the long arm of which was nearly the same length as that of the *D* chromosomes, whereas its short arm corresponded to the long arm of the *G* chromosomes. It is thus probable that this abnormal autosome was formed as a result of a translocation between a *D* and a *G* chromosome. A niece of the proband (III–1) was diagnosed as having Turner's syndrome. Her karyotype had 45 chromosomes with one element missing from group *C*, which was consistent with the diagnosis of *X* monosomy.

The pattern of chromosome replication was investigated autoradiographically in cultured leucocytes from the proband with the *B*/*C* translocation (for procedure, see Sofuni and Sandberg[5]). In thirty-eight out of fifty metaphase cells, one chromosome of group *C* was especially heavily labelled. Karyotype analyses of twelve labelled cells indicated that the late replicating *X* chromosome was probably not involved in the translocated chromosomes (Fig. 2).

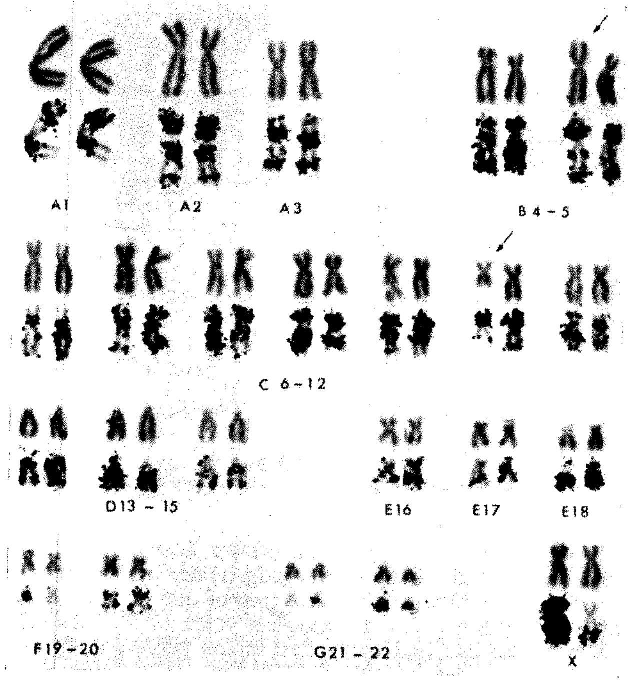


Fig. 2. Karyotype from the proband with *B*/*C* translocation. The chromosomes were photographed before and after autoradiography. Arrows indicate translocation chromosomes. Note the usual pattern of heavy labelling of one *X* chromosome, interpreted as showing that it is not involved in the translocation. One of the translocated chromosomes shows a labelling pattern similar to that of the normal *B*5 chromosome, except for the translocated segment (see text).

In group *B*, where one chromosome was missing, two of the remaining three chromosomes were relatively late replicating, and were identified as *B*4 chromosomes. The remaining chromosome in this group exhibited the expected labelling pattern of the normal *B*5 chromosome: generally early replicating but with a late replicating short arm. One of the translocated chromosomes had a labelling pattern similar to that of the *B*5 chromosome, except for the translocated segment which was early replicating (Fig. 2). The identification of the missing element in group *C* was difficult, for individual elements of the *C* chromosomes are not readily identifiable on the basis of the labelling pattern. The autoradiographic findings support the interpretation derived from karyotype analysis that the translocation probably occurred between a *B*5 and a *C* chromosome.

Recent cytogenetic studies have shown that translocations such as those represented by *G*/*G*, *D*/*G*, *D*/*D*, *C*/*E*, *C*/*D* and *B*/*C* can be transmitted through several generations and, moreover, are often evident in women who have repeated abortions[6-11].

Many individuals with balanced translocations seem to be phenotypically normal[6-12]. In gametogenesis (without nondisjunction) in these carriers, four types of gamete are theoretically possible: (1) normal, (2) balanced, (3) unbalanced with excess genetic material or (4) unbalanced with a deficient amount of genetic material. These four types of gamete from the proband with the *B*/*C* translocation and her spouse with the *D*/*G* translocation are shown in Fig. 3, along with the zygotes that would be formed by fusion of the various gametic types from each parent. Fusion of gametes I and II from the *B*/*C* parent with V and VI from the *D*/*G* parent would produce zygotes with a normal karyotype (A), one balanced translocation (B,C) and two balanced translocations (D). A, B and C are viable, but whether D would be is questionable, because presumably the double translocation would impair the genetic apparatus of the cell, perhaps eventually producing a non-viable foetus that would be lost by spontaneous abortion. The zygotes produced by a trisomic gamete from one parent and a normal gamete from the other (I and VII or III and V) would be partially trisomic for either the *G* chromosome (G) or the *C* chromosome (E). The former, with excess *G* material, might be a translocation Down's syndrome. Whether the trisomic gamete from the *B*/*C* translocation parent is viable, and if so, able to produce a viable foetus, in view of the large amount of reduplicated *C* material, is highly questionable. Most combinations of balanced and unbalanced gametes (F, H–P), together with the products of nondisjunction between the translocation chromosomes and their homologous partners, would be lethal[8,13].

We conclude that the frequent spontaneous abortions probably result from parental translocations. The occurrence of abortions in the first trimester indicates that the abortuses were probably chromosomally abnormal, although we do not have karotypes to support this view directly. Other studies have shown that the average length of gestation for abortuses with abnormal chromosomes is significantly shorter than for chromosomally normal abortuses[14-16].

It has recently been reported that the frequency of spontaneous abortions in sixty-three families with Robertsonian translocations (*D*/*D*, *D*/*G*, *G*/*G*) did not differ significantly from that in a general population[17], that some translocation carriers give birth to normal children and never experience abortion, and that even abortuses in families with translocations may be karyotypically normal[18]. It is therefore possible, in families where only one parent has the translocation, that abortions may result from some factor other than the chromosome aberration. In the family studied by us, however, both parents have different translocations, and it seems likely that these were responsible for the abortions.

In the case of *X* monosomy (III–1), the presence of the translocation might somehow predispose to nondisjunction of other normal chromosomes—in this case the sex-determinants—resulting in either trisomy or monosomy. Such an effect has been suggested by others where a *D*/*D* translocation was associated with *G*-trisomy Down's syndrome[19,20].

We thank Professor Akira Tabuchi (Department of Obstetrics and Gynecology, Hiroshima University Medical School) for his support, and Professor Sajiro Makino (Department of Zoology, Hokkaido University) and Dr Arthur D. Bloom (Department of Human Genetics, University of Michigan Medical School) for reading the manuscript. The Atomic Bomb Casualty Commission is supported by the US Atomic Energy Commission, the Japanese National Institute of Health and the US Public Health Service.

TETSUJI KADOTANI
KOSO OHAMA

Department of Obstetrics and Gynecology,
Hiroshima University School of Medicine.

TOSHIO SOFUNI
HOWARD B. HAMILTON

Cytogenetics Laboratory,
Department of Clinical Laboratories,
Atomic Bomb Casualty Commission,
Hiroshima, Japan.

Received October 6, 1969.

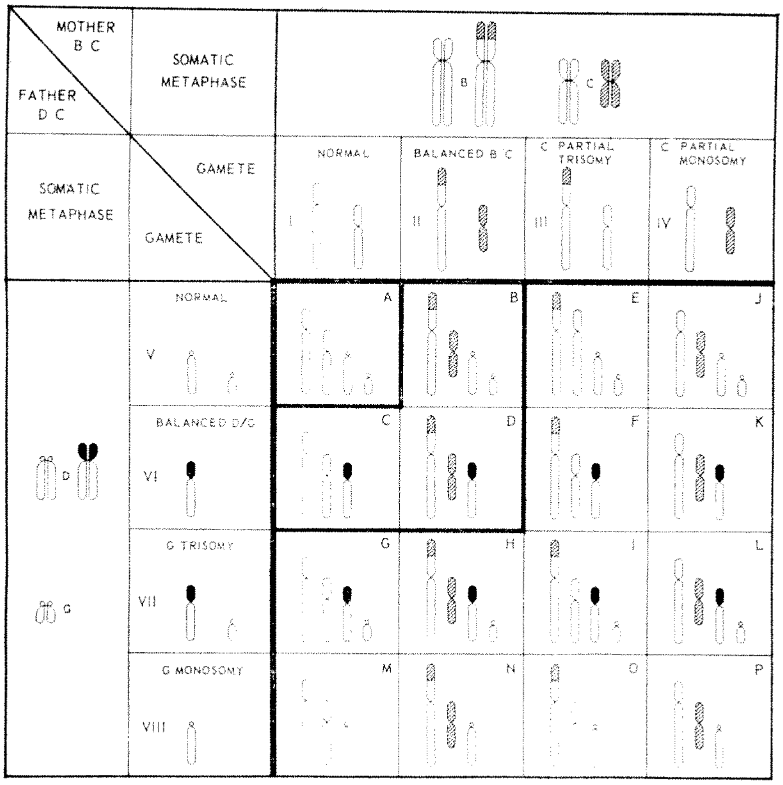


Fig. 3. Diagrammatic illustration of possible zygote formation from parents with *B/C* and *D/G* translocations. I–IV, Maternal gametes; V–VIII, paternal gametes; A, normal zygote; B, balanced zygote identical with maternal configuration; C, balanced zygote identical with paternal configuration; D, balanced zygote with both parental translocations; E–P, unbalanced zygote. Note G, with partial trisomy of a *G* chromosome, could be a "Down-like" syndrome (see text).

[1] *Bull. World Health Organization*, **34**, 765 (1966).
[2] Carr, D. H., *Amer. J. Obstet. Gynec.*, **97**, 283 (1967).
[3] Makino, S., Ikeuchi, T., Sasaki, M., Muramoto, J., Shimba, H., Fujimoto, S., and Matsuda, S., *Proc. Japan Acad.*, **43**, 552 (1967).
[4] Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Exp. Cell Res.*, **20**, 613 (1960).
[5] Sofuni, T., and Sandberg, A. A., *Cytogenetics*, **6**, 357 (1967).
[6] Forssman, H., and Lehmann, O., *Acta Paediat.*, **51**, 180 (1962).
[7] Walker, S., and Harris, R., *Ann. Hum. Genet.*, **26**, 151 (1962).
[8] Jacobsen, P., Dupont, A., and Mikkelsen, M., *Lancet*, ii, 584 (1963).
[9] Makino, S., Aya, T., and Sasaki, M., *Proc. Japan Acad.*, **41**, 746 (1965).
[10] Punnett, H. H., Pinsky, L., DiGeorge, A. M., and Gorlin, R. J., *Amer. J. Hum. Genet.*, **18**, 572 (1966).
[11] Kadotani, T., and Ohama, K., *Proc. Japan Acad.*, **44**, 397 (1968).
[12] Penrose, L. S., Ellis, J. R., and Delhanty, J. D. A., *Lancet*, ii, 409 (1960).
[13] Hamerton, J. L., Cowie, V. A., Giannelli, F., Briggs, S. M., and Polani, P. E., *Lancet*, ii, 956 (1961).
[14] Szulman, A. E., *New Engl. J. Med.*, **727**, 811 (1965).
[15] Carr, D. H., *Obstet. Gynec.*, **26**, 308 (1965).
[16] Kadotani, T., and Pergament, E., *J. Hiroshima Obstet. Gynec. Soc.*, **6**, 36 (1968).
[17] Hamerton, J. L., *Cytogenetics*, **7**, 260 (1968).
[18] Kohno, S., and Maruyama, W., *Jap. J. Hum. Genet.*, 285 (1969).
[19] Hamerton, J. L., Giannelli, F., and Carter, C. O., *Cytogenetics*, **2**, 194 (1963).
[20] Slavin, R. E., Kamada, N., and Hamilton, H. B., *Jap. J. Hum. Genet.*, **12**, 17 (1967).

Presence of an Additional Metacentric Chromosome in AKR/T1ALD Leukaemic Cells

THE cells of human and murine leukaemias are usually either diploid or very close to diploidy, even when their karyotypes show some specific morphological abnormalities. In a study of spontaneous and transplanted AKR leukaemias, Wakonig-Vaartaja and Stich[1] found that most of the mitoses had a normal diploid set of forty chromosomes although there were forty-one chromosomes in one-third of the cases. Identical observations have been reported by Kurita and Yosida[2].

In 1967, Leonard and Deknudt[3] isolated and established an AKR substrain with only thirty-eight chromosomes (AKR/T1ALD). We maintain this substrain in our laboratory. The apparent decrease in the genetic content of AKR/T1ALD is the result of the rejoining of the acrocentrics of pair 4 with pair 13. Thus the chromosome set has thirty-six acrocentrics and two submetacentrics. This Robertsonian translocation which appeared spontaneously in the AKR strain does not change the chromosomal content and does not involve any gross phenotypic change, and in our animal colony the percentage of leukaemias in the AKR/T1ALD substrain does not differ significantly from the incidence of this disease in the AKR strain.

There is accumulating evidence that cells with a chromosomal imbalance are very sensitive to the chemical and physical agents which induce chromosomal abnormalities[4]. Radiation experiments were thus carried out with the T1ALD substrain and it was discovered that an additional metacentric chromosome frequently occurred in the cells of spontaneous and radiation induced leukaemias. This article describes the various patterns in the chromosome alterations.

All mice used in the experiments belonged to the AKR strain (forty chromosomes) and to the AKR/T1ALD substrain (thirty-eight chromosomes). An X-ray machine was used in the following conditions: 225 kV peak, 11 mA, through 2 mm Al and 0·3 mm Cu added filters. The dose rate at 35 cm was about 100 rad per min. Male and female T1ALD were exposed either to an acute whole body irradiation of 900 rad or to four weekly exposures of 175 rad each. In the first case, the mice were 60–70 days old and in the second they were 33–36 days old at the time of the first exposure. All the mice which had received 900 rad were restored with 0·7–1 × 10^7 bone marrow cells which had been obtained from young AKR or AKR/T1ALD donors. The animals exposed to fractionated sublethal irradiation were divided into three groups: the first was not treated with bone marrow; the second was given 0·7 – 1 × 10^7 AKR bone marrow cells; and the third received the same amount of AKR/T1ALD cells. Control and irradiated animals received water and food until they showed obvious leukaemic symptoms. They were then injected intraperitoneally with 125 mg of colchicine and were killed 1·5 h later. Thymus and femoral marrow cells were suspended in saline and were given a hypotonic shock (1 per cent sodium citrate) and processed[5]. The slides were stained with Giemsa. Metaphases were either photographed or analysed directly under the microscope. For each mouse, fifty metaphase plates of the thymic and bone marrow cells were analysed for chromosome counts. The thymus, spleen, lymph nodes and liver were examined histologically and bone marrow smears were examined cytologically.

Table 1 summarizes our findings. The percentages of extra metacentric chromosomes and acrocentrics in leukaemic cells were established exclusively from the thymic lymphosarcomas which are characteristic of these lymphoid leukaemias.

Ten of the fourteen leukaemic T1ALD had a third metacentric chromosome. One extra acrocentric chromosome was found in 3 per cent of T1ALD and in 40 per cent of AKR; more than one acrocentric appeared in 0·7 and 17 per cent of each substrain. In T1ALD mice which had received 900 rad and in isogenic T1ALD marrow, the frequency of cells with three metacentric chromosomes was the same as in the controls (24 per cent), but the number of cells with one or more extra acrocentric chromosome increased up to 14 and 4·8 per cent respectively. After restoration by AKR bone marrow, all leukaemias were of the donor (AKR) type as identified by the absence of metacentric chromosomes. But an extra acrocentric chromosome was seen in 32 per cent of the cells and more than one extra occurred in 6 per cent; this percentage was lower than in spontaneous AKR leukaemias.

Table 1

Strain	X-ray dose in rads	Origin of marrow	No. of mice Total	No. of mice With third meta-centric	Percentage of mitosis with extra Meta-centric	Percentage of mitosis with extra Acrocentric 1	Percentage of mitosis with extra Acrocentric 1
T1ALD	—	—	14	10	24·2	3·1	0·7
AKR	—	—	6	0	—	40	16·6
T1ALD	900	T1ALD	15	10	24	14	4·8
T1ALD	900	AKR	11	0	—	32	6
T1ALD	175 × 4	—	17	7	26·7	24·6	2·9

After fractionated irradiation (4 × 175 rad), the percentage of leukaemic cells with a third metacentric was 27 per cent in T1ALD and only 6 per cent in the animal restored with T1ALD bone marrow. It should be noted that in these four experimental groups, samples from different animals correspond to frequencies of additional metacentric chromosomes ranging from 2 to 100 per cent. No matter which group they belonged to, all the mice with a third metacentric chromosome in their thymic cells showed the same abnormality in their bone marrow cells; but in this last tissue the presence of normal cells of another haemopoietic series meant that an accurate estimate of their frequency could not be made, but if the bone marrow is completely invaded by leukaemic cells, the incidence of this abnormality in the marrow seems to be similar to the thymic one. In all the fourteen control T1ALD mice, and in the ten T1ALD mice irradiated with 900 rad and restored with T1ALD bone marrow, the relative length and the centromeric indices of the third metacentric were identical with those of the normal metacentric pair, but in the animals which had received 4 × 175 rad this third metacentric was normal in only two cases and was shorter than normal in all other instances.

This third metacentric chromosome was only found in leukaemic cells, and it was never seen in normal mice of the same age. But it must be noted that its presence in leukaemia is not usual because only 59 per cent of the animals in our experiment had this abnormality in their leukaemic cells and when it occurred it was often in very few of the cells. The influence of irradiation seems negligible because the percentages were the same in the controls (24 per cent), in mice which had received 900 rad (24 per cent), and in mice which had received 4 × 175 rad (27 per cent). In the radiation induced chimaeras which were obtained by the injection of AKR cells after a 900 rad exposure, all the leukaemias were of the donor type. It can therefore be assumed that this also occurs when the restoration is carried out with T1ALD cells, and in those cells which have not been irradiated, the third metacentric chromosome is as frequent as in the controls. Moreover, the incidence of this abnormality is similar in the controls and in animals in which the leukaemic process has either been stimulated by four irradiations or inhibited by 900 rad with subsequent restoration[6]. Thus there seems to be no direct correlation between the leukaemogenesis and the presence of an extra marker although it is a characteristic of most leukaemic cells.

The age of mice at which leukaemia develops does not influence the frequency of the abnormality. The mean survival time of the mice with a third metacentric chromosome was not statistically different from the survival time of the mice which did not show it; it was respectively 229 and 216 days for controls, 263 and 259 for the animal irradiated with 900 rad and 209 days in the group exposed to 175 × 4 rad.

The mechanism of formation of the third metacentric chromosome is at present unknown. But, because the extra metacentrics are identical with the normal pair in the controls and in the lethally irradiated animals, it can reasonably be assumed that they originate from a non-disjunction or a duplication. This assumption, however, does not explain why many of these extra metacentrics are shorter than normal in the irradiated groups and the radiation seems to be responsible for this structural change of the third metacentric chromosome.

It is striking that the frequency of extra acrocentric chromosomes is significantly higher in AKR than in T1ALD and comparable with the frequency with which the third metacentric marker is observed in T1ALD. The presence of such isolated extra chromosomes in specific pairs may be ascribed either to non-disjunction or to selective duplication. The theory of selective duplication has been substantiated by Lejeune *et al.*[8] using a leukaemic patient and using *in vitro* organ cultures[9]. These results may be extended to the production of a third metacentric chromosome in the T1ALD leukaemic cells. The reported findings do not support the theory of selective duplication more than the theory of reciprocal clones induced by non-disjunction, but with respect to this last possibility, no cells were observed with only one metacentric chromosome. Such monosomic clones, however, are at such a selective disadvantage that they are unlikely to show up. Finally, it is worth noting that this trisomy selectively applies to a chromosomal pair which has been previously modified by a translocation.

We thank Professor P. C. Koller for his help and advice in the preparation of this manuscript. This work was supported by a grant from INSERM.

E. LEGRAND
J. F. DUPLAN

Laboratoire Pasteur,
26 Rue d'Ulm,
75 – Paris 5°.

Received September 22; revised December 23, 1969.

[1] Wakonig-Vaartaja, R., and Stich, H. F., *J. Nat. Cancer Inst.*, **25**, 295 (1960).
[2] Kurita, Y., and Yosida, T. H., *Gann*, **52**, 257 (1961).
[3] Leonard, A., and Deknudt, G. H., *Nature*, **214**, 504 (1967).
[4] Koller, P. C., *Europ. J. Cancer*, **3**, 279 (1967).
[5] Ford, C. E., in *Tissue Grafting and Radiation* (Academic Press, New York, 1966).
[6] Duplan, J. F., *Nouv. Revue Fr. Hémat.*, **2**, 551 (1962).
[7] Lejeune, J., and Berger, R., *CR Acad. Sci., Paris*, **263**, 1880 (1966).
[8] Lejeune, J., Berger, R., Haines, M., Lafourcade, J., Vialatte, J., Satge, P., and Turpin, R., *CR Acad. Sci., Paris*, **256**, 1195 (1963).
[9] De Grouchy, J., and Wolff, E., *Europ. J. Cancer*, **5**, 159 (1969).

Probable Occurrence of Infectious Bovine Rhinotracheitis Virus in Tanzania in Wildlife and Cattle

INFECTIOUS bovine rhinotracheitis (IBR), first described in the United States as a respiratory disease of feedlot cattle[1,2], has been identified in many countries including Tchad[3] and South Africa[4]. Serological evidence suggests that the virus is widespread in the Cameroons, the Central African Republic[5] and Nigeria[6]. There are, however, no published reports of the disease in East Africa.

At these laboratories we have therefore started to investigate the possible occurrence of IBR virus in Tanzania and its significance in animal disease in this country. A serological survey for the detection of IBR neutralizing antibody against the Oxford strain of IBR virus (provided by Dr J. H. Darbyshire of the Central Veterinary Laboratory, Weybridge, Surrey) has been started on sera of cattle and wildlife. This study began in the Masailand area of Tanzania.

Serum-virus neutralization tests based on the technique of Mohanty *et al.*[7] were carried out at a final serum dilution of 1/8 against 100 $TCID_{50}$ IBR virus in the inoculum; for each serum two tubes of primary calf kidney monolayers were used as the indicator system. Final readings were carried out when control cultures showed a maximum cytopathic effect, usually 5–6 days after inoculation, using a cytopathic effect scoring system[8] whereby neutralization is indicated by a quantitative estimation of inhibition of cytopathic effect. Neutralization titres were calculated according to the method of Reed and Muench[9].

Table 1. DISTRIBUTION OF IBR NEUTRALIZING FACTOR IN WILDLIFE AND CATTLE OF TANZANIA

Species	No. tested	No. positive	Per cent positive
Buffalo calf	3	1	33
Buffalo	52	38	76
Eland	46	0	0
Elephant	5	0	0
Giraffe	3	0	0
Impala	21	0	0
Thompson gazelle	29	0	0
Warthog	1	0	0
Wildebeeste	56	2	3·4
Cattle	61	52	85

Neutralization titres expressed as reciprocals.

Table 2. RESULTS OF THE TITRATION OF THE IBR NEUTRALIZING FACTOR IN SERA OF BUFFALO AND CATTLE

Reciprocal *S/N* titre	Nos. of positive sera Buffalo	Cattle
8–16	0	5
> 16–32	4	8
> 32–64	12	3
> 64–128	3	2

The results of the neutralization tests against the species tested so far are summarized in Table 1. Almost all positive sera were obtained from buffalo, but two wildebeeste also gave positive reactions. Eighty-five per cent of the sera collected at a local abattoir from cattle from the Ngorongoro area also gave positive neutralization for IBR virus. In buffalo and cattle the neutralization titre of positive sera was as high as 1/128 (Table 2). Rabbit hyperimmune serum collected after five serial intravenous inoculations of IBR virus during 4 months showed a titre of 1/180.

The neutralization factor thus detected is probably specific antibody against IBR virus or an antigenically closely related virus; this is indicated by the fact that it is stable to heat and trypsin and seems to be associated with the γ-globulin fraction of the serum protein. (Serum γ-globulins were precipitated by ammonium sulphate[10].)

This work indicates that IBR probably occurs in the wild life of Tanzania. This is very important, especially where the buffalo (*Syncercus caffer*) is concerned. Whether the antibody detected in buffalo is associated with any definite clinical disease in this species has yet to be determined.

M. M. RWEYEMAMU

Central Veterinary Laboratory,
Temeke, PO Box 9254,
Dar es Salaam,
Tanzania.

Received November 12, 1969.

[1] Miller, N. J., *J. Amer. Vet. Med. Assoc.*, **126**, 463 (1955).
[2] McKercher, D. G., *Amer. J. Vet. Res.*,, **18**, 246 (1957).
[3] Provost, A., Borredon, C., and Fereol, C., *Rev. Elev.*, **17**, 187 (1964).
[4] Maré, C. J., and van Rensburg, S. J., *J. South African Vet. Med. Assoc.*, **32**, 201 (1961).
[5] Provost, A., and Borredon, C., *Vet. Rec.*, **77**, 1598 (1965).
[6] Zwart, D., *Bull. Epizoot. Dis. Africa*, **14**, 405 (1966).
[7] Mohanty, S. B., and Lilie, M. G., *Amer. J. Vet. Res.*, **26**, 892 (1965).
[8] Rweyemamu, M. M., and Johnson, R. H., *Brit. Vet. J.*, **124**, 9 (1968).
[9] Reed, L. J., and Muench, H., *Amer. J. Hyg.*, **27**, 493 (1938).
[10] Wootton, I. D. P., *Micro-analysis in Medical Biochemistry*, fourth ed. (Churchill, London, 1964).

Effect of Foetal Hypophysectomy on Catecholamine Levels in the Lamb Adrenal during Prolonged Gestation

DESTRUCTION of the pituitary gland of the foetal lamb *in utero* results in an indefinite prolongation of pregnancy[1]. This seems to be caused by a reduction in the secretion from the foetal adrenal cortex, for parturition can be induced in either post-mature hypophysectomized lambs or premature normal foetuses by infusions of ACTH or of large doses of cortisol into the foetus[2]. There is, however, a close association between the adrenal cortex and the concentration of adrenaline in the adrenal medulla[3-6]. In the lamb the rapid growth of the adrenal cortex immediately before birth is accompanied by a slight rise in the proportion of adrenaline in the gland[7]; absence of growth of the adrenal cortex after hypophysectomy might therefore affect catecholamine content, or more specifically the ratio of adrenaline to noradrenaline in the medulla, particularly during the period of prolonged gestation. This possibility has been investigated in the present experiments in which foetal hypophysectomy *in utero* was carried out at 93–105 days gestation in sixteen Welsh ewes.

The technique for electrocoagulation of the foetal pituitary[8] was modified so that the electrode could be inserted stereotaxically. The ewes were anaesthetized with sodium pentobarbitone and the foetal head was held rigidly within the uterus by means of a simple head-holder. The horizontal bars were inserted into the temporal fossae which could be palpated behind the post orbital processes through the uterine wall, while the vertical movement was controlled by a clamp over the upper jaw. The coordinates for positioning the electrode with the micro-manipulator mounted on the head-holder were determined from foetal skulls. The completeness of hypophysectomy was checked by histological examination of the contents of the pituitary fossa and adjacent tissues.

Postoperative recovery was uneventful in all sixteen animals. Ten of the hypophysectomized foetuses were examined 7–33 days after the normal date of parturition (147 days) and two were used 25 and 30 days after hypophysectomy, that is, at 128 and 131 days of gestation. Four animals were born spontaneously at the usual time despite electrocoagulation of the pituitary region. The adrenal glands were removed as rapidly as possible from all animals, under sodium pentobarbitone anaesthesia. The right gland was used for catecholamine determinations according to the method described previously[7] and the left gland was examined histologically. The results were compared with data from previous work on the same breed[7] and on a further series of normal foetuses near term, at birth and at 2–3 weeks of age.

The percentage of adrenaline in the post-mature foetuses was extremely low compared with that from normal unoperated foetal and postnatal lambs (Fig. 1*a*). The wide individual variation found in normal foetuses and the increase in the percentage of adrenaline shortly before term or soon after birth was not present after hypophysectomy; indeed, the percentage of adrenaline (20 per cent) was lower than that found early in gestation. Even 25–30 days after the operation the percentage of adrenaline had fallen and was below that at 90–100 days.

The contrast between the adrenal weights of the two series was most striking (Fig. 1*b*) and the changes were parallel to those in the percentage of adrenaline in the glands. Owing to the steep rise in adrenal weight in the control animals near term, due to growth of cortical tissue, parallel to those in the percentage of adrenaline in the gland rather than the concentration per g of adrenal tissue have been used as a basis for comparison (Table 1). The total catecholamine content of the gland after prolonged gestation was remarkably similar to the mean value found in the normal animals at term. In the hypophysectomized

Table 1. EFFECT OF HYPOPHYSECTOMY ON THE DEVELOPMENT AND CATECHOLAMINE CONTENT OF ADRENAL GLANDS

Animals	Age	No.	Mean adrenal wt (mg)	Mean catecholamine content (μg/gland ± *S.E.*) Adrenaline (A)		Noradrenaline (N)		Total	Mean percentage A
Hypophysectomized foetuses	154–180 days gestation	10	135 ± 3·8	108 ± 10·3	$P < 0.01$	414 ± 21·0	$P < 0.01$	522 ± 59	20·8
Control lambs	145 days gestation or newborn	6	328 ± 31·9	248 ± 27·8		281 ± 36·5		526 ± 28	47·0
	2–3 weeks after birth	3	397	391		260		651	60·0
* Unsuccessfully hypophysectomized foetuses	Newborn	4	200 ± 11·2	235 ± 52·0		250 ± 61·0		485	48·5

* Some anterior pituitary tissue attached to stalk (parturition 147–150 days).

animals, however, the mean noradrenaline levels were much higher and the adrenaline levels correspondingly lower than the values obtained from control lambs at birth or 3–4 weeks later.

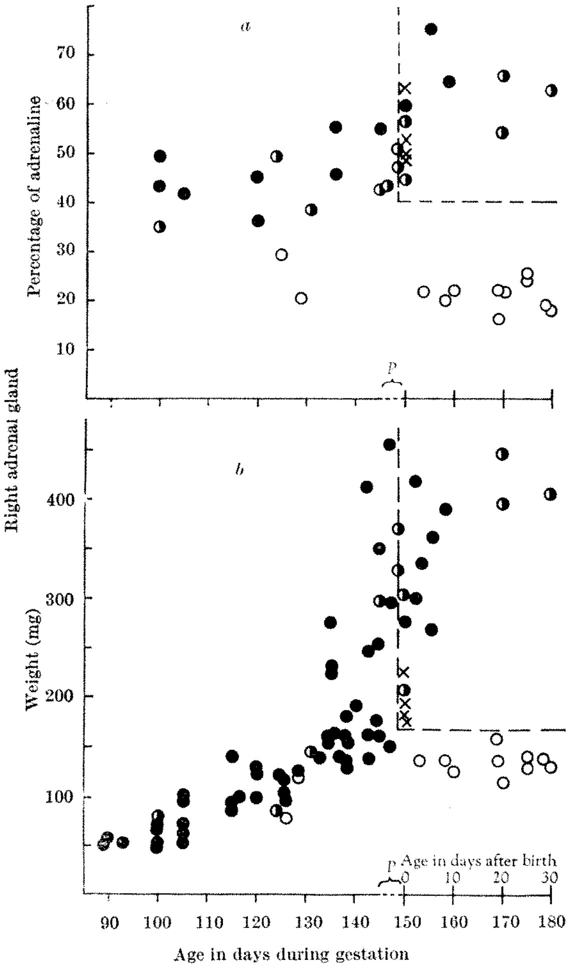


Fig. 1. Changes in (*a*) percentage of adrenaline in adrenal glands and (*b*) adrenal weights of hypophysectomized and control lambs during gestation and early post-natal life. ●, Unoperated control animals[7]. ◐, Unoperated control animals, present series. ○, Foetuses hypophysectomized at 93–105 days gestation, in which gestation was prolonged and germinated artificially. ×, Incompletely hyphophysectomized foetuses in which parturition (*P*) occurred at the normal time (147–150 days gestation). All points within the interrupted lines were obtained from lambs after birth.

In the four lambs in which parturition occurred spontaneously at the usual time in spite of electrocoagulation of the pituitary, the adrenal weight reached the minimum found after normal birth, largely because of growth of the cortex. The proportion of adrenaline in the adrenal medulla of these foetuses (Fig. 1) and the catecholamine content (Table 1) did not differ significantly from the control animals at term. Histological examination of the pituitary region in these animals showed that although the major part of the anterior pituitary had been destroyed the pituitary stalk was invariably present and had a small amount of anterior pituitary tissue attached to it.

The present findings suggest that after hypophysectomy the foetal adrenal medulla becomes unable to methylate noradrenaline; synthesis apparently continues and the stores of noradrenaline increase while those of adrenaline fall. These changes are intimately associated with the lack of adrenal cortical growth, but whether the adrenal medulla as well as the cortex is involved in the termination of gestation remains to be elucidated.

R. S. COMLINE
MARIAN SILVER

Physiological Laboratory,
University of Cambridge.

I. A. SILVER

Department of Anatomy,
University of Cambridge.

Received August 7; revised November 10, 1969.

[1] Liggins, G. C., and Kennedy, P. C., *J. Endocrinol.*, **40**, 371 (1968).

[2] Liggins, G. C., *J. Endocrinol.*, **42**, 323 (1968).

[3] Shepherd, D. M., and West, G. B., *Brit. J. Pharmacol. Chemother.*, 6, 665 (1951).

[4] Coupland, R. E., *The Natural History of the Chomaffin Cell* (Longmans, London, 1965).

[5] Jost, A., *Recent Progress in Hormone Research*, **22**, 541 (1966).

[6] Wurtman, R. J., and Axelrod, J., *J. Biol. Chem.*, **241**, 2301 (1966).

[7] Comline, R. S., and Silver, M., *J. Physiol.*, **156**, 424 (1961).

[8] Liggins, G. C., Kennedy, P. C., and Holm, L. W., *Amer. J. Obstet. Gynec.*, **98**, 1080 (1967).

Gonadotrophin Inhibition by the Synergistic Action of Vasopressin and Oxytocin

GONADOTROPHIN inhibitory activity has been demonstrated in urine[1,2], serum[3] and in pineal extracts[4-6]. The similarity between the inhibitors from urine and the pineal gland[4,5], and the absence of inhibitory activity from urine from patients with sexual precocity resulting from pinealoma[7], suggests that the urinary factor is derived from the pineal gland. There are, however, several substances with gonadotrophin-inhibitory activity in the pineal gland. Melatonin prevents growth of the ovary and inhibits oestrus in rats[8], but in short term experiments, this substance differed from urinary extracts in having no effect on exogenously administered gonadotrophin[4]. Serotonin and adrenaline are gonadotrophin-inhibitory[9] and are present in the pineal gland[10]. But these substances could not be detected in potent pineal and urinary extracts[6]. A polypeptide, identified as arginine-vasotocin, has been found in the bovine pineal bland[10]. This compound inhibited the uterine weight response to pregnant mare serum gonadotrophin[11]. Because arginine-vasotocin has both pressor and oxytocic activities[10], it was thought that useful information might be obtained by studying the gonadotrophin inhibitory properties of vasopressin and oxytocin individually. I report here the results of such experiments, in particular, a synergistic effect between the two substances to produce gonadotrophin inhibition similar to that observed with urinary extracts.

The experimental procedure has been described[2,9]. The gonadotrophin-inhibitory properties of vasopressin and oxytocin were evaluated by the mouse uterus method using immature, albino mice from an inbred colony. They were 20–22 days old and initially weighed 8–11 g. Although the mean body weights of the groups in each experiment were the same, they varied from experiment to experiment and therefore responses to the same treatment in different experiments cannot be compared. Food and water were given *ad libitum*. The experimental groups consisted of six to eleven animals. All injections were given subcutaneously in aqueous solution except oestrone which was administered in oil. The total dose of human chorionic gonadotrophin ('Pregnyl', Organon) was 0·4 IU and that of follicle-stimulating hormone (FSH-P, Armour) was equivalent to 400 μg NIH—FSH—S1. The animals of further groups were injected at different sites with HCG (0·4 IU) and FSH (50 μg equivalents), while oestrone was given to a total dose of 0·3 μg.

Vasopressin ('Pitressin', Parke Davis) and oxytocin ('Syntocinon', Sandoz) were used in preliminary experiments to determine the minimal effect dose of each. This was found to total 15 IU for vasopressin and 6 IU for oxytocin. Some mice were given a mixture of 0·3 IU vasopressin and 0·3 IU oxytocin. These substances were tested for HCG inhibitory activity after they had been boiled for 1 h under reflux. Injections were at the same time but at a different site from those of the gonadotrophins or oestrone.

Treatment was given for 3 days using the following volumes: HCG, 0·2 ml., 0·1 ml. and 0·1 ml.; FSH, 0·2 ml., 0·2 ml. and 0·1 ml.; vasopressin and oxytocin, 0·2 ml. daily; and oestrone and vasopressin–oxytocin mixture, 0·1 ml. daily.

On the fourth day, the mean body and uterine weights from the test groups were compared with those given gonadotrophins (or oestrone) and distilled water, using the Student *t* test.

Table 1. EFFECT OF VASOPRESSIN (15 IU) ON THE MOUSE UTERINE WEIGHT RESPONSE TO GONADOTROPHINS AND OESTRONE

Experiment	No. of animals	Mean uterine weight (mg + *S.E.*)	*P*
HCG 0·4 IU + water	11	31·9 ± 4·6	= 0·05
HCG 0·4 IU + vasopressin*	11	21·3 ± 2·8	
HCG 0·4 IU + boiled vasopressin	10	23·8 ± 3·9	> 0·1
FSH 400 μg + water	10	14·1 ± 0·9	> 0·1
FSH 400 μg + vasopressin	9	15·1 ± 1·2	
Oestrone 0·3 μg + water	11	22·5 ± 1·5	> 0·1
Oestrone 0·3 μg + vasopressin	11	21·7 ± 0·9	
HCG 0·4 IU FSH 50 μg } + water	9	37·9 ± 4·3	> 0·1
HCG 0·4 IU FSH 50 μg } + vasopressin*	8	29·4 ± 4·2	

* Significantly lower final body weight than group given distilled water.

Table 2. EFFECT OF OXYTOCIN (6 IU) ON THE MOUSE UTERINE WEIGHT RESPONSE TO GONADOTROPHINS AND OESTRONE

Experiment	No. of animals	Mean uterine weight (mg ± *S.E.*)	*P*
HCG 0·4 IU + water	11	32·5 ± 1·5	
HCG 0·4 IU + oxytocin*	11	15·7 ± 1·4	< 0·001
HCG 0·4 IU + boiled oxytocin*	11	22·7 ± 3·3	< 0·02
FSH 400 μg + water	10	11·7 ± 1·0	> 0·1
FSH 400 μg + oxytocin	10	14·3 ± 1·3	
Oestrone 0·3 μg + water	11	27·2 ± 1·9	> 0·1
Oestrone 0·3 μg + oxytocin*	11	29·2 ± 1·8	
HCG 0·4 IU FSH 50 μg } + water	10	28·2 ± 3·3	> 0·1
HCG 0·4 IU FSH 50 μg } + oxytocin	10	27·4 ± 2·8	

* See Table 1.

The results are shown in Tables 1–3. Vasopressin and oxytocin, given separately or as a mixture, did not affect the uterine weight response to FSH or oestrone. On the other hand, they reduced the potency of HCG although this reduction was only just significant with vasopressin. Boiling removed the inhibitory activity from vasopressin but not from oxytocin or from the vasopressin–oxytocin mixture. HCG could, however, be protected from the inhibitory effects of the materials by administration of FSH. It was noted that some of the treatment groups failed to gain weight normally.

The urinary gonadotrophin inhibitor (GIM) is heat stable and reduces the potency of HCG but not of FSH or oestrone in the mouse uterus assay[2,7]. Similar results were obtained with crude extracts of bovine pineal glands[4,6]. In the present investigations, pharmacological doses of vasopressin and oxytocin (15 IU and 6 IU, respectively) were also found to inhibit gonadotrophin activity. In the case of vasopressin, this inhibitory effect was destroyed by boiling (Table 1). When the neurohypophysial hormones were mixed, there was a synergistic effect, and inhibition could be demonstrated with a dose as low as 0·3 IU (Table 3). This dose of arginine vasotocin has been found to be gonadotrophin-inhibitory[11]. As mentioned earlier, arginine–vasotocin is a polypeptide isolated from the pineal gland with both pressor and oxytocic activities[10]. The results of the present investigation suggest that the inhibitory properties of arginine–vasotocin, and therefore of the pineal extract, result from the combined effect of its pressor and oxytocic activities rather than from the polypeptide specifically. A further possibility is that the synergistic effect is caused by an altered rate of absorption of oxytocin from the injection site following vascular changes induced by vasopressin.

Table 3. EFFECT OF VASOPRESSIN–OXYTOCIN (V–O) MIXTURE (0·3 IU) ON THE MOUSE UTERINE WEIGHT RESPONSE TO GONADOTROPHINS AND OESTRONE

Experiment	No. of animals	Mean uterine weight (mg ± *S.E.*)	*P*
HCG 0·4 IU + water	10	46·7 ± 4·5	
HCG 0·4 IU + v–o mixture	10	31·9 ± 3·7	= 0·025
HCG 0·4 IU + boiled v–o mixture*	10	25·2 ± 3·7	< 0·005
FSH 400 μg + water	10	15·3 ± 0·7	> 0·1
FSH 400 μg + v–o mixture	10	14·0 ± 0·6	
Oestrone 0·3 μg + water	9	27·7 ± 2·9	> 0·1
Oestrone 0·3 μg + v–o mixture	10	30·6 ± 1·8	
HCG 0·4 IU FSH 50 μg } + water	10	44·0 ± 3·6	> 0·1
HCG 0·4 IU FSH 50 μg } + v–o mixture	10	47·0 ± 5·2	

* See Table 1.

The inhibitory properties of the neurohypophysial hormones are very similar to those of the urinary gonadotrophin inhibitor, suggesting that their modes of action are related. There is some evidence that the latter inhibits the endogenous gonadotrophin secretion necessary for the mouse uterine weight response to HCG and PMS[2,12]. This hypothesis could also be applied to the neurohypophysial hormones because of the protection afforded to HCG by giving exogenous FSH. Brinkley and Nalbandov have also reported inhibition of endogenous gonadotrophin secretion by oxytocin[13]. The similarity between the neurohypophysial hormones and urinary gonadotrophin inhibitor is also found when ovarian ascorbic acid is studied: vasopressin[14] and gonadotrophin inhibitor[6] both cause its depletion.

Several interpretations of the similar activities of neurohypophysial hormones and urinary gonadotrophin inhibitor are possible. First, their effects may be independent and both may produce a non-specific response to the injection of toxic material. This has been proposed as the mode of action of urinary gonadotrophin inhibitor[9] and in this investigation some of the animals given vasopressin and oxytocin failed to gain weight normally. Second, endogenous vasopressin secretion is part of the response to noxious stimuli[15] and it is likely that this is accompanied by oxytocin release[16]. Urinary gonadotrophin inhibitor activity may be mediated by this mechanism, but it is difficult to compare a physiological response with the effect of the pharmacological doses used in this investigation. Finally, it is possible that urinary gonadotrophin inhibitor contains neurohypophysial hormones. It will be necessary to demonstrate the presence of both vasopressin and oxytocin because it has already been shown that the amount of the former is less than 0·1 IU per l. urine[17].

This study was supported by the North West Cancer Research Fund. I thank Mrs J. Sullivan for technical assistance.

L. J. Hipkin

University of Liverpool Endocrine Unit,
Liverpool Clinic,
Myrtle Street, Liverpool 7.

Received August 25, 1969.

[1] Futterweit, W. S., Margolis, S. A., Soffer, L. J., and Dorfman, R. I., *Endocrinology*, **72**, 903 (1963).

[2] Hipkin, L. J., *Acta Endocrinol.*, **59**, 417 (1968).

[3] Reiss, M., Pearse, J. J., Davis, R. H., Hillman, J. C., and Sideman, M. B., *J. Endocrinol.*, **29**, 83 (1964).

[4] Soffer, L. J., Fogel, M., and Rudavsky, A. Z., *Acta Endocrinol.*, **48**, 561 (1965).

[5] Yamashita, I., Hillman, J. C., and Reiss, M., *J. Endocrinol.*, **33**, 223 (1965).

[6] Hipkin, L. J., thesis, Univ. Liverpool (1969).

[7] Fogel, M., and Soffer, L. J., *Amer. J. Obstet. Gynec.*, **98**, 85 (1967).

[8] Wurtman, R. J., Axelrod, J., and Chu, E. W., *Science*, **141**, 277 (1963).

[9] Hipkin, L. J., *Endocrinology*, **84**, 482 (1969).

[10] Milcu, S. M., Pavel, S., and Neacsu, C., *Endocrinology*, **72**, 563 (1963).

[11] Pavel, S., and Petrescu, S., *Nature*, **212**, 1054 (1966).

[12] Hipkin, L. J., *Nature*, **221**, 473 (1969).

[13] Brinkley, H. J., and Nalbandov, A. V., *Endocrinology*, **73**, 515 (1963).

[14] Giuliani, G., Martini, L., Pecile, A., and Fochi, M., *Acta Endocrinol.*, **38**, 1 (1961).

[15] Mirsky, A., Stein, M., and Paulisch, G., *Endocrinology*, **55**, 28 (1954).

[16] Van Dyke, H. B., *Clinical Endocrinology* (edit. by Astwood, E. B.), **1**, 68 (Grune and Stratton, New York, 1960).

[17] Mayer, F. S., *Acta Endocrinol.*, **35**, 568 (1960).

Eight Types of Bisalbuminaemia

As our knowledge of human albumin variants increases, their description as electrophoretically "slow", "faster" and so on is becoming inadequate for classification. The largest comparative series[1] found five different migration rates in sera from nineteen families. Electrophoretic mobility is not the only criterion, and an earlier classification[2] used eight, variations of mobility in different electrophoretic media and dye-binding properties among them. Such observations will not establish the identity of two albumins but may prove their non-identity.

We have attempted a fuller description of bisalbuminaemia in eight unrelated families. Four are published variants, albumin Reading[3] (No. 4 in this series), albumin Naskapi[4] (No. 7), No. 5 (A.D.M. of Cooke, Cleghorn and Lockey[5]) and 6 (Payne and Dickinson[6]), one (No. 8) is awaiting publication (Rawstron and Mellor, to be published), and three are unpublished. No. 7 is North American Indian, No. 8 is Spanish, and the rest are British.

All sera were examined by three electrophoretic methods, on Whatman 3*MM* filter paper at pH 8·6 with bromophenol blue staining[7], on cellulose acetate at pH 8·6 and staining with Ponceau-*S* (ref. 8), and by disc electrophoresis in 7 per cent acrylamide gel in a discontinuous system of pH 8·3→8·9→8·3 with Ponceau-*S* staining[9]. These techniques of electrophoresis were also used with sera pre-stained with a quantity of bromophenol blue sufficient only to bind the albumin. Specificity of binding was achieved by incubation with 200–250 μg dye per ml. of serum at 37° C for 10 min shortly before filter paper or cellulose acetate electrophoresis, or by adding 2 μg to the 300 μg of serum protein used as the sample in disc electrophoresis, but omitting to add the tracking dye usual in this method. Sera 1–7 were also separated by horizontal starch gel electrophoresis in Poulik's pH 8·65 buffer, with naphthalene black staining[10]. Disc electrophoresis results are given in the 0–100 mm notation[9] (see Fig. 1), and relative mobilities in cellulose acetate are expressed in the same terms.

As Table 1 shows, all of the eight variants are distinguishable. All differ in several particulars, though the results of some tests show parallel tendencies and suggest that a short list of tests will serve to characterize variants. Only half the albumins resolve on filter paper; where they do, their type (fast or slow) follows that of cellulose acetate. The albumin of serum 2 gives two bands in acrylamide but does not resolve in starch gel; for the other sera, resolution in starch follows that in acrylamide. Generally, it seems that for each double albumin the difference

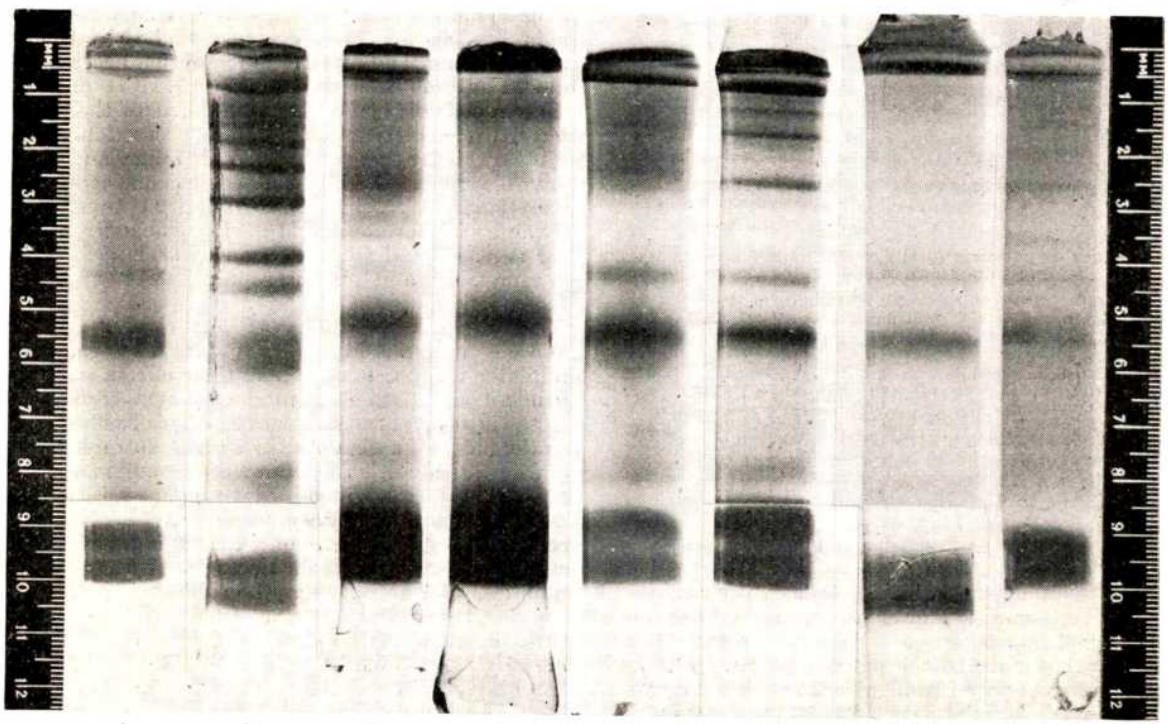


Fig. 1. Disc electrophoretic protein patterns of sera from eight families with different albumin variants. 0, Cathode end of pattern; 100, advancing edge of normal albumin band. Where the photographic contrast between the albumins and the rest of the pattern is too great, single negatives are printed by two processes and the prints joined at 85 mm.

Table 1. CHARACTERIZATION OF EIGHT ALLOALBUMINS

Serum No.	1	2	3	4[3]	5[5]	6[6]	7[4]	8[7]
Name of alloalbumin				Al Reading			Al Naskapi	
Electrophoresis:								
C.A.	85	113	107	108	86	86	110	90
F.P.	S	F	1	1	S	S	1	1
ACR.	89–95, 96–100	95–100, 101–105	85–100	82–100	86–92, 94–100	85–91, 94–100	94–100, 101–105	89–95, 95–100
ST.	2	1	1	1	2	2	2	
Bromophenol blue uptake:								
C.A.	A > S	F > A	F > A	F (Re)	A ≫ S	A	F(Na) > A	A
F.P.	A > S	F > A	—	—	A > S	A > S	—	—
ACR.	A, S	F sl. > A	—	—	S	A, S	F (Na)	A, S

Electrophoresis: C.A., position of alloalbumin in cellulose acetate pattern (> 100 is faster, < 100 slower than normal albumin A). F.P., type of alloalbumin in filter paper pattern (F, fast; S, slow; 1, no separation of bands). ACR., position of albumins in acrylamide gel pattern (type assumed identical with C.A.). ST., number of bands in starch gel pattern. Bromophenol blue uptake: presence of stain on fast (F), slow (S) and normal (albumin A) bands.

of dye uptake between bands is in the same direction in all three media. But while on cellulose acetate and filter paper the slow alloalbumins take up less bromophenol blue than the normal band and fast variants take up more, acrylamide does not show this regularity.

The present tests may be developed to give more precise information. Millimetre positions in the cellulose acetate separations tend to be higher for shorter lengths of pattern, and this variation (within ±5 per cent) would be eliminated by adopting a standard running length. 0–100 mm values for filter paper separations could be obtained from replicate runs. Our starch gel separations have not generally been clear-cut enough for this notation (only serum 1 could be assigned band measurements, at 90–95, 96–100), and Ashton and Braden's lithium buffer[4] may be preferable for these separations.

Our results suggest a greater variety of human albumins than can be classified on published data. A set of tests, both electrophoretic and of the dye-binding type, gives more information and may enable variants to be equated with some degree of confidence. This would require agreement on a rational choice of tests and on standardized conditions for carrying them out.

We thank Miss Patricia Orr, Mr P. M. G. Broughton, Drs K. B. Cooke, W. G. Dangerfield, F. G. Herman, J. Kohn, Liisa Melartin, R. B. Payne, J. R. Rawstron and K. E. Spittlehouse for gifts of sera, Dr F. H. Grimbleby for advice on electrophoresis and Mr L. F. Williams for the photographs.

A. L. TÁRNOKY
B. DOWDING
A. L. LAKIN

Royal Berkshire Hospital and
University of Reading.

Received August 4; revised October 28, 1969.

[1] Weitkamp, L. R., Shreffler, D. C., Robbins, J. L., Drachmann, O., Adner, P. L., Wieme, R. J., Simon, N. M., Cooke, K. B., Sandor, G., Wuhrmann, F., Braend, M., and Tárnoky, A. L., *Acta Gen., Basel*, **17**, 399 (1967).

[2] Tárnoky, A. L., *Proc. Assoc. Clin. Biochem.*, **4**, 12 (1966).

[3] Tárnoky, A. L., and Lestas, A. N., *Clin. Chim. Acta*, **9**, 551 (1964).

[4] Melartin, L., and Blumberg, B. S., *Science*, **153**, 1664 (1966).

[5] Cooke, K. B., Cleghorn, T. E., and Lockey, E., *Biochem. J.*, **81**, 39P (1961).

[6] Payne, R. B., and Dickinson, J. P., *Nature*, **215**, 536 (1967).

[7] Tárnoky, A. L., *Clinical Biochemical Methods*, 144 (Hilger, London, 1958).

[8] Kohn, J., in *Chromatographic and Electrophoretic Techniques* (edit. by Smith, I.), second ed., **2**, 84 (Heinemann, London, 1968).

[9] Tárnoky, A. L., and Dowding, B., *Clin. Biochem.*, **1**, 48 (1967).

[10] Smith, I., in *Chromatographic and Electrophoretic Techniques* (edit. by Smith, I.), second ed., **2**, 217 (Heinemann, London, 1968).

Nuclear Relaxation in Phospholipids and Biological Membranes

ATTEMPTS have recently been made to apply high resolution proton magnetic resonance spectrometry to the problem of molecular association in biological membranes and in dispersions of phospholipids in water[1–4]. On the high resolution scale, line broadening gives the appearance of weak absorption by protons of fatty acid molecules. Because broad lines could result from restricted molecular motion and might indicate that fatty acid chains are immobilized in these systems, a central problem in the interpretation of spectra is the relationship between molecular mobility and linewidth. In steady-state absorption nuclear magnetic resonance (NMR) the apparent transverse relaxation time, T_2, is given by the reciprocal of the linewidth. Often the T_2 thus obtained is an inverse measure of correlation time and reflects molecular mobility. This interpretation is valid for isotropic solutions of small molecules, where rapid motion produces line narrowing and large values of T_2.

The interpretation of linewidths in terms of correlation times is also valid for isotropic solutions of detergent micelles[5] and for serum lipoproteins[4–7], but extension of these ideas to anisotropic particulate systems such as cell membranes or phospholipids dispersed in water may not be justified. Other line broadening mechanisms can introduce spurious results in the sense that measured linewidths do not truly reflect the state of molecular mobility. Field-dependent broadening has already been demonstrated in phospholipids in the lamellar phase[8]. The observed linewidths are several hundred Hz, but the values extrapolated to zero external field are approximately 10–20 Hz. Similar field-dependent broadening also occurs with sonicated phospholipids in water and is more accentuated for the absorption lines of the fatty acid methylene protons than for those of the polar ends of the molecules[9]. Such phenomena most likely arise from internal magnetic field gradients caused by local variations in diamagnetic susceptibility[9].

Field-dependent and susceptibility-dependent line broadening is well known in other materials, both particulate and liquid, and is especially pronounced with particulate systems[10–14]. As with phospholipids, the linewidth is a linear function of field strength. Local internal magnetic field gradients have been shown to be present, and in some cases diffusion within these gradients plays a part. In such conditions, broadening due to field inhomogeneities occurs and short transverse relaxation times obtained from linewidths are erroneous.

Values of T_2 independent of field inhomogeneities can be obtained by pulsed spin-echo methods[10]. It would be expected, then, that for phospholipids the equivalent linewidths calculated from spin-echo measurements of T_2 would be considerably smaller than those determined by other methods. If broadening mechanisms similar to those in phospholipids are also present in biomembranes, they, too, should yield narrow equivalent lines by spin-echo techniques.

We have examined, without using sonication, erythrocyte ghosts and total ghost lipids by two kinds of pulsed NMR: free induction decay and the Meiboom–Gill modification of the Carr–Purcell spin-echo method[15,16]. In the first approach, the decay of the transverse magnetization is observed after a 90° pulse (a 90° pulse is one which rotates the magnetization vector by 90°). T_2 measured by this technique is similar to that calculated from linewidths, and is subject to the same broadening mechanisms. In the second method a 90° pulse is followed by a train of 180° re-focusing pulses, and T_2 is obtained from the envelope of resultant echoes. This latter T_2 is independent of field inhomogeneities, whether from the external laboratory magnet or from gradients within the particles. The results show that line broadening arising from field

Table 1. RELAXATION TIMES DETERMINED BY THE FREE INDUCTION DECAY (FID) AND CARR-PURCELL (CP) METHODS

	T_2 (ms)	$\Delta\nu$ (Hz)
Ghost lipids	0·56 ± 0·3 (FID)	1,100
	110 ± 10 (CP)	5·8
Ghosts	1·4 ± 0·2 (FID)	460
	75 ± 1 (CP)	8·4

inhomogeneities occurs with both membranes and extracted lipids. The transverse relaxation times obtained with the spin-echo technique are long, suggesting a high degree of molecular mobility.

Erythrocyte ghosts were prepared from fresh citrated beef blood by lysis in phosphate buffer[17], and were then lyophilized from distilled water and resuspended in deuterium oxide containing 0·1 M NaCl. Lipids were extracted from lyophilized ghosts with chloroform–methanol (2 : 1), lyophilized from benzene, and also suspended in 0·1 M NaCl-^2H_2O. For both systems the concentrations were approximately 25 per cent w/v. Measurements were made at 60 MHz and room temperature with a 'Magneon SE-30' high-power spin-echo spectrometer; 5 mm tubes were used for free induction decay, and 12 mm tubes for Carr–Purcell runs. Oscilloscope traces were photographed on 'Polaroid' film, digitized with an Autotrol Corp. Model 3400 digitizer, recorded on paper tape, and fitted by a computerized least-squares routine. One dominant relaxation time was present in each case. Relaxation times determined by the two methods are listed in Table 1, together with equivalent full linewidths at half-height calculated from the relation $\Delta\nu = 1/\pi T_2$.

As expected, linewidths calculated from free induction decay experiments are several hundred Hz, in agreement with published steady-state absorption spectra. Those obtained from the Carr–Purcell programme, however, are only a few cycles and are typical of liquid-like systems. These narrow lines are similar to those obtained by extrapolation of phospholipid absorption spectra to zero field. The true correlation times appear to be short and are approximately the same in both lipid dispersions and membranes. We interpret these results to mean that in both systems absorption lines are inhomogeneously broadened by magnetic field gradients. Interpreted in terms of molecular motion, broad lines obtained by absorption NMR are misleading.

Preliminary experiments with intact ghosts which were not lyophilized and with sonicated ghosts also show disparities between free induction decay and Carr–Purcell results. Minor changes are caused by freeze-drying; and these are under investigation. The phenomena described are not confined to erythrocyte ghosts: membranes of *Mycoplasma laidlawii* and *Escherichia coli* also appear to exhibit broadening due to internal magnetic field inhomogeneity.

We thank Dr R. H. Lawler, Dr R. Kedzie and Mr D. Longland for discussions and technical aid. This work was supported by a grant from the US Public Health Service.

STANLEY KAUFMAN
JOSEPH M. STEIM
JULIAN H. GIBBS

Department of Chemistry,
Brown University,
Providence, Rhode Island, 02912.

Received November 10, 1969.

[1] Chapman, D., and Penkett, S. A., *Nature*, **211**, 5055 (1966).
[2] Chapman, D., Kamat, V. B., de Gier, J., and Penkett, S. A., *Nature*, **213**, 74 (1967).
[3] Chapman, D., Kamat, V. B., de Gier, J., and Penkett, S. A., *J. Mol. Biol.*, **31**, 101 (1968).
[4] Steim, J. M., in *Molecular Association in Biological and Related Systems*, **84**, 259 (Amer. Chem. Soc. Adv. Chem. Series, 1968).
[5] Clifford, J., and Pethica, B. A., *Trans. Faraday Soc.*, **60**, 1483 (1964).
[6] Steim, J. M., Edner, O. J., and Bargoot, F. G., *Science*, **162**, 909 (1968).
[7] Chapman, D., Leslie, R. B., and Hirz, R., *Nature*, **221**, 260 (1969).
[8] Penkett, S. A., Flook, A. G., and Chapman, D., *Chem. Phys. Lipids*, **2**, 273 (1968).
[9] Sheard, B., *Nature*, **223**, 1057 (1969).
[10] Zamir, D., and Cotts, R. M., *Proc. Colloq. Ampère*, **13**, 276 (1964).
[11] Drain, L. E., *Proc. Phys. Soc.* (*London*), **80**, 1380 (1962).
[12] Drain, L. E., *J. Phys. Chem. Solids*, **24**, 379 (1963).
[13] Zamir, D., and Cotts, R. M., *Phys. Rev.*, **134**, A666 (1964).
[14] Zamir, D., Wayne, R. C., and Cotts, R. M., *Phys. Rev. Lett.*, **12**, 512 (1964).
[15] Carr, H. Y., and Purcell, E. M., *Phys. Rev.*, **94**, 630 (1954).
[16] Meiboom, S., and Gill, D., *Rev. Sci. Instrum.*, **29**, 688 (1958).
[17] Dodge, J. T., Mitchell, C., and Hanahan, D. J., *Arch. Biochem. Biophys.*, **100**, 119 (1963).

Formation of Epithelial Basement Membrane is restricted by Scurvy *in vitro* and is stimulated by Vitamin C

ALTHOUGH scurvy was probably the first clearly defined nutritional disease and active antiscorbutic material was the first of all the vitamins to be crystallized and synthesized, the biochemical function of ascorbic acid in synthesis of collagen has only recently been identified. Some of the proline residues of the collagenous polypeptide are hydroxylated to form hydroxyproline, and ascorbic acid is a cofactor for the hydroxylating enzyme[1]. Most pathological findings in scurvy can be attributed to a restriction in ability to synthesize collagen of bone, teeth and other connective tissues. Some manifestations occasionally found in infantile scurvy are more prominent in adult scurvy and are difficult to explain by a restriction in ability to synthesize collagen of connective tissues. For example, the cutaneous haemorrhages or "scorbutic spots" appearing spontaneously about hair follicles are one of the cardinal signs of adult scurvy[2].

Epithelial and vascular basement membrane contains a protein similar or identical in composition to collagen of bone and connective tissue[3]. The purpose of this report is to present evidence that the synthesis of epithelial basement membrane is dependent on ascorbic acid and to suggest that some of the manifestations of scurvy may be attributed to a restriction in synthesis of basement membranes.

Cell cultures were initiated from explants of a transplantable parietal yolk sac carcinoma of the mouse, LS402A VI, shown to synthesize epithelial basement membrane both *in vivo* and in cell culture[4,5]. The cultures were grown at 37° C in Dulbecco and Vogt's modification of Eagle's medium supplemented with 10 per cent foetal bovine serum and equilibrated with 10 per cent CO_2 in air. In these conditions the cells do not form a confluent monolayer in the culture vessel, but grow as separated colonies on the glass surface. After the cells within a colony proliferate and become densely packed, spherical masses of cells approaching a millimetre in diameter detach and float in the nutrient medium. These cellular masses enlarge further while floating. A "stationary phase" of growth is difficult to identify.

From histological evidence, epithelial basement membrane is more abundant in the cellular masses that are detached or nearly detached than in the cells attached to the glass substrate. It was therefore decided to examine the influence of ascorbic acid on the ability of the detached cellular masses to synthesize hydroxyproline of protein.

The cell masses were removed from the nutrient medium by centrifugation, were resuspended in fresh medium and two aliquots were reincubated at 37° C in the presence of 5 μCi/ml. of ^{3}H-L-proline (304 mCi/mmole, obtained from Nuclear Chicago, Chicago, Illinois). One aliquot was incubated with 125 μM sodium ascorbate and the other aliquot without. After incubation for 3 h the cells were again separated by centrifugation, washed with 0·01 M L-proline in ethanol, and an aliquot taken for analysis of cellular protein[6]. The remainder was hydrolysed for determination of radioactivity in proline and hydroxyproline in the cells[7]. In order to estimate the amount of radioactively labelled proline and hydroxyproline secreted

Table 1. EFFECT OF ASCORBIC ACID ON APPEARANCE OF RADIOACTIVITY IN PROLINE AND HYDROXYPROLINE OF PROTEIN IN CELLS AND MEDIUM OF CULTURED PARIETAL YOLK SAC CARCINOMA

		Without ascorbate	With ascorbate
Cells	mg protein	32·1	31·5
	DPM in proline	$8{\cdot}6 \times 10^6$	$7{\cdot}9 \times 10^6$
	DPM in hydroxyproline	$1{\cdot}2 \times 10^5$	$1{\cdot}8 \times 10^5$
Medium	DPM in proline	$2{\cdot}7 \times 10^4$	$2{\cdot}8 \times 10^4$
		$3{\cdot}3 \times 10^4$	$2{\cdot}8 \times 10^4$
	DPM in hydroxyproline	$1{\cdot}5 \times 10^3$	$2{\cdot}6 \times 10^3$
		$1{\cdot}5 \times 10^3$	$3{\cdot}0 \times 10^3$

by the cells into the nutrient medium, protein was precipitated from aliquots of nutrient medium by adding 4 volumes of absolute ethanol. The precipitate was washed with 0·01 M L-proline in ethanol and was hydrolysed for determination of radioactivity in proline and hydroxyproline[7].

The results of this experiment (see Table 1) indicate that ascorbic acid stimulates the appearance of hydroxyproline in cellular protein by about 50 per cent. Hydroxyproline secreted by cells into the nutrient medium was stimulated nearly two-fold by the vitamin. Ascorbic acid did not, however, affect the amount of radioactive proline in either cells or medium. Additional experiments indicated that the effects of the vitamin were reproducible. Thus ascorbic acid enhances the synthesis of hydroxyproline by cultured parietal yolk sac carcinoma cells making basement membrane. The enhancement by ascorbic acid is quantitatively similar to that reported for cultured fibroblastlike cells during collagen production[8]. Only about 1·5 per cent of the radioactive proline incorporated into protein of parietal yolk sac carcinoma is, however, hydroxylated to form hydroxyproline, whereas a much larger fraction is reported to be hydroxylated by fibroblast-like cells.

In order to determine whether the antigenic components of basement membrane are increased by ascorbic acid, the tumour cells were grown on 22 × 40 mm coverslips in medium devoid of ascorbic acid or supplemented with 125 μM sodium ascorbate. The medium was changed on alternate days. One week later the coverslips were removed and rinsed in phosphate-buffered saline. They were then exposed for 30 min at room temperature to antibasement membrane rabbit globulin conjugated with fluorescein isothiocyanate, were again rinsed with saline and were then mounted. These conjugated antibodies were prepared to basement membrane of the same parietal yolk sac carcinoma grown in ascitic form[4]. The antibodies thus prepared react with epithelial basement membrane, vascular basement membrane and reticulin. These preparations have been previously used in the study of epithelial basement membrane[5]. The mounted coverslips in my experiments were randomized and coded, then examined independently by two observers who did not know the coding. The amount of fluorescence over cell masses producing epithelial basement membrane was scored on a scale of 0 to 4+ and the results are recorded in Table 2. A substantially greater fluorescence was recorded by both observers for the cultures grown in the presence of ascorbic acid.

Table 2. EFFECT OF ASCORBIC ACID ON FLUORESCENCE IN CULTURES OF PARIETAL YOLK SAC CARCINOMA EXPOSED TO ANTIBASEMENT MEMBRANE RABBIT GLOBULIN CONJUGATED WITH FLUORESCEIN ISOTHIOCYANATE

Without ascorbate		With ascorbate	
Observer No. 1	Observer No. 2	Observer No. 1	Observer No. 2
0	0	+ +	+ +
+	±	+	+
+ +	+	+ + +	+ +
+	±	+ + + +	+ + +
+ +	+	+ + +	+ +
±	±	+ + + +	+ + +

These observations suggest that the ability of cells to form a basement membrane is restricted when they are grown in medium devoid of vitamin C. Addition of the vitamin stimulates synthesis of hydroxyproline, a component of basement membrane protein, to an extent quantitatively similar to the stimulation of hydroxyproline synthesis by fibroblasts that form collagen. Antigenic components of epithelial basement membrane (presumably including basement membrane protein) produced in cell culture are also stimulated by the vitamin. Some of the pathological manifestations of vitamin C deficiency may well be explained by inability to synthesize epithelial and vascular basement membranes.

This study was supported by funds from the National Institutes of Health, US Public Health Service. I thank Paul Nakane and G. Barry Pierce for supplying the conjugated antibodies and for assistance with the immunofluorescence procedures.

ROBERT E. PRIEST

Department of Pathology,
University of Colorado Medical Center,
Denver,
Colorado 80220.

Received October 17, 1969.

[1] Udenfriend, S., *Science*, **152**, 1335 (1966).
[2] Follis, R. H., *Deficiency Disease* (Thomas, Springfield, Illinois, 1958).
[3] Kefalides, N. A., *Biochemistry*, **7**, 3103 (1968).
[4] Pierce, G. B., Midgley, A. R., Sri Ram, J., and Feldman, J. D., *Amer. J. Path.*, **41**, 549 (1962).
[5] Midgley, A. R., and Pierce, G. B., *Amer. J. Path.*, **43**, 929 (1963).
[6] Oyama, V. I., and Eagle, H., *Proc. Soc. Exp. Biol. and Med.*, **91**, 305 (1956).
[7] Juva, K., and Prockop, D. J., *Anal. Biochem.*, **15**, 77 (1966).
[8] Priest, R. E., and Bublitz, C., *Lab. Invest.*, **17**, 371 (1967).

Allosteric Interactions in $(Na^+ + K^+)$-ATPase

SODIUM and potassium ions are both required to activate the $(Na^+ + K^+)$-dependent–ATPase and each inhibits activation by the other[1]. The K^+ activates the turnover of enzyme hyperbolically at low Na^+ concentrations, but with increasing concentrations of Na^+ the activation curve becomes sigmoid[2]. Similarly at low concentrations of K^+, the Na^+ activation curve is hyperbolic but at high concentrations of K^+ it becomes sigmoid[1]. These data indicate the presence of at least two Na^+ and/or K^+ sites, respectively, but do not indicate the mechanism of interaction between these ions or sites[3]. Priestland and Whittam[2], and others, have suggested that direct competition between the cations for the activating sites can account for the sigmoidal activation curves in the presence of high concentrations of the other cation. Alternatively, Robinson[4] has argued that the sigmoidal activation curves, which are consistent with the heterotropic effects of an allosteric activator[5], are evidence for allosteric interactions in the $(Na^+ + K^+)$-ATPase. In support of this hypothesis he has presented evidence for a cooperativity in the K^+ activation of the *p*-nitrophenylphosphatase activity of the $(Na^+ + K^+)$-ATPase[4]. As Garrahan[6] has pointed out, however, these data are not conclusive.

In support of Robinson's interpretation we present more evidence for cooperative interactions between the Na^+ binding sites of this enzyme. In the presence of Mg^{2+} and P_i a readily reversible binding of ^3H-ouabain to this enzyme was observed[7,8]. The equilibrium level of this binding was lowered by the addition of Na^+, and zero in the presence of high Na^+. At low concentrations of ouabain ($2{\cdot}5 \times 10^{-7}$ M) the Na^+ inhibition curve was almost hyperbolic and the apparent K_m for Na^+ was 2·5 mM. A Hill plot of these data gave a slope of $n = 1{\cdot}11$. When the concentration of ouabain in the medium was increased, the apparent K_m for Na^+ increased and the inhibition curve became markedly sigmoid. At $2{\cdot}5 \times 10^{-5}$ M ouabain the apparent K_m for Na^+ was about 70 mM and n increased to 2·5. These observations suggest a cooperative interaction between the Na^+ sites and seem to represent a "standard example of a heterotropic allosteric interaction"[4,8].

This interpretation is strongly supported by the experiment in Fig. 1, which shows that the cooperativity of the Na⁺ sites is sensitive to a sulphydryl reactant. The (Na⁺+K⁺)-ATPase retains a considerable portion of its Mg^{2+} and P_i-dependent ouabain binding after treatment with ethacrynic acid[9]. After such treatment, however, the Na⁺ sites cannot cooperate as effectively as before, and this is indicated by the changed shape of the inhibition curve and the altered slope of the Hill plot in Fig. 1. Because Na⁺ accelerates inhibition of this enzyme by ethacrynic acid[10] and was used to accelerate inhibition in this particular enzyme, it is unlikely that ethacrynic acid acted directly at the Na⁺ sites. Further, the apparent affinity of the enzyme for Na⁺ was little changed. Thus it seems that ethacrynic acid, interacting with this enzyme at other than the Na⁺ sites, has reduced the ability of these sites to cooperate. The effect resembles the classical desensitization of an allosteric enzyme[11]. It shows that the interaction between the Na⁺ sites is effected by the protein (or lipoprotein) structure of the enzyme, and is therefore allosteric[12].

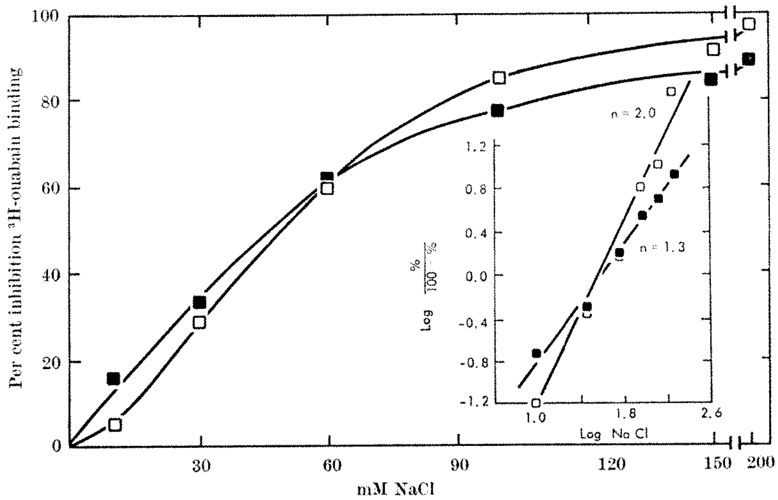


Fig. 1. (Na⁺+K⁺)-ATPase was treated with 10 mM ethacrynic acid for 40 min in 100 mM Na⁺ at 37° C, *p*H 7·6±0·1. The enzyme was then washed three times in buffer by alternate centrifugation and resuspension to remove unreacted ethacrynic acid. (Na⁺+K⁺)-ATPase activity was 79 per cent inhibited: Mg^{2+} and P_i dependent ouabain binding was 38 per cent inhibited. ³H-ouabain 2·5 × 10⁻⁶ M was allowed to bind to both untreated (□) and treated (■) enzymes in the presence of 4·0 mM Mg^{2+}, 1·0 mM H_3PO_4 and NaCl as indicated. Other conditions were as in ref. 8. Binding was plotted as a percentage of the value at 0 mM NaCl. Hill plot slopes were fitted by the method of least squares. In another experiment with a more inhibited enzyme the slope of the Hill plot shifted from 2·38 to 1·10.

This work was supported by a grant from the Medical Research Council of Canada.

T. TOBIN
S. P. BANERJEE
A. K. SEN

Department of Pharmacology,
Medical Sciences Building,
University of Toronto,
Toronto 181, Ontario.

Received September 12; revised November 3, 1969.

[1] Skou, J. C., *Biochim. Biophys. Acta*, **23**, 394 (1957).
[2] Priestland, R. M., and Whittam, R., *Biochem. J.*, **109**, 369 (1968).
[3] Sachs, J. R., and Welt, L. G., *J. Clin. Invest.*, **46**, 1433 (1967).
[4] Robinson, J. D., *Nature*, **220**, 1325 (1968).
[5] Monod, J., Wyman, J., and Changeux, J. P., *J. Mol. Biol.*, **12**, 88 (1965).
[6] Garrahan, P. J., *Nature*, **222**, 1000 (1969).
[7] Tobin, T., and Sen, A. K., *Abstr. Fourth Intern. Cong. Pharmacol.*, 199 (1969).
[8] Tobin, T., and Sen, A. K., *Biochim. Biophys. Acta* (in the press).
[9] Tobin, T., Banerjee, S. P., and Sen, A. K., *Irish J. Med. Sci.* (in the press).
[10] Banerjee, S. P., and Sen, A. K., *Fed. Proc.*, **28**, 1846 (1969).
[11] Gerhart, J. C., and Pardee, A. B., *J. Biol. Chem.*, **237**, 891 (1962).
[12] Monod, J., Changeux, J. P., and Jacob, F., *J. Mol. Biol.*, **6**, 306 (1963).

Exchange Diffusion

C. and D. van Breeman have reported[1] that radioactive calcium passes more slowly through an artificial membrane when calcium is absent from the *trans* side of the membrane than when it is present. Following a general practice, they call the phenomenon exchange diffusion. Because the membrane was a porous structure they say that exchange diffusion was demonstrated in the absence of carriers, although exchange diffusion was previously regarded as evidence for the existence of carriers. I wish to point out that the apparent contradiction arises because "exchange diffusion" is currently used to name two different phenomena.

Consider a solute passing passively through a membrane. If the individual molecules of solute move independently of one another in the membrane, the ratio of the fluxes of solute in opposite directions must be equal to the ratio of the electrochemical activities of the solute on each side. This is the Independence or Ussing relationship[2-6] and is particularly valuable in non-equilibrium conditions: if experimentally the flux ratio should differ from the electrochemical activity ratio, there must be some interaction and exchange of energy between the solute molecules during passage.

Ussing[3] originally used "exchange diffusion" to describe what happens when the flux ratio is closer to unity than the electrochemical activity ratio, as, for example, when glucose passes through the human red cell membrane[7]. It means that when a solute molecule passes through the membrane it must assist the movement of one or more molecules of the same solute in the opposite direction. The "mobile" carrier[3,8] was proposed to explain this type of interaction. Because all the suggested mechanisms for exchange diffusion seem to be variants of the "mobile" carrier hypothesis, exchange diffusion in Ussing's sense is regarded as strong evidence for the existence of carriers.

But if there are interactions between different solutes within the membrane (sodium and potassium, for example), if there is an active pumping of the solute or if the solute molecules are moved by a flow of solvent, no conclusions about interactions between like solute molecules can be drawn from the flux ratio. In such conditions a fall in the flux when the solute is removed from the *trans* side of the membrane[8] has been taken as evidence for exchange diffusion. The magnitude of the fall was considered to be equal to the exchange diffusion flux before the solute was removed from the *trans* side of the membrane. This test requires that the membrane potential should remain unchanged if the solute is electrically charged. But it also assumes[10] (1) that any exchange diffusion is caused entirely by a carrier system in which the unladen form is immobile: the carrier system can thus only mediate a one-for-one exchange; (2) that removing the solute from the *trans* side of the membrane interrupts the one-for-one exchange but has no other effects on the membrane permeability. Neither of these assumptions is universally true, and so it is theoretically possible for the flux to fall when the solute is removed even though exchange diffusion of Ussing's type is not occurring; or there might be no change, even an increase, in the flux in the presence of exchange diffusion. Unfortunately, however, the limitations of the test have been overlooked, and many now talk of exchange diffusion as if it were synonymous with a fall in the flux when the solute is removed from the *trans* side of the membrane. The term thus describes two different phenomena which are not necessarily related.

I therefore suggest that "exchange diffusion" should be reserved for the appropriate deviation, in passive conditions, of the flux ratio from the independence relationship. The term seems to be justified by the thermodynamic significance of the observations. On the other hand, the fall in the flux of a solute when the solute is removed from the *trans* side of the membrane has of itself no clear thermodynamic significance; it need neither mean that exchange diffusion is taking place nor require the existence of carriers. One should therefore say simply that the flux decreased when the solute was removed from the *trans* side of the membrane.

H. G. BRITTON

Department of Physiology,
St Mary's Hospital Medical School,
London W2.

Received October 20; revised December 5, 1969.

[1] van Breeman, C., and van Breeman, D., *Nature*, **223**, 898 (1969).
[2] Behn, U., *Ann. Phys. Chem. Neue Folge*, **62**, 54 (1897).
[3] Ussing, H. H., *Acta Physiol. Scand.*, **19**, 43 (1949).
[4] Britton, H. G., *Nature*, **198**, 190 (1963).
[5] Britton, H. G., *Nature*, **205**, 1323 (1965).
[6] Britton, H. G., *Nature*, **209**, 296 (1966).
[7] Rosenberg, T., and Wilbrandt, W. J., *J. Gen. Physiol.*, **41**, 289 (1957).
[8] Widdas, W. F., *J. Physiol.*, **118**, 23 (1952).
[9] Keynes, R. D., *J. Physiol.*, **114**, 119 (1951).
[10] Britton, H. G., *J. Theor. Biol.*, **10**, 28 (1966).

Demonstration of Sodium and Potassium Conductance Changes during a Nerve Action Potential

WE wish to report a new method with which we have been able to measure the sodium and potassium conductance changes postulated by Hodgkin and Huxley[1] in the course of a single action potential. To measure the membrane conductance at any desired time during the course of the action potential, we have designed a membrane potential control system which can be turned on and off quickly with an electronic switch.

The electronic switch consists of two transistors (PNP and NPN). The emitters of these transistors are electrically connected together to the output of the control amplifier. The collector of the PNP transistor makes electrical contact with the current supplying wire, which is inside the axon. The collector of the NPN transistor is connected through a resistance to the input of the control amplifier. Both bases are electrically connected together and to a positive constant voltage which holds the PNP transistor "closed" and the NPN transistor "open"; application of a rectangular negative voltage pulse to the bases reverses this situation and the membrane potential control is turned on during the pulse.

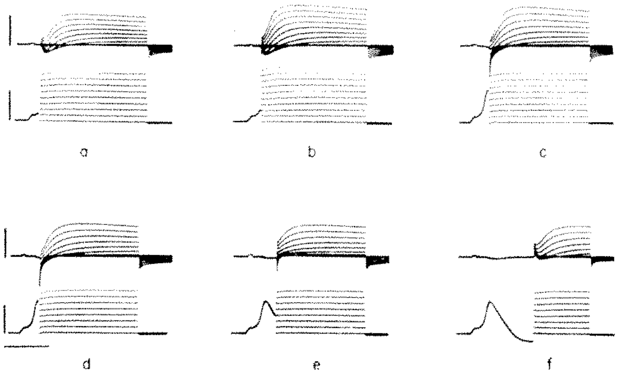


Fig. 1. Upper traces represent records of ionic currents; lower traces represent records of membrane potential before and during the period of membrane potential control. Compensated feedback was used to correct for the voltage drop through the resistance in series with the axolemma. It was set to compensate for only 4 Ω·cm². Ordinate for current traces: 2·68 mA/cm². Ordinate for membrane potential traces: 100 mV. Abscissa: 4 ms. The axon was internally perfused with 550 mM KF and externally perfused with potassium-free artificial seawater at 8° C. The resting potential was −60 mV.

Giant axon fibres from the squid *Dosidicus gigas* were used in this work (for details of the combined perfusion technique and voltage clamp procedure, see refs. 2 and 3).

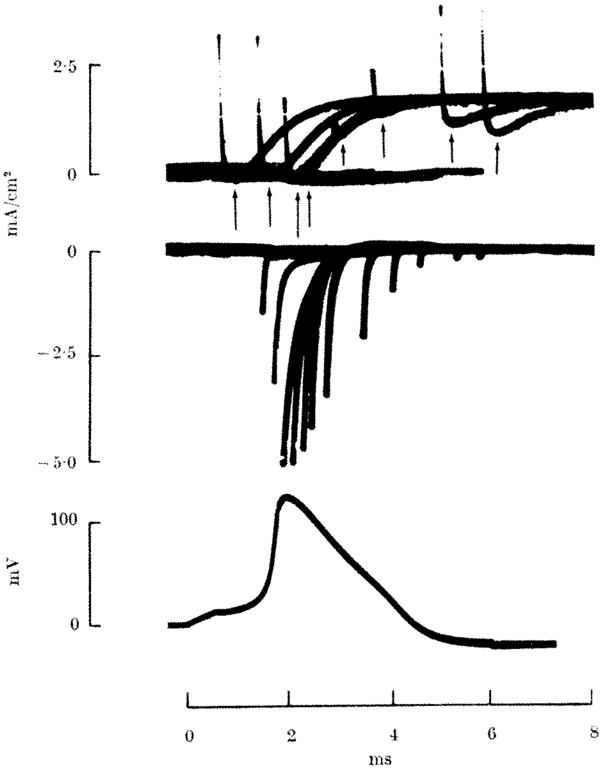


Fig. 2. Upper traces represent unretouched records of ionic currents when the membrane potential during the control period was 60 mV. Lower traces represent records of the ionic currents when the membrane potential during the control period was −80 mV. The capacitative current transients in the upper traces are apparent because a Z-input intensifier was used with the oscilloscope. No series resistance compensation was used. The resting potential was −60 mV. The temperature of the external seawater was 8° C.

Fig. 1 illustrates the results obtained with this switch. Membrane currents are shown in the upper traces; membrane potentials before and during the time in which the membrane potential was under control are shown in the lower traces. The individual traces were taken at intervals of 1 s for depolarizations from the resting potential to −40 through 80 mV in 20 mV steps. Capacitative current transients lasted for about 20 µs and are not apparent in these current records. From these records we have determined six (*a–f*) current–voltage relationships measured 50 µs after the control system was turned on. The slopes of these current–voltage curves were found to be approximately linear and represent the instantaneous conductance at various times during the action potential. The time course of the instantaneous conductance measured in this way is essentially the same as found by Cole and Curtis[4] utilizing bridge techniques and as calculated by Hodgkin and Huxley[1].

The total membrane conductance during a membrane action potential can be split into its "sodium" and "potassium" conductance by the following method. If the potential is switched to −80 mV, which is approximately equal to the potassium equilibrium potential, there is no potassium current, and the current is almost entirely in the sodium channel. Similarly by switching to 60 mV, which is approximately the equilibrium potential of the sodium channel, there is no sodium current, and the current is almost entirely in the potassium channel. Fig. 2 shows the current records obtained when the membrane potential during the control period was either the potential at the peak of the action potential (60 mV, upper traces) or the potential at the underswing of the action potential (−80 mV, lower traces). The points indicated by the arrows on the upper set of transients are proportional to the potassium conductance and show its time course. The beginning of each transient on the lower set of membrane current records is proportional to the sodium conductance and shows its time course.

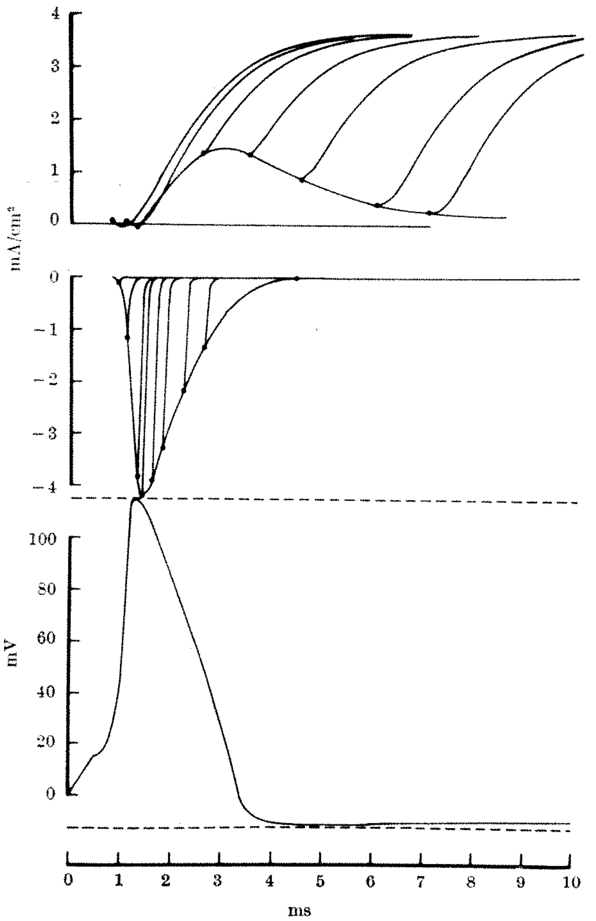


Fig. 3. Ionic currents and membrane action potential calculated utilizing the Hodgkin and Huxley equations[1]. The curve drawn through the points on the upper curves (initial potassium currents) is proportional to the potassium conductance change. The curve drawn through the points on the centre curves (initial sodium currents) is proportional to the sodium conductance change. Resting potential equal to −60 mV. The temperature was 6·3° C.

The results of a similar experiment performed on the model axon are shown in Fig. 3. Ionic currents were calculated utilizing the Hodgkin and Huxley equations[1], but some of the parameters used to compute the action potential were determined for a *Dosidicus* axon. The upper set of curves represents the total ionic currents when the potential during the control period is equal to 55 mV (peak of the action potential) and the lower set of curves represents the total ionic currents when the potential during the control period is equal to −71 mV. The calculated ionic currents are remarkably similar to those shown in Fig. 2 which were obtained on a real axon. Fig. 3 also shows the envelope curves drawn through the points of the initial potassium and sodium currents. The quantitative comparison will be published later, but it is clear that the calculated time course of the potassium or sodium conductance change agrees closely with the measured time course.

We thank Dr C. Armstrong for suggestions during the course of this work and Professor A. L. Hodgkin for comment.

E. Rojas*
F. Bezanilla

Laboratorio de Fisiología Celular,
Facultad de Ciencias,
Universidad de Chile.

R. E. Taylor

Laboratory of Biophysics,
National Institutes of Health,
Bethesda, Maryland 20014.

Received November 19, 1969.

* Present address: Churchill College, Cambridge.

[1] Hodgkin, A. L., and Huxley, A. F., *J. Physiol.*, **117**, 500 (1952).
[2] Rojas, E., and Canessa-Fischer, M., *J. Gen. Physiol.*, **52**, 240 (1968).
[3] Atwater, I., Bezanilla, F., and Rojas, E., *J. Physiol.*, **201**, 657 (1969).
[4] Cole, K. S., and Curtis, H. J., *J. Gen. Physiol.*, **22**, 649 (1939).

Histochemical Differentiation of Motor Neurones and Interneurones in the Anterior Horn of the Cat Spinal Cord

The neurones of the anterior horn are a heterogeneous population of physiologically different cells. Alpha and gamma motor neurones, Renshaw cells and interneurones are recognized components[1]. Alpha motor neurones have been divided further into F and S types according to the fast and slow contractile properties of the muscle fibres that they innervate[2]. Anatomic investigations, largely by retrograde chromatolysis, have shown that the different neurones are intermingled, and that the larger are mainly alpha motor neurones while the smaller are a heterologous group of gamma motor neurones, Renshaw cells and interneurones[3,4]. Staining techniques selective for the Golgi body differentiate motor neurones from interneurones by the direction of their axons, but standard histological methods result in uniform staining without significant morphological distinction. We have used histochemical staining reactions to attempt neuronal differentiation in the anterior horn.

The histochemical techniques for phosphorylase, diphosphopyridine and triphosphopyridine diaphorases and α-glycerophosphate menadione-mediated dehydrogenase have been described previously[5]. Those for succinate, malate, lactate and glucose-6-phosphate dehydrogenases, with minor modifications, were performed as summarized by Pearse[6]. The tissue investigated was the lumbar seven spinal cord segment of the cat, fresh frozen and serially sectioned at 20 μm.

Marked differences in the activities of phosphorylase and succinate dehydrogenase were found among the neurones in the anterior horn (Fig. 1*a* and *b*). Consistent patterns of cell distribution for each enzymatic reaction were found according to cell size and area of grey matter (Fig. 2). In the ventrolateral pool the large (more than 50 μm) and medium (30 to 50 μm) neurones were high in phosphorylase and low in succinic dehydrogenase. The small (less than 30 μm) neurones were negative for phosphorylase but exhibited high activity of succinic dehydrogenase. These results were confirmed for individual neurones when identified in serial sections, histochemical staining providing clear separation of two types of neurones otherwise lying interspersed. The staining reactions were located in the perikaryon and, with succinate dehydrogenase, also in (or on) the dendrites. Neurones of the ventromedial grey matter were uniformly high in succinic dehydrogenase and negative for phosphorylase. This was true even in medium sized neurones. Exceptional medium and even small size neurones with high activity in both enzymes were found in the ventromedial area. The axon exit zone of the anterior grey matter (Fig. 2) demarcated the different histochemical features of the ventromedial and ventrolateral areas.

According to previous anatomical knowledge mentioned, the larger neurones in the anterior horn are alpha motor neurones and the smaller are gamma motor neurones, Renshaw cells and interneurones. The neurones of type "S" (slow) motor units are considered to be smaller than those of type "F" (fast) motor units[2,7]. Characterization of anterior horn neurones in the middle of the size spectrum has not been possible with current physiological techniques.

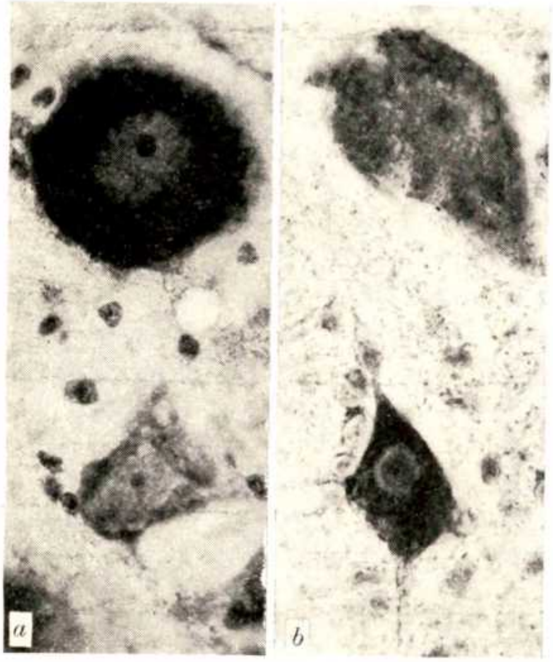


Fig. 1. Differences in the intensity of staining reactions ("darkness") for (*a*) phosphorylase and (*b*) succinate dehydrogenase in large and small neurones of the ventrolateral grey matter. Because in each reaction a counterstain of contrasting colour (gallocyanin for phosphorylase and saffranin for succinate dehydrogenase) was required to demonstrate enzymatically poorly reactive neurones, the marked contrast between strongly and weakly reacting neurones of the original slides and colour photographs could not be faithfully reproduced in black-and-white photographs. × 480.

These reactions for phosphorylase and succinate dehydrogenase separate the neurones of the ventrolateral grey matter into two groups with diameter larger and smaller than approximately 30 µm. It may be concluded that the largest type "F" alpha motor neurones belong to the group with high phosphorylase and low succinate dehydrogenase activities and the smallest gamma motor neurones, Renshaw cells and interneurones correspond with the reverse histochemical group with low or no phosphorylase and high succinate dehydrogenase activities. The type "S" alpha motor neurones and perhaps some interneurones of the ventrolateral grey matter could belong to either or both histochemical groups.

The histochemical demonstration of high level of phosphorylase in alpha motor neurones suggests that they are more dependent on glycogen metabolism than other spinal neurones.

Other histochemical reactions used in this study failed to show differences among the neurones of the L-7 cord segment. The activities of NADH and NADPH diaphorases, and of lactate and malate dehydrogenases, were equally strong in all neurones. The reactions for glucose-6-phosphate and menadione-mediated α-glycerophosphate dehydrogenases were uniformly negative.

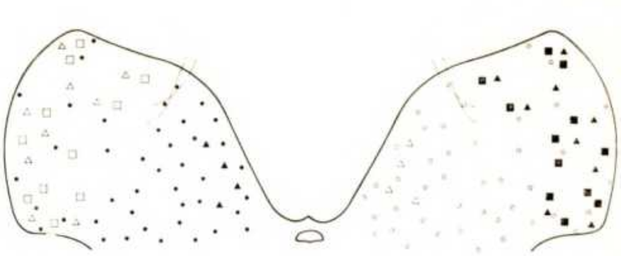


Fig. 2. Diagram of the anterior horns representing the histochemical reactivity (high = black, and low = white) for succinate dehydrogenase (left horn) and phosphorylase (right horn) in large (squares), medium (triangles) and small (circles) size neurones. The axon exit zone is represented by crossed lines.

This work was supported by a special fellowship award from the US National Institutes of Health.

J. FERNANDEZ-CAMPA
W. KING ENGEL

Medical Neurology Branch,
National Institute of Neurological Diseases and Stroke,
National Institutes of Health,
Bethesda, Maryland 20014.

Received August 1, 1969.

[1] Eccles, J. C., *Anesthesiology*, **28**, 31 (1967).
[2] Burke, R. E., *J. Physiol.*, **193**, 141 (1967).
[3] Romanes, G. J., *Prog. Brain Res.*, **11**, 93 (1964).
[4] Nyberg-Hansen, R., *Exp. Neurol.*, **13**, 71 (1965).
[5] Engel, W. K., and Brooke, M. H., *Neurological Diagnostic Techniques* (edit. by Fields, W. S.), 90 (Thomas, Springfield, 1966).
[6] Pearse, A. G. E., *Histochemistry*, 910 (Little Brown, Boston, 1960).
[7] Henneman, E., Sourjen, G. G., and Carpenter, D. O., *J. Neurophysiol.*, **28**, 599 (1965).

Effects of Lithium on Amino-acids in Mouse Brain *in vivo*

LITHIUM exerts marked effects on the function of brain and peripheral nerve *in vivo* and *in vitro* and has been used successfully in the treatment of manic-depressive psychoses[1,2]. Although the effects of lithium treatments on monoamine metabolism have been studied[3,4], the relationships which may exist between lithium injections and the content of free amino-acids of brain remain unexplored. Recently, it was shown that injection of lithium directly into the amygdala of monkeys which were awake exerts very potent and reproducible effects on the EEG. After these effects had subsided they could be re-induced by direct injections of L-glutamate into the same sites[5]. It was therefore postulated that glutamate exerts its pharmacological action in animals which were awake by increasing the permeability of CNS neurones to sodium or to lithium (the latter not normally being present)[5]. Our experiments were conducted to show whether lithium injections affect the content of glutamate in the brain.

Young, male, Swiss albino mice (ICR strain), weighing about 20 g, were decapitated at various times after short-term intraperitoneal injections of LiCl (1·0 M) in doses of 0·1–2·0 ml. per mouse. Their brains were excised, homogenized in nine volumes of ice-cold ethanol (v/v) and centrifuged for 1 h at 20,000g (refs. 6 and 7). Aliquots of the clear supernatant fluid representing 10 mg brain tissue were subjected to an accelerated system of automated amino-acid analysis[8-10]. For studies on the effects of lithium on the synthesis of amino-acids in brain, 1·0 µCi/g

Table 1. EFFECTS OF SHORT-TERM LITHIUM INJECTIONS ON THE CONTENT OF CERTAIN AMINO-ACIDS OF MOUSE BRAIN

Amount LiCl injected in (ml./mouse*)	Time of decapitation†	Amino-acids of brain (μmole/g fresh wt.)					
		Aspartate	Glutamine, serine and asparagine	Glutamate	Glycine	α-Alanine	GABA
None	—	3·7 ± 0·14 (6)	5·3 ± 0·37 (6)	14·6 ± 0·16 (6)	0·98, 1·05	0·69, 0·69	2·9 ± 0·04 (6)
0·1	1 h	3·6 ± 0·08 (4)	5·6 ± 0·22 (4)	13·8 ± 0·40 (4)	1·01 ± 0·02 (4)	0·69 ± 0·03 (4)	2·9 ± 0·06 (4)
0·1	2 h	3·5, 3·8	4·8, 6·3	13·7, 14·8	1·12, 1·22	0·67, 0·83	3·0, 3·1
0·25	1 h	3·4 ± 0·14 (4)	5·7 ± 0·27 (4)	12·6 ± 0·06 (4) ‡	1·00 ± 0·02 (4)	0·66 ± 0·06 (4)	2·9 ± 0·04 (4)
0·5	20 min	3·8 ± 0·05 (4)	5·8 ± 0·42 (4)	12·8 ± 0·23 (4) ‡	1·00 ± 0·06 (4)	0·72 ± 0·02 (4)	3·1 ± 0·14 (4)
0·5	1 h	3·5, 3·8	5·5, 5·6	13·0, 13·1	1·03, 1·10	0·68, 0·72	2·8, 3·0
1·0–2·0	3 min after death	3·4 ± 0·17 (6)	5·4 ± 0·26 (6)	13·6 ± 0·41 (6)	1·04 ± 0·02 (6)	1·09 ± 0·03 (5) ‡	4·1 ± 0·08 (6) ‡

* Intraperitoneal injections of 1·0 M LiCl.

† Brains were removed within 1 min after mice were killed except when 1·0 and 2·0 ml./mouse of LiCl were injected; in the latter cases all mice died within 5–10 min after injection.

‡ $P < 0{\cdot}001$, by t test.

Results are means ± $S.E.$; numbers of mice are in parentheses.

(of mouse) of $^{14}C_6$-D-glucose (110 mCi/mmole) was injected intraperitoneally 30 min before mice were killed. Amino-acid analyses were carried out as described and in some cases the effluent from the analyser was coupled to a fraction collector so that radioactivity in the amino-acids could be monitored[9,10]. Radioactivity was measured with a Packard scintillation counter and corrections for quenching were made by a method of external standardization.

It is clear that cerebral glutamate is decreased with injections of 0·25 and 0·5 ml./mouse of LiCl and that this change occurs rapidly (see Table 1). When mice were given very large doses of LiCl (1·0 and 2·0 ml./mouse) and allowed to die from the toxic effects before their brains were excised, glutamate levels were not changed though the well known post-mortem increases in GABA[6] and α-alanine (for example, ref. 11) occurred. All mice became sedated within a few minutes after injection of 0·25–2·0 ml. of LiCl, but a detailed study of this behavioural change was not made. The total amount of radioactivity of $^{14}C_6$-D-glucose which was found in the brain was not significantly decreased by LiCl injections (0·25 or 0·5 ml./mouse) given either 10 min after or 30 min before glucose (four mice in each case and four control mice), but the radioactivity in all amino-acids was decreased by about 10–20 per cent. There was no selective action of LiCl on the synthesis of glutamate.

The significant decrease in cerebral glutamate caused by short-term LiCl treatments may be related to the mechanism responsible for the sedative action of lithium in animals and to its prophylactic effect on manic-depressive psychotics, but further and more detailed studies involving chronic treatments are required. The fact that glutamate was the only amino-acid affected by lithium treatment could lend support to its suggested role as an excitatory transmitter[12], although the possibility exists that the change is a result of, rather than the cause of, sedation.

This study was supported by a grant from the US Public Health Service.

F. V. DEFEUDIS
J. M. R. DELGADO

Department of Psychiatry,
Yale University,
New Haven, Connecticut.

Received July 16; revised December 17, 1969.

[1] Schou, M., *Pharmacol. Rev.*, **9**, 17 (1957).
[2] Schou, M., *J. Psychiat. Res.*, **6**, 67 (1968).
[3] Schanberg, S. M., Schildkraut, J. J., and Kopin, I. J., *Biochem. Pharmacol.*, **16**, 393 (1967).
[4] Schildkraut, J. J., Schanberg, S. M., and Kopin, I. J., *Life Sci.*, **5**, 1479 (1966).
[5] Delgado, J. M. R., and DeFeudis, F. V., *Exp. Neurol.*, **25**, 255 (1969).
[6] Lovell, R. A., and Elliott, K. A. C., *J. Neurochem.*, **10**, 479 (1963).
[7] DeFeudis, F. V., thesis, McGill Univ. (1968).
[8] Spackman, D. H., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1190 (1958).
[9] DeFeudis, F. V., Delgado, J. M. R., and Roth, R. H., *Nature*, **223**, 74 (1969).
[10] DeFeudis, F. V., Delgado, J. M. R., and Roth, R. H., *Brain Res.* (in the press).
[11] DeFeudis, F. V., and Elliott, K. A. C., *Canad. J. Physiol. Pharmacol.*, **46**, 803 (1968).
[12] Krnjević, K., and Phillis, J. W., *J. Physiol.*, **165**, 274 (1963).

Two Innate Releasing Mechanisms subserving the Same Motor Pattern System

ANIMALS respond to an external stimulus situation with the biologically appropriate motor pattern. The response is triggered by a filter system which is called here an innate releasing mechanism (IRM)[1,2] if it functions independently of any experience with the appropriate stimulus situation. The mobbing response of breeding pied flycatchers (*Ficedula h. hypoleuca*) to owls and shrikes is highly stimulus specific; moreover, it is independent of individual experience with these predators (ref. 3, and our unpublished results). The question, then, is whether these two predators are recognized by means of two different IRMs (first hypothesis), or whether a more general IRM is used (second hypothesis).

To decide between the two models, a number of special experiments were designed. In the course of 11 years, seventy different enemy dummies were shown to over 2,200 breeding pied flycatchers at a standard distance from their nest box (study areas in Berlin, around Frankfurt/Main, and near Brunswick). The mobbing response was measured by counting the calls during a standard period. Each bird was tested once only, to circumvent biasing after-effects. The results obtained will be stated here only in qualitative terms; the quantitative details will be published elsewhere.

The first method was to present dummies combining owl and shrike characters with releasing value. For the sake of comparison we selected enemies of equal size, the pigmy owl (*Glaucidium passerinum*) and the red-backed shrike (*Lanius collurio*). The total colour pattern of the male shrike or its most effective part was transferred to a wooden painted model of the owl, and vice versa. Experiments allowed for the fact that such transference of "donor" sign stimuli took place, in some cases, at the expense of sign stimuli of the "host" dummy. The behaviour capacity of the birds would have allowed an increase in response strength[4]. The visual change interferes, however, with the original appearance of the "host" object and thereby decreases its releasing value. At the very least it leaves it unchanged. According to the "law of heterogenous summation", the sign stimuli entering one and the same IRM should summate[5], though not necessarily in an algebraic manner[4]. The result therefore favours the first hypothesis by exclusion of the second.

The second method was to compare dummies of both predator species altered identically. The rationale behind this method is to modify both the shrike and the owl with respect to some character shared by both. If the amount and/or the direction of the resulting change in the strength of the response is different in each case, the first hypothesis would be supported. This follows because the two presumed IRMs could then be distinguished, at least, on the basis of their differential evaluation of that changed common character, depending on the accompanying stimuli[6]. For the comparison to be conclusive, it was essential that the scaling of mobbing motivation should be known, as reflected in the measured response[4]. The only character thus tested yielded a result that clearly favoured the first model.

The third method used stimulus specific habituation to either of the two avian predators. The response to both the owl and the shrike can be habituated by ineffective models of either type, thereby avoiding any possible change of the mobbing motor system as a consequence of the stimulation[7]. By this "reaction-free" habituation, the response to the shrike could be entirely eliminated without interference with the response to the owl, which maintained the full releasing value. This experiment reveals more directly than the two first methods the high degree of functional independence of the two channels under consideration, thus supporting the first hypothesis too.

The model of at least two IRMs converging before or on the mobbing motor system is in harmony with two other findings. First, there is interaction between the sign stimuli within both the owl and the shrike, the respective releasing values not being added algebraically (unpublished). Second, the recognition of both predators in two subspecies of the pied flycatcher matches closely the differential distribution of shrikes in the two areas concerned[3].

The organization of the IRM coping with the resting avian predator into operationally defined functional subunits outlined here shows that "the IRM" is not as unitary either as critics of the concept have propounded[8,9] or as many investigators using it believe it is.

The work was supported by grants from the Deutsche Forschungsgemeinschaft to E. C.

E. Curio
R. Blaich

Arbeitsgruppe für Verhaltensforschung,
Abteilung für Biologie,
Ruhr-Universität Bochum,
463 Bochum.

N. Rieder

Zoologisches Institut der Universität,
75 Karlsruhe.

Received August 11; revised December 4, 1969.

[1] Tinbergen, N., *The Study of Instinct* (Oxford, 1951).
[2] Marler, P. R., in *Current Problems in Animal Behaviour* (edit. by Thorpe, W. H., and Zangwill, O.), 150 (Cambridge, 1960).
[3] Curio, E., *Proc. Thirteenth Intern. Orn. Cong.*, 206 (1963); *Experientia*, **17**, 188 (1961).
[4] Curio, E., Blaich, R., and Rieder, N., *Z. Vergl. Physiol.*, **62**, 301 (1969).
[5] Seitz, A., *Z. Tierpsychol.*, **4**, 40 (1940).
[6] Curio, E., *Experientia*, **17**, 516 (1961).
[7] Curio, E., *Verh. Deutsch. Zool. Ges.*, **13**, 153 (1967).
[8] Quine, D. A., and Cullen, J. M., *Ibis*, **106**, 145 (1964).
[9] Hinde, R. A., *Animal Behaviour* (McGraw-Hill, 1966).

Learning of Leg Position in Chronic Spinal Rats

Is the brain necessary for learning? Pavlov[1] concluded that the cortex is essential for conditioning; yet Culler and Mettler[2] reported conditioning a decorticate dog, while Shurrager *et al.*[3–5] report having conditioned dogs and cats caudal to spinal cord transections. Franzisket[6,7] believes that it is possible to condition the withdrawal reflexes of spinal frogs.

Such evidence has not been widely accepted. Other similar experiments have failed[8,9], and alternative interpretations can be made[10]. One difficulty has been the absence of appropriate control animals[10].

Chamberlain *et al.*[11] have studied a phenomenon that resembles learning in spinal rats; and the conditioning of foot withdrawal has been reported for chronic spinal kittens[12] and acute spinal cats[13]. A. A. B. and Dawson[14] discussed an effect akin to conditioning in chronic spinal kittens. Horridge[15,16] devised an ingenious paradigm for studying how insects learn leg position when their heads are removed or part of their nervous system is isolated. His method is particularly useful because it includes a yoked control, and we have used it in a modified form to study chronic spinal rats.

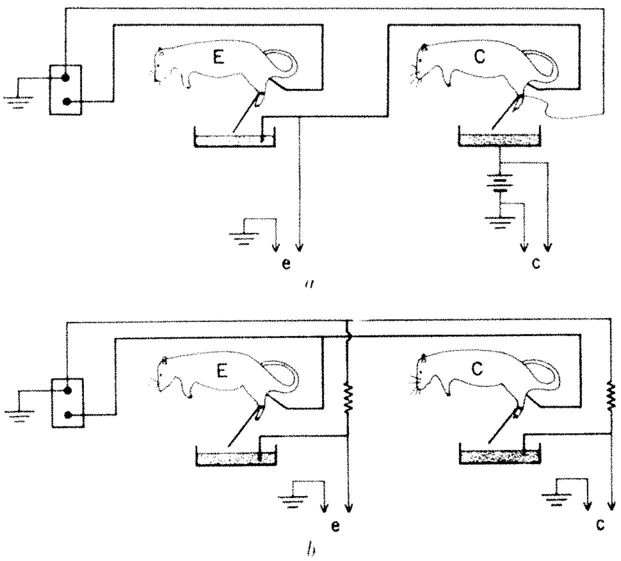


Fig. 1. *a*, The experimental situation; *b*, the testing situation.

In the first step (Fig. 1*a*) the experimental animal serves as an electrical switch for the control animal. The position of the experimental animal's leg determines whether it is shocked; there is no relation between leg position and shock for the control.

In the second step (Fig. 1*b*), the aim is to verify learning by testing for improved performance (savings) resulting from previous experience. Each animal is electrically independent of the other and the leg position of each animal determines whether it is shocked. If learning occurs, two effects would be expected. (1) In the training situation, the experimental animal should hold its leg so as to avoid shock, whereas the control animal should not change its normal leg position. (2) In the testing situation, the experimental animal should avoid shock before the control animal does. Horridge found these results with headless cockroaches and locusts; we have tried to obtain them for chronic spinal mammals.

The spinal cords of mature female rats were transected between L_1 and L_2 under barbiturate anaesthesia supplemented with atropine sulphate. The dorsal and ventral roots exposed by the transection were cut. The small amount of bleeding that occurred was controlled with saline-soaked cotton, thrombin solution or gelatine sponge. When the transection was complete, the bottom and sides of the vertebral canal were seen to be free of neural tissue at the site of the transection. This procedure was the first check that the cord was cut completely; the fact was verified at autopsy. Several days after surgery, two animals were fastened snugly in wire mesh tubes. Holes had been cut in the tubes so that their hind legs could hang freely. The same hind leg on each animal was extended dorsally and taped out of the way. On the contralateral feet, electrodes of fine silver wire were inserted under the skin over the dorsal surface of the metatarsal bones. They were then connected in the training situation as shown in Fig. 1*a*. All connexions except the foot electrodes were of insulated copper wire. The containers of electrolyte solution were filled with 10 per cent acetic acid. The shocks were then delivered by a commercially available stimulator. Sometimes the natural tonus of the leg muscles was so great that the legs were weighted slightly; if so, identical weights were always applied to both animals. A multichannel recording device of high input impedance was connected to the apparatus at points *e* and *c* for separate recordings of the withdrawals of each animal. Because the input impedance of the device was high, it did not influence the responses of either animal.

Pilot experiments convinced us that the phenomena we were looking for could not be obtained easily if the animals could remove their electrodes from the electrolyte by extension of the leg. Typically, the animals would extend the legs backwards about once a second for 10 to 20 min until habituation or fatigue occurred. We tried several methods designed to make the animals remove their electrodes by flexion. The simplest effective way we found was to tie a ligature firmly around the foot at the level of the tarsal bones and then to fasten it at a point in front of the animal such that the leg hung naturally but could not move caudally. All our animals' legs were tied in this way and levels of the electrolyte containers were carefully adjusted so that both animals' electrodes were inserted the same distance into the fluid. The exact distance varied between 1 and 3 mm.

Both experimental and control animals received the same pattern and intensity of current in the training situation. Any subsequent differences between animals therefore occurred because experimental animals always received shocks when their legs were in a particular position, whereas control animals received shocks unrelated to leg position. Rather, the control animals received shocks at times determined by the position of the experimental animal's leg. For these reasons, the exact stimulus parameters are not important because they could not have produced subsequent differences in behaviour. The stimulator was turned on and the stimulus parameters set so that both animals began flicking their legs out of the water. If the experimental animal stopped flexing its leg, the stimulus parameters were increased. After a training period of 10–30 min, the two animals were reconnected in the testing situation (Fig. 1*b*): each was shocked independently whenever its own foot was lowered far enough for the attached electrode to enter the conducting solution. The levels of the containers were again adjusted with each foot electrode an equal distance into the solution, and the stimulator was set to make both animals flick their electrodes out of the solution.

We had hoped to put each pair of animals through the procedure twice, using different pairs of legs, with a few days betweeen tests. We also planned to exchange the experimental and control animals the second time around. Chronic spinal mammals are, however, prone to sickness and we refused to start with a pair in which either animal showed any signs of disease or in which hind leg withdrawal reflexes did not appear normal. Only two pairs of animals stayed in good condition long enough to be used twice. They showed the desired results on both sets of rear legs: after exposure to the experimental situation using the right legs, the experimental animals held their electrodes out of the solution, whereas the control animals did not. During the testing situation, the experimental animals showed savings, that is, they held their electrodes out of the solution sooner and for a longer time than the controls. Finally, two days later, both pairs were tested with the left legs, using the former experimental animals as controls and vice versa. The results were the same as before. Five pairs of rats were put through the procedure once only, and they all gave similar results.

Our results are significant at the 0·01 level, according to the Fisher exact probability test[17]. They demonstrate that repetitive application of shocks when the leg is in a given position produces changes absent when the same shocks are applied without relation to leg position. It seems fair to conclude that the isolated spinal cord is capable of associating leg position with shock. In psychological terms, the phenomenon seems to be a form of escape and avoidance learning by an isolated and rather simple part of the nervous system. A physiological, anatomical or biochemical analysis of this phenomenon will probably be quite complex, in spite of the relatively small number of neurones involved.

Our research was partially supported by a fellowship from the US National Institute of Mental Health (A. A. B.), a traineeship grant from the US National Science Foundation to Harvard University, and by grants to C. G. Gross from the US National Institute of Mental Health and the US National Science Foundation. We thank the faculty and staff of the Department of Psychology at Harvard University for help and advice, especially Dr C. G. Gross for his discussion and support, and D. Bender and P. Rogers for technical assistance.

A. A. BUERGER
A. FENNESSY

Department of Psychology,
Harvard University,
Cambridge, Massachusetts 02138.

Received August 27, 1969.

[1] Pavlov, I. P., *Conditioned Reflexes*, 228 (Oxford University Press, London, 1927).
[2] Culler, E., and Mettler, F. A., *J. Comp. Psychol.*, **18**, 291 (1934).
[3] Shurrager, P. S., and Culler, E. A., *Amer. J. Physiol.*, **123**, 186 (1938).
[4] Shurrager, P. S., and Shurrager, H. C., *J. Exp. Psychol.*, **36**, 347 (1946).
[5] Dykman, R. A., and Shurrager, P. S., *J. Comp. Physiol. Psychol.*, **49**, 26 (1956).
[6] Franzisket, L., *Naturwissenschaften*, **37**, 90 (1950).
[7] Franzisket, L., *Anim. Behav.*, **11**, 318 (1963).
[8] Forbes, A., and Mahan, C., *J. Comp. Physiol. Psychol.*, **56**, 36 (1963).
[9] Kellogg, W. N., Pronko, N. H., and Deese, J., *Science*, **103**, 49 (1946).
[10] Kellogg, W. N., Deese, J., Pronko, N. H., and Feinberg, M., *J. Exp. Psychol.*, **37**, 99 (1947).
[11] Chamberlain, T. J., Rothschild, G. H., and Gerard, R. W., *Proc. US Nat. Acad. Sci.*, **49**, 918 (1963).
[12] Buchwald, J. S., and Schramm, S., *Physiologist*, **8**, 115 (1965).
[13] Fitzgerald, F. A., and Thompson, R. F., *Psychon. Sci.*, **8**, 213 (1967).
[14] Buerger, A. A., and Dawson, A. M., *Exp. Neurol.*, **23**, 457 (1969).
[15] Horridge, G. A., *Nature*, **193**, 697 (1962).
[16] Horridge, G. A., *Proc. Roy. Soc.*, B, **157**, 33 (1962).
[17] Siegel, S., *Nonparametric Statistics*, 96 (McGraw-Hill, New York, 1956).

Movement Control in a Repetitive Motor Task

IN experiments that used a pencil and graph paper targets, we have been able to demonstrate what we believe to be the intermittency of visual feedback control in a simple motor task. The intermittency hypothesis was first advanced by Craik[1]. It was developed by E. R. F. W. Crossman and P. J. Goodeve in their theoretical analysis of movement control (unpublished), showing that an intermittent servo mechanism could produce results obtained by Fitts and others that related the speed of movement to the terminal accuracy.

We have attempted to show the intermittent nature of visual feedback in a simple speed and accuracy experiment by interrupting the visual feedback loop. In our experiment the subject aims with a pencil at a vertical line target about two feet in front of his face, from a home position near his shoulder. The accuracy of movement is registered by the root mean square of the distribution of pencil marks about the target. Various speeds are achieved by asking the subject to make repeated movements in time with a metronome.

The pencil has a "wing" of cardboard taped to it, near the point, and when the wing breaks an infrared beam the illumination of the target and the room is extinguished. It returns as the subject leaves the target. The infrared beam can be moved to various distances from the target.

We assumed that if error feedback were sampled, then a delay would occur while processing went on until a motor correction occurred. In this case the accuracy of hitting the target should depend on whether the light was turned off nearer or farther from the target than a distance corresponding to the corrective reaction time

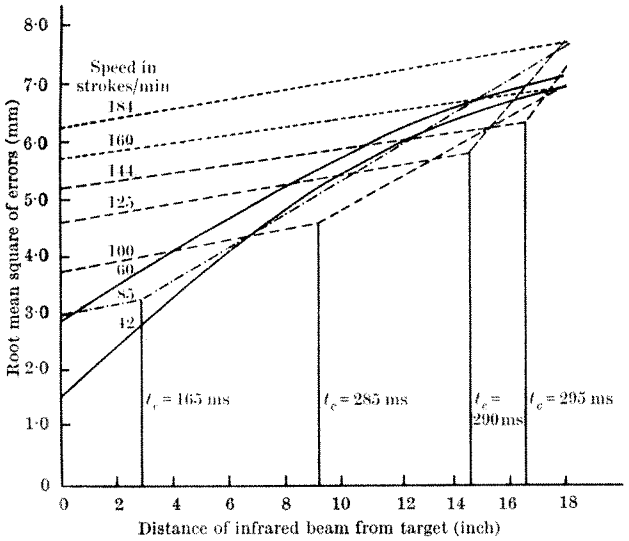


Fig. 1. Effect of extinguishing the light at different distances from the target at different speeds.

t_c. The effect on the accuracy should be greater when t_c is exceeded.

Fig. 1 shows the relationship we found between the root mean square of the errors on target at different speeds, and the distance of the infrared beam from the target. For the faster movements, 700 ms and less, the expected result is obtained. For the slowest speeds, with movement times of 1 s and more, the lines show no discontinuity and curve in the opposite direction. We believe the explanation is that the subject, in the very unnatural condition of aiming slowly in the dark, adopts a different strategy from the strategy he uses when working more quickly.

We have recorded the time from breaking the beam to hitting the target in all our experiments. These data yield a set of approach curves, giving the time to impact at any distance and speed. We can simply read off from them the time to impact corresponding to each break in the curves in Fig. 1.

Our estimates of t_c appear in Fig. 1. There seems to be an anomalous result at a speed of 700 ms movement time, but a mean corrective reaction time of 290 ms is found at the higher speeds. This value is of the order of that found by Keele and Posner[2] and Vince[3]. It thus seems reasonable to identify the discontinuities in three of our curves as evidence of delayed visual feedback processing.

The equal slopes in the non-visual parts of these curves look interesting, but we cannot yet explain them.

Our research is supported by a grant from the Medical Research Council.

W. D. A. Beggs
C. I. Howarth

Department of Psychology,
University of Nottingham.

Received September 10; revised December 8, 1969.

[1] Craik, K. J. W., *Brit. J. Psychol.*, **38**, 56 (1947).
[2] Keele, S. W., and Posner, M. I., *J. Exp. Psychol.*, **77** (1), 155 (1968).
[3] Vince, M. A., *Quart. J. Exp. Psychol.*, **1**, 85 (1948).

Water Uptake from Subsaturated Atmospheres: its Site in Insects

Although the uptake of water from subsaturated atmospheres by some arthropods is well established, neither its mechanism nor its site has been conclusively established. Uptake may occur over the general body surface and the mechanism involve both cuticle and epidermis: much of the evidence is summarized by Beament[1-3]. Conclusive location of the site of uptake would be an important step in the elucidation of the mechanism. I deal here with a test of the assumption that uptake takes place over the general body surface, attempting to locate the site of uptake more precisely.

The firebrat *Thermobia domestica* (Packard) is capable of absorbing water from atmospheres down to 50 per cent relative humidity (RH)[4,5]. In my experiments, 2 days of desiccation in dry air at 37° C depleted the water content of the insects, and the mean weight loss of twenty-six insects was 17·1 ± 1·5 per cent (*S.D.*) of their initial weight. The experimental treatments were then carried out under carbon dioxide anaesthesia. After recovery from anaesthesia, subsequent gains were measured after 1 day of exposure to 83 per cent RH at 37° C.

Control insects were handled under anaesthesia as though being operated on, but given no further treatment. Gains were 17·1 ± 6·2 per cent of their initial weight, returning them almost exactly to their initial weight.

In a series of sham operations, paraffin wax, heated so that it just melted, was applied to different regions of the body. Subsequent gains were 18·2 ± 1·2 per cent after treatment of the mid-dorsal surface of the abdomen, 13·5 ± 3·2 per cent for insects with their mouths blocked, and 11·0 ± 4·7 per cent after treatment of the posterior ventral surface of the abdomen. These gains differ significantly neither from those of the control insects nor from one another (t tests, $P > 0·05$ in all cases).

The main operation was to block the anus with molten wax, with care to avoid damage to surrounding tissues as far as possible. No treated insect subsequently showed any gain. Losses amounted to a further 3·4 ± 1·5 per cent of the initial weight after 1 day and continued subsequently. Occlusion of the anus clearly prevents uptake of water from the atmosphere.

This was further confirmed by making continuous weight recordings at 20·5° C of individual insects given successive treatments. Gains in 84 per cent RH were recorded before and after treatment of the dorsal abdomen; but only losses were recorded when the anus was blocked.

These experiments point conclusively to the anal area as a localized site for the uptake of water from the atmosphere. The failure of oral blocking to prevent uptake precludes the reingestion of hygroscopic faeces as a mechanism of water uptake and confirms previous work[2,3]. Blocking of the anus does, however, cut off the rectum from the exterior, and it is probable that this explains the effect of this treatment. The rectum in *Tenebrio* equilibrates the air spaces in its lumen to 88 per cent RH[6], which is also the lowest humidity from which this insect can absorb water. Preliminary experiments with *Tenebrio* indicate that uptake is prevented when the anus is blocked, and recommences when the block is removed. Taken together with the results in *Thermobia* it would seem that the rectum is the site of uptake, being connected to the atmosphere through the valve-like anus. The rectum is known to be involved in the reabsorption of water from the faeces and is under physiological control in the insect[7-14]. Preliminary observations on *Thermobia* show that it has the complex fine structure associated with water-absorbing tissues. The involvement of the rectum in the uptake of water from the atmosphere could prove on further investigation to be of more general distribution.

One implication of these findings is that the general body surface does not take part in uptake, the cuticle of these areas being involved only in the restriction of transpiration. Lowered water potentials in the cuticle of living insects[15,16] may therefore not be of significance in the uptake mechanism, but may only reflect an added device for restricting transpiration. A further implication is that the site of uptake is separate from the site of restriction of transpiration, as foreshadowed in earlier work on *Thermobia*[5].

I thank the Agricultural Research Council for a grant in support of this investigation.

J. NOBLE-NESBITT

School of Biological Sciences,
University of East Anglia, Norwich.

Received August 25; revised October 27, 1969.

[1] Beament, J. W. L., *Biol. Rev.*, **36**, 281 (1961).
[2] Beament, J. W. L., *Adv. Insect Physiol.*, **2**, 67 (1964).
[3] Beament, J. W. L., *Symp. Soc. Exp. Biol.*, **19**, 273 (1965).
[4] Beament, J. W. L., Noble-Nesbitt, J., and Watson, J. A. L., *J. Exp. Biol.*, **41**, 323 (1964).
[5] Noble-Nesbitt, J., *J. Exp. Biol.*, **50**, 745 (1969).
Grimstone, A. V., Mullinger, A. M., and Ramsay, J. A., *Phil. Trans. Roy. Soc.*, B, **253**, 343 (1968).
[7] Wigglesworth, V. B., *Quart. J. Micros. Sci.*, **75**, 131 (1932).
[8] Maddrell, S. H. P., *J. Exp. Biol.*, **40**, 247 (1963).
[9] Phillips, J. E., *J. Exp. Biol.*, **41**, 15 (1964).
[10] Berridge, M. J., and Gupta, B. L., *J. Cell Sci.*, **2**, 89 (1967).
[11] Mills, R. R., *J. Exp. Biol.*, **46**, 35 (1967).
[12] Wall, B. J., *J. Insect Physiol.*, **13**, 565 (1967).
[13] Cazal, M., and Girardie, A., *J. Insect Physiol.*, **14**, 655 (1968).
[14] Mordue, W., *J. Insect Physiol.*, **15**, 273 (1969).
[15] Winston, P. W., *Nature*, **214**, 383 (1967).
[16] Winston, P. W., and Beament, J. W. L., *J. Exp. Biol.*, **50**, 541 (1969).

Alarm Pheromone and Avoidance Conditioning in Goldfish, *Carassius auratus*

WHILE the alarm reaction in fish is believed to be produced by a chemical substance (pheromone) secreted by specialized cells in the epidermis and released by epidermal damage, relatively little systematic research has been devoted to the extent, nature or essence of the reaction and its relationship to the relevant chemical substance[1]. Our investigation represents an exploration of the effect of alarm pheromone on avoidance conditioning. Fifteen 2 inch goldfish, *Carassius auratus*, were kept in a thirty gallon aquarium divided into thirds by wire barriers. In accordance with other reports, the fish were deliberately well fed[2]. Massed avoidance conditioning (all trials on one day) was studied in a Lafayette Instrument aquatic conditioning unit (volume of approximately one gallon; inside dimensions, 3·5 × 6 × 9 inches). Lights at each end of the conditioning tank served as the conditional stimuli (CS) and electric shock as the unconditional stimulus (US). Shock was adjusted for each fish and varied from 5 to 7 a.c.

A successful avoidance response involved swimming out of the lighted side of the tank into the dark side within the CS–US interval of 7 s. Following the 7 s CS, the CS remained on and shock was delivered through electrodes at the top and bottom of the tank on the lighted side only for 23 s. The next trial started 30 s after shock offset. Thus the intertrial interval was a constant 1 min. The experimenter manually switched the apparatus so that, regardless of the fish's position (if, for example, it swam through the divider during the intertrial interval), the CS (light) would be presented on the side of the tank that the fish was occupying at the start of each trial. In other words, this is a two-way shuttle avoidance paradigm.

Thus a typical avoidance paradigm was followed (delay procedure, CS–US interval of 7 s, 1 min intertrial interval) with trials continuing until a criterion of twelve successful avoidances out of fifteen consecutive trials was attained. Six of the fish received training trials in aquarium water, four were conditioned in aquarium water into which 50 ml. of pheromone solution had been thoroughly mixed, and five were left undisturbed in one end section of the aquarium. The alarm pheromone was prepared by scaling a freshly decapitated goldfish into 50 ml. of aquarium water.

Fish conditioned in plain water reached criterion in an average of 65·5 trials (range 49–84). Fish conditioned in water containing pheromone reached criterion in an average of 45·5 trials (range 12–94). Using the Wald–Wolfowitz Runs test, this was found to be a significant difference ($P < 0·05$). It should be pointed out that the extreme case in the pheromone group (ninety-four trials) was operating "near" criterion throughout, and all the other pheromone fish reached criterion in thirty-eight trials or less. To assess the effects of pheromone on activity and to observe the "fright" or alarm reaction more carefully (with video tape), 100 ml. of pheromone solution was prepared by scaling two freshly killed goldfish into 100 ml. of aquarium water. The solution was dumped into one corner of the section of the aquarium containing the five previously undisturbed fish. As soon as several of the fish happened to swim into the area in which the solution had been dumped, they displayed agitated, non-directional, jerky movements with fins fully erected. Soon afterwards, all the fish began doing this and within seconds all sank to the bottom of the tank in the corner farthest removed from the one into which the pheromone solution had been dumped. The form of the reaction agrees with other reports[2]. The fish remained clumped together in this fashion and did not resume their normal free-swimming patterns for several hours.

The pheromone's effect on avoidance conditioning cannot be explained from this brief experiment. It is probably not the result of an increase in general activity or reactiveness to stimuli in general as shown by the large tank experiment. Observation did not reveal any differential sensitivity to electric shock in the two groups. As shown by the large tank experiment, alarm pheromone seems to produce not only "fright" and "alarm" behaviour but also flight or withdrawal. In our apparatus, avoidance involved swimming from a lighted chamber on presentation of the CS into a dark one. Even though this is a somewhat unnatural situation for a fish to be in, it is probably similar enough to swimming into dark recesses and crevices, as many kinds of fish do when frightened or threatened. So perhaps the increased "fearfulness" resulting from the alarm pheromone in the water interacts with the CS–US pairing to result in an enhancement of avoidance conditioning.

G. McA. KIMBRELL*
MARK R. WEINROTT
EDWARD K. MORRIS, JUN.

Department of Psychology,

JENNIFER SCHEID
DEBORAH SANGSTON

Department of Botany,
Denison University,
Granville,
Ohio 43023.

Received July 4; revised December 1, 1969.

* To whom requests for reprints should be sent.

[1] Gleason, K. K., and Reynierse, J. H., *Psychol. Bull.*, **71**, 58 (1969).
[2] Thinnes, H., and Vandenbussche, E., *Anim. Behav.*, **14**, 296 (1966).

Serum Lipids and the Death of Spawning Pacific Salmon

DURING a recent expedition of the R/V Alpha Helix (1968), we had the opportunity to compare blood serum lipids of pink salmon (*Oncorhynchus gorbuscha*) collected at two points in their spawning migration. Fish were sampled from commercial purse seines near Namu, which is located on the British Columbia coast at the entrance to the Burke Channel; and again 60 miles from the sea in a small freshwater stream (a tributary of Thornsons Creek) near Bella Coola, at the head of the same channel. The fish were spawning at this latter site and were obtained by dip net. The principal difference between the Namu and Thornsons Creek fish was that the latter had undertaken a migration of 60 miles under total fasting conditions. The Namu fish were in good condition while those in the freshwater stream had lost on average one-third of their weight and were close to death. Other studies[1-4] have demonstrated that salmon lose weight during the spawning migration. It is known that lipids are the principal substrate in the energy metabolism of fish and that 91 to 96 per cent of the fat reserve in the salmon is utilized during this migration[1]. Ultrastructural observations (to be published) confirmed that lipid deposits had disappeared from the back muscle of the salmon after migration.

By thin layer chromatographic analysis a large triglyceride component was consistently detected in individual as well as pooled serum samples for both male and female pink salmon from Namu whereas little or no triglyceride could be detected in similar serum samples of spawned fish. Because circulating triglycerides must be an important energy source in fasting fish, a more detailed analysis of the major classes of serum lipids for representative groups of fish from the two sites was undertaken.

Blood (10 to 15 ml.) was drawn from the caudal vein of freshly caught fish and allowed to clot for 30 min in 12 ml. conical centrifuge tubes. Serum was collected from above the sedimented clot after centrifuging for 15 min at 3,000 r.p.m. Lipids were extracted from 2 ml. serum samples by the method of Folch *et al.*[5]. The concentration of total lipid in serum was calculated from the weight of the extracted lipids which were made to 2·0 ml. in chloroform and stored at 2°–4° C. Aliquots of the chloroform solutions were analysed: total cholesterol by the method of Abell *et al.*[6] and phospholipid phosphorus by the procedure of Rouser *et al.*[7]. After separation by thin layer chromatography, triglycerides and free fatty acids were analysed by the methods of Stern and Shapiro[8] and of Duncombe[9], respectively. Thin layer chromatography was also used to separate the free and esterified cholesterol, the relative proportions of which were then calculated from data by the Abell method.

Table 1. AVERAGE CONCENTRATIONS OF MAJOR BLOOD SERUM LIPIDS IN PINK SALMON COLLECTED AT TWO POINTS ON THEIR SPAWNING ROUTE

	Lipids (mg/100 ml. ± *S.D.*)	
	Ocean coast	Spawning ground
	mg/100 ml. (± *S.D.*)	
Total cholesterol	267 (± 31)	237 (± 22)
Phospholipids	994 (± 139)	410 (± 56)
Triglycerides	375 (± 117)	71 (± 11)
Free fatty acids	26 (± 5·6)	16 (± 3·5)
Total lipids	1,986 (± 328)	640 (± 121)

Results are from duplicate analyses of eight representative fish at each location.

Table 1 includes data from four males and four females of the post-spawning specimens and two males and six females of the pre-spawning fish. For the variables which were particularly significant in this study, that is, serum triglycerides and total serum lipids, sex did not appear to be an important factor. The data for phospholipids were derived by multiplying lipid phosphorus in 100 ml. of serum by a factor of twenty-five. The free fatty acid values were calculated as stearic acid, the triglycerides as triolein. Only one or two of the serum lipid samples showed an even faintly detectable level of diglycerides and none showed monoglycerides by thin layer chromatography.

The cholesterol levels of the two groups of fish were fairly comparable. The ocean fish averaged 54 per cent esterified and 46 per cent free cholesterol; the post-spawning fish had 60 per cent esterified and 40 per cent free cholesterol. The serum free fatty acids of the ocean fish averaged somewhat higher than those for post-spawning fish, but this resulted primarily from two fish in the ocean group with higher levels (44 and 52 mg/100 ml.). Bilinski and Gardner[10] have shown that starvation of rainbow trout over a 70 day period brings about no marked change in plasma free fatty acids. The vanishing levels of triglycerides in the post-spawning fish may, however, signal a failing supply of energy to vital organs and muscles. This depression was confirmed for many fish in addition to the sixteen for which data are provided. There is no plausible food supply which could feed millions of mature salmon in the spawning streams. Our data regarding loss of weight and marked depression of serum lipids support the conclusion that these fish are starving.

We thank the Department of Fisheries, Canada, for cooperation and O. K. Kimsquit and E. G. Trams for technical assistance. This work was supported in part by a grant from the US Public Health Service and the US National Science Foundation.

STUART PATTON

Division of Food Science,
Pennsylvania State University,
University Park, Pennsylvania 16802.

G. F. CROZIER

Marine Science Institute,
University of Alabama,
Bayou La Batre, Alabama 36509.

A. A. BENSON

Scripps Institution of Oceanography,
University of California,
San Diego,
La Jolla, California 92037.

Received September 4; revised November 5, 1969.

[1] Tashima, L., and Cahill, jun., G. F., in *Handbook of Physiology* (edit. by Renold, A. E., and Cahill, jun., G. F.), **5**, 55 (Amer. Physiol. Soc., Washington, 1965).

[2] Idler, D. R., and Tsuyuki, H., *Canad. J. Biochem. Physiol.*, **36**, 783 (1958).

[3] Idler, D. R., and Bitner, I., *J. Fish. Res. Board Canada*, **16**, 235 (1959).

[4] Hoar, W. S., in *The Physiology of Fishes* (edit. by Brown, M. E.), **1**, 304 (Academic Press, New York, 1957).

[5] Folch, J., Lees, M., and Sloane-Stanley, G. H., *J. Biol. Chem.*, **226**, 497 (1957).

[6] Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E., *J. Biol. Chem.*, **195**, 357 (1952).

[7] Rouser, G. N., Siakotos, A. N., and Fleischer, S., *Lipids*, **1**, 85 (1966).

[8] Stern, I., and Shapiro, B., *J. Clin. Path.*, **6**, 158 (1953).

[9] Duncombe, W. G., *Biochem. J.*, **88**, 7 (1963).

[10] Bilinski, E., and Gardner, L. J., *J. Fish. Res. Board Canada*, **25**, 1555 (1968).

Host Response of Tobacco to *Verticillium dahliae* (tob.) Kleb.—a Cytological Method of assaying Resistance

A WILT disease of tobacco caused by *V. dahliae* constitutes an economic problem in New Zealand. During a histological study of the progress of the pathogen inside the host tissue, the interplay of various mechanical defence mechanisms such as gummosis and tylosis was observed in the vessels of roots and stems. In the meristematic regions of the root-tips, however, a reduction in cell division was revealed by the number of metaphases in the infected resistant variety as compared with non-infected plants or infected susceptible plants. This observation was subsequently confirmed, and I report the findings here.

In the experiments, I used plants of two varieties of tobacco—a biotype of McNairs 121 (MN), which is a resistant type, and Virginia Gold (VG), a susceptible variety. The plants were grown to transplanting stage (6–8 weeks old), and after most of the fibrous root system had been severed they were washed, surface sterilized in 5 per cent sodium hypochlorite solution and transferred to a standard water culture solution in glass tubes. After 6–7 days in a growth chamber the roots emerged. A control sample of the extreme tips of 2–3 roots was fixed in 3 : 1 alcohol–acetic acid solution. The water culture solution was then replaced by a spore suspension (8×10^6 spores per ml.) of *V. dahliae*, grown on potato dextrose agar for 4 days. Samples of root-tips were taken at 4, 8, 16 and 24 h, and fixed for 2 h in the alcohol–acetic acid mixture. The plants were retransferred to water culture solution and further samples of root tips were taken on the third and fifth days.

After fixation, root-tips were transferred to a mixture of nine parts of acetic acid–orcein[1] (0·5 per cent orcein in 45 per cent (by volume) acetic acid) and 1 part of 1 M HCl and heated gently over a spirit lamp. The extreme tip of the individual root was squashed under a cover slip which was sealed with a rubber solution. The number of cells at the metaphase stage was counted under a microscope (×250). The thickness of the root-tips was taken into consideration in averaging the results, which are summarized in Table 1.

Table 1. EFFECTS OF SPORE SUSPENSION OF *V. dahliae* ON ROOT MERISTEM OF TOBACCO

No. of sample	Time	McNairs 121 (MN resistant) (No. of cells showing metaphase)	*Virginia Gold (VG susceptible) (No. of cells showing metaphase)
1	0 h	180 (control)	160 (control)
2	4 h	45 (spore suspension) †	170 (spore suspension)
3	8 h	50 ,, ,, †	150 ,, ,,
4	16 h	80, 15 ,, ,,	60, 15 ,, ,,
5	24 h	100, 10 ,, ,,	50 ,, ,,
6	72 h	200 (water culture)	180 (water culture)
7	120 h	260 ,, ,,	200 ,, ,,

Figures recorded here are an average of 20 experiments of 10 plants each.

* 5 Virginia Gold plants showed reduction in metaphase like that of McNairs 121.

† Significantly different from control: $P < 0{\cdot}01$.

Within 4 h of infection the rate of cell division had dropped by more than 50 per cent in the resistant (MN) variety, compared with the controls taken from the same plants or from non-infected plants. By contrast, in infected Virginia Gold (susceptible) the number of dividing cells remained unchanged. The phenomenon was consistent in all replicate experiments for up to 8 h in the spore suspension. After 16 h, however, variable cell counts were recorded for both resistant and susceptible varieties; some roots showed very low numbers and some high numbers. By this time, the spores were germinating, with a variable effect on the root growth. Once returned to water culture, however, all roots reverted to the normal rate of cell division, and, in fact, the number was much higher in MN than in controls. This experiment was repeated with two other varieties which gave similar results. Cell-free filtrates of a spore suspension of *V. dahliae* held for 4 h before filtration were as effective in reducing cell division as the spore suspension itself. The fluctuations resulting from germinating spores were thereby eliminated.

I suggest that the phenomenon of rapid repression of cell division after 4–8 h of immersion in a spore suspension could be used as a test for the resistance of plants before they are transplanted to the field. The technique is relatively rapid once the active root system has been formed. Care must be taken, however, to minimize contamination from other microorganisms. The same plants could be used in subsequent field trials.

As far as I know, this is the first report of the partial inhibition of cell division in root meristem of *Verticillium*-infected host. The view that host–parasite reaction results in a change in nucleic acid and protein synthesis is widely held[2-4]. Patil and Diamond[5] have reported a higher rate of protein synthesis in a resistant variety of potato infected with *Verticillium*. Hadwiger and Schwochan[6] proposed an induction hypothesis for host resistance responses on the basis of their own results and those of others. The observation of partial inhibition of cell division in the resistant McNairs is probably a part of this "induction" process. Fungal metabolites temporarily repress such of cells' differentiation processes as are shown by the rate of cell division in the roots. This hypersensitive reaction is possibly necessary for gene activation which results in a higher rate of protein synthesis. In the susceptible VG the reaction is either delayed or diminished. It must be emphasized, however, that resistance in tobacco is polygenic[7]. Variable resistance is therefore expected within a single variety until selection is complete.

I am now pursuing both biochemical studies to identify the chemical nature of the inhibitor and a comparison of the rate of protein synthesis.

H. K. MAHANTY

Cawthron Institute,
Nelson,
New Zealand.

Received September 2; revised November 24, 1969.

[1] La Cour, L., *Stain Tech.*, **16**, 169 (1941).
[2] Hadwiger, L. A., and Fulger, S., *Phytopathology*, **57**, 1005 (Abst.) (1967).
[3] Shaw, M., *Canad. J. Bot.*, **45**, 1205 (1967).
[4] Williams, P. H., *Phytopathology*, **56**, 521 (1966).
[5] Patil, S. S., and Diamond, A. E., *Phytopathology*, **56**, 894 (1966).
[6] Hadwiger, L. A., and Schwochan, M. E., *Phytopathology*, **59**, 223 (1969).
[7] Wright, D. S. C., *NZ J. Agric. Res.*, **11**, 655 (1968).

Trends in Montane Grasslands in Snowdonia, expressed in Terms of "Relative Entropy"

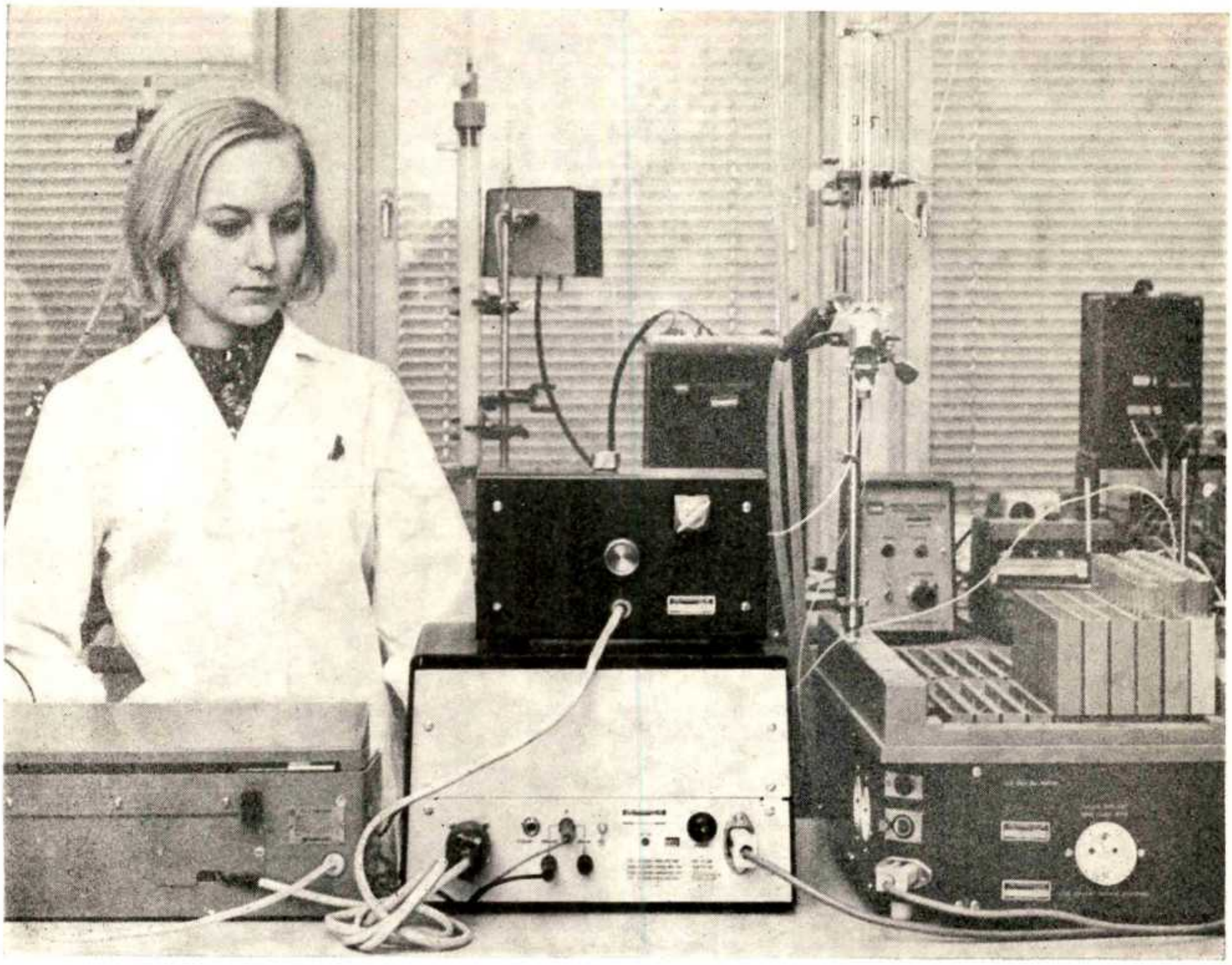
BETWEEN 1957 and 1960 a series of experiments was set up at four sites in Snowdonia, North Wales, to study the influence of sheep grazing on two widely distributed plant communities characterized by the dominance within them of *Nardus stricta* (on soils of peaty podsol/peaty gley character), and *Agrostis tenuis* and *Festuca ovina* (on soils of brown earth character), respectively. Some of the experiments were established by M. J. Chadwick[1]. The experimental sites cover an altitude range of 1,300–3,000 feet O.D. (396–914 m) and, at each site, three treatments are set out in a randomized design incorporating four replicates per treatment. The experimental treatments were: (i) Normal grazing: open to the sheep from the surrounding mountain pastures (most of the breeding ewes and yearling sheep are removed from the mountain in winter); (ii) winter protection from grazing: the plots were protected by fencing from October to April each year; normal grazing took place during the summer; (iii) no grazing; the plots were ungrazed throughout the period of the experiment. (The approximate densities of the sheep populations grazing the experimental plots, expressed in ewe units/acre (hectares), are set out in Tables 1 and 2.)

Each block of replicates covers 576 square yards (481 m²) and the individual replicates are 48 square yards (40 m²) in area. The vegetation has been sampled by means of point quadrats in all years except 1964.

One hundred randomly placed vertical point quadrats (each consisting of a pin 35 cm × 2 mm) are recorded within each replicate, so that the whole experimental block at each site contains 1,200 points. The individual point quadrats are located by coordinates given from tables of random numbers and each species hit at a particular point is recorded. The data are thus summarized as percentage hits of each species.

Changes in the species composition of the vegetation types have been recorded in this way for a period of ten years. Changes that have taken place in the absence or diminution of grazing have received particular attention. They may well be relevant to problems of managing the vegetation in the future. The detailed features of vegetational trends have been described by J. Dale and his results will be published elsewhere.

We have also followed changes with time and treatment in the pattern of species in the plant communities and used an "index" to summarize and order some of the information about the vegetational changes. We have applied the concept of "entropy" in the statistical sense to the study of multicomponent systems (as outlined by Miller and Kahn[2]) of which the plant communities are instances.



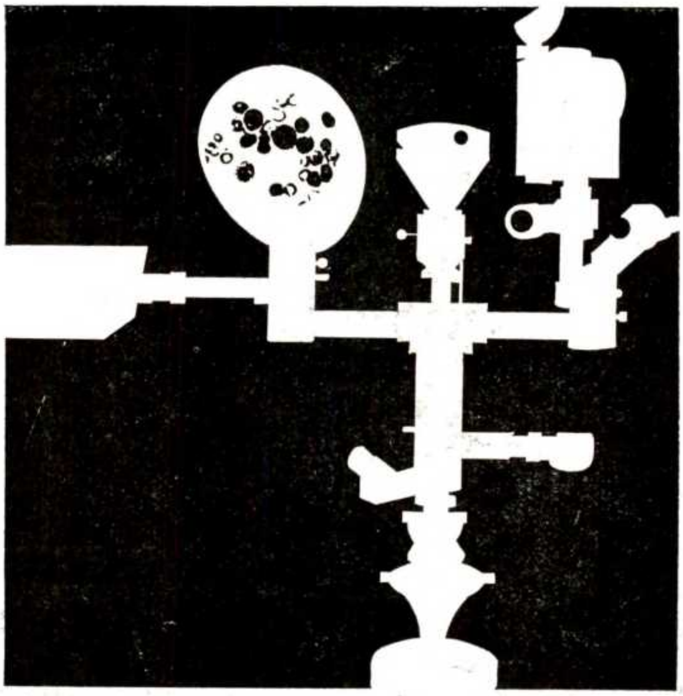
The other side of the UVICORD

Once an LKB Uvicord ultraviolet monitor has been installed people seldom look at the back—that's why we are giving you the chance to see it before yours is installed.
LKB designed the Uvicord to be fully compatible with all kinds of recorders; you will find three signal outlets on the back. One of these will fit your recorder, no matter what type it may be. A further example of the versatility of LKB instrumentation, which will operate in any system your laboratory may possess. A factor worth considering, when you weigh up the other features: stable base line, high sensitivity and sharply-defined bandwidth.

LKB IN THE SERVICE OF SCIENCE

LKB INSTRUMENTS LTD. ● LKB HOUSE ● 232 ADDINGTON ROAD
● S. CROYDON, SURREY. CR2 8YD ● TEL: 01-657-0286
SALES AND SERVICE THROUGHOUT THE WORLD: STOCKHOLM, WASHINGTON, THE HAGUE, COPENHAGEN, ROME, VIENNA

8300-UK5



This is what your microscope would look like

if you were to use at one and the same time all of the reproduction methods offered by WILD.

Even then it would be incomplete, because we also offer:

- The **"Variomag" zoom adapter,** which can be used with our M 12 or M 20 microscopes. The scale of magnification can be steplessly altered, so that features of interest can be made to fill the format available.
- We also have an **image converter unit,** which will extend the wavelength range of the visible spectrum into the infra-red and ultra-violet regions. It can be used to make things visible which are normally invisible.
- There are some more accessories available for projection work with prisms and mirrors.
- We have a lot of different cameras; if desired they can be made fully automatic, or they can be used with our new exposure meter "S".

WILD
HEERBRUGG

Wild Heerbrugg Ltd.,
CH-9435 Heerbrugg/Switzerland
Optical Precision Instrument Makers

Further particulars from Wild Heerbrugg (U.K.) Ltd. 49-51 Church Street, Maidstone Kent
Telephone: Maidstone 56736/7/8

Miller and Kahn applied information theory to mapping multicomponent systems in geology, using an "entropy" function defined by Shannon[3] and developed by Pelto[4]. Pelto's function is

$$100\,H_r = \frac{-100 \sum\limits_{i=1}^{N} P_i \ln P_i}{H_m}$$

and

$$H_m = -\Sigma \frac{1}{N} \ln \frac{1}{N} = \ln N$$

where $100\,H_r$ = "relative entropy" on a percentage basis
H_m = maximum "entropy"
N = number of components or species
P_i = the proportion of the ith component or species

The "relative entropy" ratio is similar to Pielou's[5,6] and can be taken as a measure of the "evenness" of the population of species in the plant community.

Functions of similar derivation to Pelto's "relative entropy" have subsequently been used in several population studies[7-10]. In **1964** we considered the possibility of presenting our vegetational data (point quadrat data expressed as percentage hits) in terms of the "relative entropy" ratio. The ratio tended to decrease with time after grazing had ceased completely. Here the ratio is used not as an aid to mapping but to express a vegetational trend in relation to time and treatment.

Our data from Cwm Idwal National Nature Reserve, North Wales, illustrate the broad vegetational trends, expressed in terms of "relative entropy", that take place in relation to time and variations in grazing pressure. The data are closely paralleled at our other sites (Tables 1 and 2). Where no grazing occurs, the vegetation on the peaty podsol/peaty gley soil shows a trend over a period of 10 years from domination by *Nardus stricta* to ericoid shrubs (*Vaccinium myrtillus*, *Erica cinerea*, and *Calluna vulgaris*) and *Molinia caerulea*. On the brown earth soil the grasses *Holcus lanatus* and *Deschampsia caespitosa* tend to become prominent after the cessation of grazing in the vegetation previously dominated by *Agrostis tenuis* and *Festuca ovina*. Where grazing has been restricted to the summer months (April–October), and where normal grazing throughout the years has remained, there is little change in the vegetation.

Table 1 shows that in the absence of grazing, "relative entropy" has decreased with time, while there is no definite trend for the two other treatments. The total number of species present in the plant community does not show a parallel change with time, although there are seasonal fluctuations and an initial increase.

Table 2 covers three other experimental sites in Snowdonia. In each case there is a decrease in the "relative entropy" of the point quadrat data over a period of 8–10 years when the vegetation is not grazed.

In practical terms a decrease in "relative entropy" probably means that there is a movement from the "uncertainty" of the grazed sites to a state of greater "certainty" where they are ungrazed. So far the applica-

Table 1. "RELATIVE ENTROPY" OF VEGETATION IN RELATION TO TIME AND GRAZING BY SHEEP AT CWM IDWAL NATIONAL NATURE RESERVE, CAERNARVONSHIRE, NORTH WALES

Altitude 1,200 feet (365 m), mean annual rainfall 100+ inches (2,540+ mm). Grid reference SH(23) 643599

Vegetation	*Nardus stricta*			*Agrostis tenuis/Festuca ovina*		
Soil type and derivation	Peaty podsol/peaty gley (pH range 3·8–4·0) Drift—mainly Ordovician acid volcanic rocks			Brown earth (pH range 4·6–4·7) Alluvial fan—mainly basic Ordovician volcanic rocks		
Treatment	1 (Free grazing)	2 (Winter protection)	3 (No grazing)	1 (Free grazing)	2 (Winter protection)	3 (No grazing)
Mean sheep population* densities in ewe units/acre (hectare)	Summer 1·12 (2·76) Winter 0·41 (1·01)	Summer 1·12 (2·76) Winter nil	Nil —	Summer 1·76 (4·34) Winter 0·58 (1·43)	Summer 1·76 (4·34) Winter nil	Nil —
Year						
1958	65·07 (19)†	64·04 (18)	73·37 (21)	71·84 (19)	68·61 (19)	73·28 (19)
1959	69·25 (23)	73·49 (24)	76·39 (24)	66·99 (23)	68·07 (19)	70·02 (21)
1960	66·61 (22)	75·26 (24)	73·69 (23)	65·07 (28)	63·99 (34)	73·23 (32)
1961	65·75 (28)	69·97 (29)	73·25 (32)	72·57 (24)	67·48 (26)	68·50 (26)
1962	68·56 (30)	72·68 (30)	70·51 (31)	66·37 (27)	63·09 (28)	66·58 (28)
1963	72·32 (32)	67·66 (27)	66·61 (28)	67·22 (29)	70·05 (28)	66·11 (29)
1964	—	—	—	—	—	—
1965	65·52 (28)	72·01 (24)	65·43 (20)	74·03 (24)	70·94 (25)	61·22 (23)
1966	68·47 (32)	79·04 (31)	64·92 (22)	67·45 (29)	70·64 (29)	58·32 (28)
1967	68·07 (27)	73·98 (24)	64·67 (22)	74·69 (26)	66·19 (25)	55·13 (25)
1968	74·69 (25)	74·06 (26)	62·89 (19)	75·01 (26)	72·38 (25)	58·23 (26)

* Sheep population densities expressed as ewe units/acre (per hectare). 1 adult sheep = 1 ewe unit; 1 lamb = 0·5 ewe unit (approximate).
† Figures in parentheses indicate number of species recorded.

Table 2. "RELATIVE ENTROPY" OF VEGETATION IN RELATION TO TIME AND GRAZING BY SHEEP IN SNOWDONIA, CAERNARVONSHIRE, NORTH WALES

		"Relative entropy"			Sheep population densities ewe units/acre (per hectare)		
Site, soils and vegetation		Treatment 1 (free grazing)	Treatment 2 (winter protection)	Treatment 3 (no grazing)	Treatment 1 (free grazing)	Treatment 2 (winter protection)	Treatment 3 (no grazing)
(i) Crib Goch* Shallow brown earth *Agrostis tenuis + Festuca ovina*	Initial	70·86	75·56	79·26	Summer 0·77 (1·90) Winter 0·30 (0·74)	Summer 0·77 (1·90) Winter Nil	Nil
	Final	70·93	72·01	61·31			
(ii) Llyn Llydaw Peaty podsol/peaty gley *Nardus stricta*	Initial	79·68	75·45	75·71	Summer 1·09 (2·69) Winter 0·35 (0·86)	Summer 1·09 (2·69) Winter Nil	Nil
	Final	86·62	82·26	60·20			
(iii) Llyn Llydaw Brown earth *Agrostis tenuis + Festuca ovina*	Initial	76·01	69·79	74·64	Summer 2·73 (6·74) Winter 0·86 (2·12)	Summer 2·73 (6·74) Winter Nil	Nil
	Final	72·13	77·27	59·04			
(iv) Pen-y-Pass Peaty podsol/peaty gley *Nardus stricta*	Initial	75·89	84·07	83·33	Summer 0·81 (2·00) Winter 0·26 (0·64)	Summer 0·81 (2·00) Winter Nil	Nil
	Final	88·19	80·22	69·39			
(v) Pen-y-Pass Gleyed brown earth *Agrostis tenuis + Festuca ovina*	Initial	71·07	75·72	74·79	Summer 1·63 (4·02) Winter 0·51 (1·26)	Summer 1·63 (4·02) Winter Nil	Nil
	Final	78·78	73·75	67·96			

* Data for Crib Goch site are for 1960 and 1968. Data for other sites are for 1958 and 1968.
(i) Altitude 2,800 feet (853 m). Mean annual rainfall 200 inches (5,080 mm). Grid ref. (i) SH(23) 621552.
(ii) and (iii) Altitude 1,450 feet (442 m). Mean annual rainfall 150 inches (3,810 mm). Grid ref. (ii) SH(23) 636547 and Grid ref. (iii) SH(23) 635552.
(iv) and (v) Altitude 1,200 feet (396 m). Mean annual rainfall 120 inches (3,048 mm). Grid ref. (iv) and (v) SH(23) 645556.

tion of the "relative entropy" or "evenness" ratio or index has promised to be valuable in analysing vegetational trends for experiments of the kind we have described, but it can probably be of wider use in ecological classification and mapping generally[10-12], as well as in the ordination of vegetational assemblages so that they may be related to environmental and other factors.

We thank members of the staff of the Nature Conservancy for their help and advice, particularly Mr M. Mountford (Biometrics) and Dr D. F. Ball (Pedology); and Mr K. B. Martin of the School of Plant Biology, University College of North Wales, Bangor, for his helpful criticism.

R. Elfyn Hughes
J. Dale

Bangor Research Station,
The Nature Conservancy,
Penrhos Road, Bangor.

Received June 23; revised November 26, 1969.

[1] Chadwick, M. J., PhD thesis (University of Wales, 1959).
[2] Miller, L R., and Kahn, J. S., *Statistical Analysis in the Geological Sciences* (John Wiley and Sons, New York and London, 1962).
[3] Shannon, C. E., and Weaver, W., *The Mathematical Theory of Communication* (University of Illinois Press, Urbana, 1964).
[4] Pelto, C. R., *J. Geol.*, **62**, 501 (1954).
[5] Pielou, E. C., *J. Theor. Biol.*, **13**, 131 (1966).
[6] Pielou, E. C., *J. Theor. Biol.*, **10**, 370 (1966).
[7] Dickman, M., *Ecology*, **49**, 1191 (1968).
[8] Landenberger, D. E., *Ecology*, **49**, 1062 (1968).
[9] Hairston, N. G., *et al.*. *Ecology*, **49**, 1091 (1968).
[10] Williams, W. T., Lambert, J. M., and Lance, G. N., *J. Ecol.*, **54**, 427 (1966).
[11] Lambert, J. M., and Williams, W. T., *J. Ecol.*, **54**, 635 (1966).
[12] Kikkowa, J., *J. Anim. Ecol.*, **37**, 143 (1968).

Extract of *Ipomoea pes-caprae* (Convolvulaceae) antagonistic to Histamine and Jelly-fish Poison

The creeper, *Ipomoea pes-caprae* (L.) Roth. of the family Convolvulaceae, which is found in abundance along the warmer seashores of both hemispheres, is used as a medicant in many parts of the world. The leaves are usually used—either boiled for external application as an anodyne in colic or rheumatism, or powdered and incorporated in an ointment said to have good local effects on bedsores—but they may also be used as an escharotic in the treatment of ulcers. Previous reports[1,2] suggest that the extracts of the plant have no pharmacological effects.

In the light of use of the plant by Thai fishermen as an antidote to jelly-fish stings and as an antipruritic agent, I examined various crude extracts and have found that there is an active principle (IPA) that is antagonistic to jelly-fish poison and is also mildly antihistaminic. This is of interest because there are no known specific antidotes for jelly-fish poison, described by Halstead[3] as toxic proteins; oral and topical antihistamine drugs are used for urticarial lesions and symptoms from contact with jelly-fish.

The water distillate from the dry ground leaves of the plant was extracted with ether. The residue was found to be slightly impure IPA and yielded about 0·05 per cent (dry basis). Infrared absorption and chemical analysis indicated that IPA is a volatile ester.

The antihistaminic activity of IPA compared with histamine dihydrochloride was evaluated *in vitro* by the Magnus method[4], in which measurement is made of the amount of the drug which relaxes histamine-induced spasm in an isolated ileum of guinea-pig immersed in Tyrode solution. The antagonistic effect of IPA compared with jelly-fish poison was evaluated by the same technique, but a dilute solution of jelly-fish poison (obtained by water extraction of the tentacles of a large jelly-fish, *Catostylus* sp.) was used to replace histamine dihydrochloride for inducing spasm in the isolated ileum of guinea-pig. (Compared with histamine dihydrochloride, the jelly-fish toxic material induced a slower contraction of the muscle, but the spasm remained much longer even after repeated washings. Moreover, an isolated guinea-pig ileum could stand many more experimental tests with histamine than with jelly-fish toxic material which paralysed the ileum after a few experiments.) The results of these experiments and similar experiments using two well known antihistamine drugs, diphenhydramine hydrochloride injection USP, BP ('Benadryl'), and antazoline methanesulphonate injection BPC ('Antistine'), are shown in Tables 1 and 2.

Table 1. DETERMINATION OF THE ANTIHISTAMINIC EFFECT OF IPA, COMPARED WITH OFFICIAL ANTIHISTAMINE DRUGS, AGAINST 2×10^{-6} HISTAMINE DIHYDROCHLORIDE ON THE ISOLATED ILEUM OF GUINEA-PIG

Description	Concentration	Antihistaminic effect (per cent)
Diphenhydramine HCl injection USP, BP	5×10^{-7}	Positive, 50
	1×10^{-6}	Positive, 99
Antazoline methanesulphonate injection BPC	5×10^{-6}	Positive, 80
	1×10^{-5}	Positive, 99
IPA	3×10^{-5}	Positive, 50
	6×10^{-5}	Positive, 99
Ethanol* (control sample)	0·3–0·6%	Negative

Table 2. DETERMINATION OF THE ANTAGONISTIC EFFECT OF IPA, COMPARED WITH OFFICIAL ANTIHISTAMINE DRUGS, AGAINST DILUTED GIANT JELLY-FISH POISONS

Description	Concentration	Antagonistic effect (per cent)
Diphenhydramine HCl injection USP, BP	4×10^{-7}	Positive, 25
	4×10^{-6}	Positive, 30
	1×10^{-5}	Positive, 90
	4×10^{-5}	Positive, 100
Antazoline methanesulphonate injection BPC	6×10^{-5}	Positive, 99
IPA	3×10^{-5}	Positive, 80
	6×10^{-5}	Positive, 99
Ethanol* (control sample)	0·3–0·6%	Negative

* The final IPA solution for this test experiment contained 0·3–0·6 per cent ethanol.

It is seen that although IPA is less effective in its antihistaminic effect than either of the two official antihistamines, it has about the same antagonistic effect against jelly-fish poison as the two drugs.

Dr Mongkol Mokkhasmit and Mr Prayuth Satrawaha of the Department of Medical Sciences, Thai Ministry of Public Health, have found that IPA has no toxic effect on mice after oral administration of 7·5 g per kg mouse.

S. Wasuwat

Technological Research Institute,
Applied Scientific Research Corporation of Thailand,
Bangkhen, Bangkok.

Received September 15; revised December 15, 1969.

[1] Christensen, B. V., and Reese, S. A., *J. Amer. Pharm. Assoc.*, **27**, 195 (1938).
[2] Cwalina, G. E., and Jenkins, G. L., *J. Amer. Pharm. Assoc.*, **27**, 585 (1938).
[3] Halstead, B. W., in *Poisonous and Venomous Marine Animals of the World*, **1**, 335 (USGPO, Washington, DC, 1965).
[4] Burger, A., in *Medicinal Chemistry*, second ed., 581 (Wiley–Interscience., NY, 1960).

Eustigmatophyceae—a New Algal Class with Unique Organization of the Motile Cell

Investigation of the cytology and ultrastructure of fifteen species drawn from twelve genera of coccoid Xanthophyceae (carried out in conjunction with a similar study of nineteen species drawn from four filamentous genera[1,2]) reveals that this algal class contains two structural series. The morphology of the motile cell in one series is unique in the plant kingdom and this series is here separated from the Xanthophyceae *sensu stricto* as a new class, the Eustigmatophyceae.

Diagnostic features of the new class are as follows:

(1) The eyespot (Figs. 1 and 3) is a large orange-red body at the anterior end of the motile cell, completely independent of the single chloroplast; it consists of an irregular group of non-membrane-bounded droplets, with no membrane around the whole complex.

(2) Zoospores of most species have only one emergent flagellum (Figs. 1–3) but a second basal body is present; the flagellum is inserted subapically, is always directed anteriorly during swimming and bears a bilateral array of stiff hairs (Fig. 2). Only one species, *Ellipsoidion acuminatum*, has the second flagellum developed as an emergent organelle; this flagellum is smooth and short, with a long hair-point, and is always directed laterally or posteriorly during swimming.

(3) A flagellar swelling (Fig. 3) is borne at the proximal end of the hairy flagellum; it consists of an expansion of the dilated flagellar sheath (see Fig. 2) and is always closely applied to the cell membrane in the region of the eyespot.

(4) The elongate chloroplast (Figs. 1 and 3) contains three-thylakoid lamellae but no girdle lamella; endoplasmic reticulum bounds the plastid but is not in direct association with the nuclear envelope.

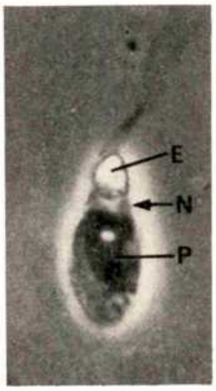


Fig. 1. Zoospore of *Vischeria stellata* (Eustigmatophyceae), showing the single flagellum with expanded sheath, the eyespot (E), the position of the nucleus (N) and the plastid (P). Light microscopy (×2,000).

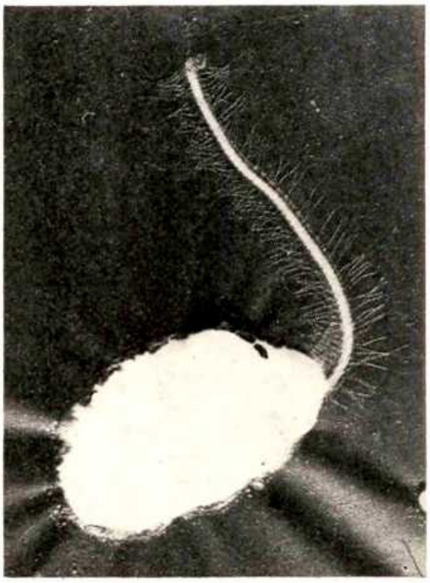


Fig. 2. Zoospore of *Vischeria punctata* (Eustigmatophyceae), showing the bilateral array of stiff hairs and the expanded sheath of the single flagellum. Electron micrograph of shadowcast whole cell, reversed print (×5,000).

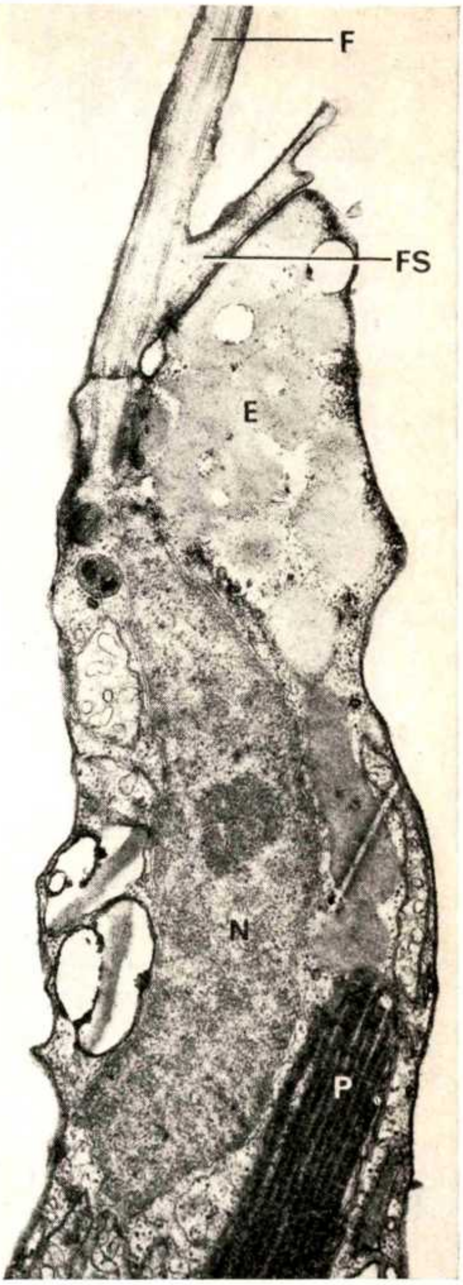


Fig. 3. L.S. anterior end of the zoospore of *Polyedriella helvetica*, showing the key features of eustigmatophycean structure: the extraplastidic eyespot (E) is separated from the plastid (P) by the nucleus (N); the single, anteriorly directed flagellum (F) bears a swelling (FS) which is adpressed to the eyespot and is an extended dilation of the expanded flagellar sheath; the plastid does not contain a girdle lamella. Electron micrograph (×20,000).

(5) Golgi bodies are absent from the motile cell but present in the vegetative cell.

(6) A unique form of pyrenoid is always present in the vegetative cell, never in the zoospore; the pyrenoid is polygonal, projects from the inner face of the lobed plastid on a narrow stalk and is surrounded by flat plates of an unidentified photosynthate which appears finely lamellate after fixation (and is not normal starch); thylakoids do not enter the pyrenoid matrix.

Species which can be placed with certainty in the Eustigmatophyceae are *Ellipsoidion acuminatum* Pascher, *Pleurochloris commutata* Pascher, *Pleurochloris magna* Boye Petersen, *Polyedriella helvetica* Vischer et Pascher, *Vischeria punctata* Vischer and *Vischeria stellata* (Poulton) Pascher; *Chlorobotrys regularis* West et West can probably be included on vegetative characters alone (no zoospores recorded). Coccoid forms which can be left with certainty in the Xanthophyceae *sensu stricto* are *Botrydiopsis alpina* Vischer, *Bumilleriopsis filiformis* Vischer, *Chlorellidium tetrabotrys* Vischer et Pascher, *Chloridella neglecta* Pascher, *Mischococcus sphaerocephalus* Vischer, *Ophiocytium majus* Naegeli, *Pleurochloris meiringensis* Vischer and *Sphaerosorus composita* Moewus, joining filamentous forms of the genera *Bumilleria*, *Heterococcus*, *Heterothrix* and *Tribonema*, and the siphoneous *Botrydium granulatum* Greville, which have all been investigated by modern techniques[1-4]. These true xanthophycean forms have the eyespot as part of a chloroplast; an anteriorly directed hairy flagellum and a posteriorly directed smooth one, the latter bearing the flagellar swelling; two or more plastids, typically with a girdle lamella; a sheath of chloroplast endoplasmic reticulum continuous with the nuclear envelope; Golgi bodies present in both motile and vegetative cells; and pyrenoids rare, but also in both zoospores and vegetative cells when present, semi-immersed, with no surrounding photosynthate (except lipid) and with normal three-thylakoid lamellae always entering the pyrenoid matrix. In addition, a pigment difference has recently been reported[5] between two true xanthophycean species (with antheraxanthin as the major xanthophyll) and two eustigmatophycean forms (with antheraxanthin replaced by a pigment corresponding to violaxanthin).

It is therefore clear that the Eustigmatophyceae possesses several unique features and is, in fact, less like the Xanthophyceae *sensu stricto* than the latter is like the other "heterokont" classes (the Phaeophyceae, Chrysophyceae *sensu* Christensen[6] and Bacillariophyceae) which share most of the features listed here for the Xanthophyceae. The possession of a typical "heterokont" anterior hairy flagellum by the Eustigmatophyceae does, however, raise a point of real taxonomic difficulty, necessitating a review of the whole concept of heterokont organization. The taxonomic status of the new class in relation to this problem will be discussed elsewhere, together with the detailed morphology of the motile cell and the ultrastructure of the individual organelles.

DAVID J. HIBBERD*
GORDON F. LEEDALE

Department of Botany,
University of Leeds.

Received June 30; revised November 13, 1969.

---

* Present address: Department of Botany, British Museum (Natural History), London.

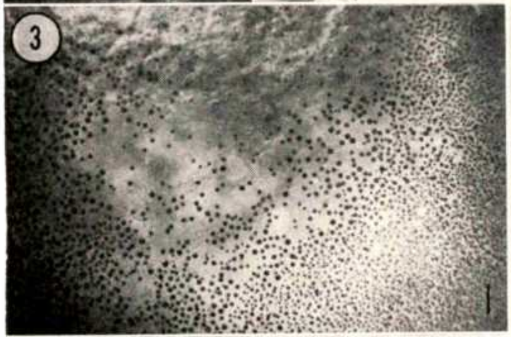
[1] Massalski, A., thesis, Univ. of Leeds (1969).
[2] Massalski, A., and Leedale, G. F., *Brit. Phycol. J.*, **4**, 159 (1969).
[3] Falk, H., *Arch. Mikrobiol.*, **58**, 212 (1967).
[4] Falk, H., and Kleinig, H., *Arch. Mikrobiol.*, **61**, 347 (1968).
[5] Whittle, S. J., and Casselton, P. J., *Brit. Phycol. J.*, **4**, 55 (1969).
[6] Christensen, T., *Alger*, in *Botanik* (edit. by Böcher, T. W., Lange, M., and Sørensen, T.), **2**, 1–178 (Munksgaard, Copenhagen, 1962).

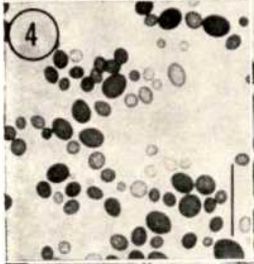
Proline in the Vesicles and Sporelings of *Valonia ventricosa* and the Concept of Cell Wall Protein

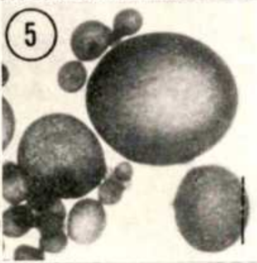
THE mature vesicle of *Valonia ventricosa* was an early source of information about the nature of cellulose[1] and the configuration of a cellulose cell wall[2,3]. Later it was used in one of the first investigations with the electron microscope of the way a plant cell wall develops at a naked protoplasmic surface[4]. The opportunity to make such a study was provided by the formation, at will, of aplanospores and by their subsequent development[5]. Aplanospores are small spherical masses of protoplasm, each with a nucleus, and they may form in very large numbers from the multinucleate, non-septate, protoplasm within a single vesicle. This system, therefore, has also provided an opportunity to allow sporelings to develop their cell walls while in contact with ^{14}C-proline in sea-water. Current methods of autoradiography and electron microscopy make possible determination of the distribution of the radioactivity fixed from ^{14}C-proline. This should test whether a developing cellulose cell wall incorporates substantial amounts of "cell wall protein" as indicated by radioactivity bound in the form of ^{14}C-proline. Also the cell wall of mature vesicles of *V. ventricosa* can easily be obtained free of cytoplasm so that it is possible to ascertain, by direct analysis, how much total protein each contains and its content of proline and/or hydroxyproline.

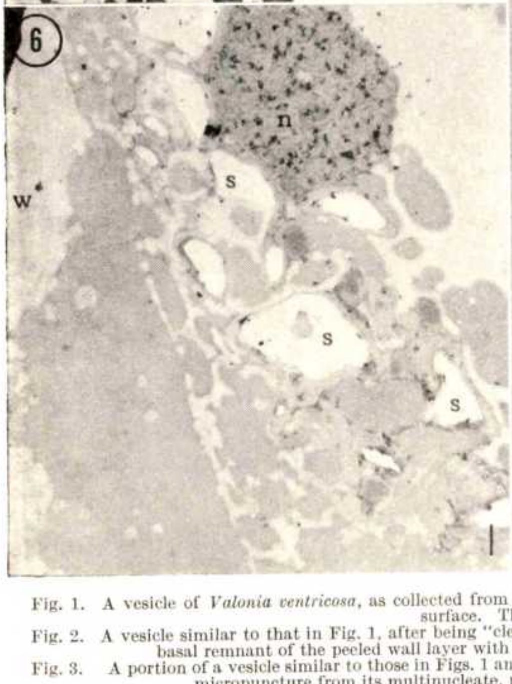
These observations are pertinent because of frequent claims that the combined hydroxyproline of higher plants is present, virtually exclusively, in a protein moiety present in their cell walls and which, in advance of its isolation and purification, was named extensin[6]. This claim has been extended to the presence of protein in cell walls of algae[7,8], along with speculations as to its function[9]. All such claims depend on proof that the analyses were performed on authentic preparations of pure cell wall, as it exists *in situ*, and with no cytoplasmic contamination. This has been demonstrated with preparations which behaved so as to indicate that as algal walls are more purified they may contain peptide but not protein[10]. A recent comprehensive study[11], of which note was taken in *Nature*[12], described the results of a combined electron microscopic and autoradiographic study which located the products of combined proline in the protoplasm, rather than in the wall of aseptically cultured cells of carrot. This work[11] had particular relevance because it involved the system which had furnished the original evidence that ^{14}C-proline absorbed by rapidly dividing cells was incorporated into a metabolically stable entity in which it was converted to hydroxyproline in the approximate overall ratio of 1 : 1[13,14]. Because of their relevance to these problems, certain findings on *Valonia* are being presented here, out of the context of the larger investigations from which they arise. Inasmuch as these observations concern a free cell culture that develops into a plant they relate to our work on morphogenesis; the evidence on the distribution of radioactivity from ^{14}C-proline between the cytoplasm and the developing cell wall is a sequel to the work on carrot cells[11]; the extent of the absorption of this solute contributes to other work on solute uptake[16], and all the results bear on continuing work on the metabolism of proline and hydroxyproline in plants (ref. 11 and communication to Amer. Soc. Plant Physiologists, August 1968, by F. C. S., R. L. M. and P. M. L.).

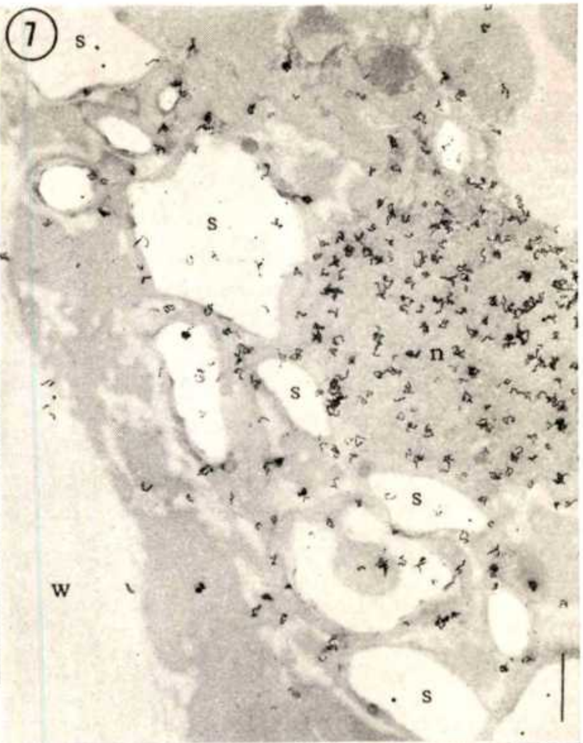
Our observations were of two kinds, being made either on mature freshly collected vesicles or on the aplanospores to which they may give rise. *Valonia ventricosa* vesicles, as they occur in their natural habitat, are commonly encrusted (Fig. 1) with calcareous material and minute organisms (including *Melobesia*) as well as other epiphytes. It has long been our practice to "clean" these vesicles before use in metabolic and cell physiological experiments. A layer of wall can be removed without irreversible damage to the vesicle (Fig. 2). The resulting "clean," iridescent wall can then be punctured and the entire cell contents washed out. In this way three fractions were obtained for analysis from each vesicle as follows. (i) The outermost layer of wall variously contaminated with other organisms from bacteria, protozoa, to other algae (Fig. 2); (ii) a relatively clean, thick, predominantly cellulosic cell wall, free of the gross contamination of the outer layers and which was washed free of cytoplasm; (iii) the whole living content of the vesicle. These various fractions were weighed, hydrolysed with acid, and submitted to amino-acid analysis by chromatographic and autoradiographic methods which











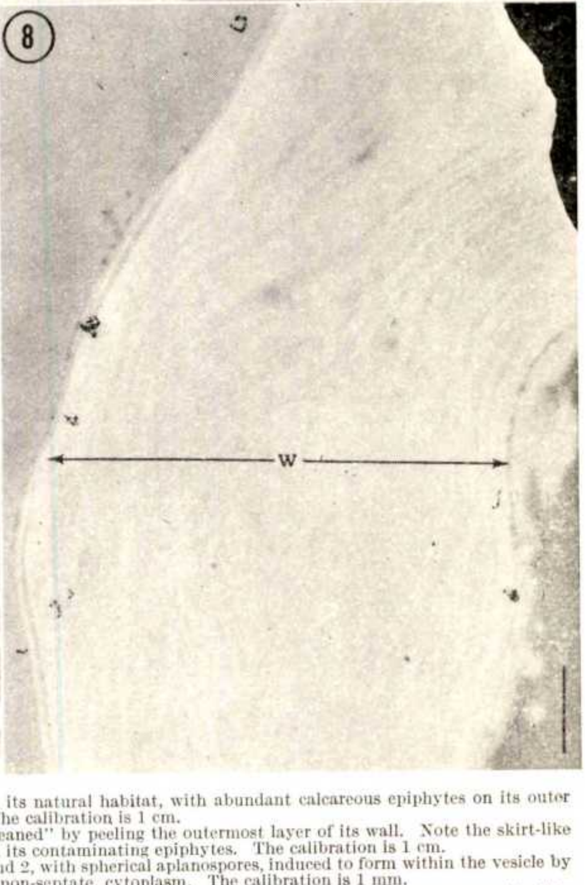


Fig. 1. A vesicle of *Valonia ventricosa*, as collected from its natural habitat, with abundant calcareous epiphytes on its outer surface. The calibration is 1 cm.

Fig. 2. A vesicle similar to that in Fig. 1, after being "cleaned" by peeling the outermost layer of its wall. Note the skirt-like basal remnant of the peeled wall layer with its contaminating epiphytes. The calibration is 1 cm.

Fig. 3. A portion of a vesicle similar to those in Figs. 1 and 2, with spherical aplanospores, induced to form within the vesicle by micropuncture from its multinucleate, non-septate, cytoplasm. The calibration is 1 mm.

Fig. 4. Newly formed aplanospores which were aseptically recovered from a mature vesicle, and which had been exposed to ^{14}C-proline seawater for approximately 8 h. At the time of first contact with ^{14}C-proline—7 h after puncture—the cellulose at the sporeling surface would be negligible[4]. The calibration is 1 mm.

Fig. 5. Sporelings from the population as in Fig. 4, but cultured for 19 days in ^{14}C-proline containing seawater. The calibration is 1 mm.

Figs. 6–8. The distribution of radioactivity in *V. ventricosa* sporelings after 19 days' growth in ^{14}C-proline seawater as revealed by electron microscope autoradiography. w, Wall; n, nucleus; s, starch grain. The calibration is 2 μm.

Fig. 6. A portion of wall and parietal protoplasm at very low magnification. Note the concentration of radioactivity in the nucleus.

Fig. 7. As Fig. 6, but at a somewhat higher magnification. Note the scattered radioactivity in the cytoplasm, the concentration in the nucleus and the virtual absence of radioactivity in the wall.

Fig. 8. A portion (area 100 μm^2) of a new cell wall conspicuously free of radioactivity although it developed during 19 days in contact with ^{14}C-proline seawater.

Table 1. THE PROTEIN CONTENT OF *Valonia ventricosa* VESICLES AND OF THEIR WALLS

	µg protein per vesicle
Total protein* in epiphytes and outer wall of a typical vesicle, 2·4 cm in diameter	551
Total protein in the wall† free of cytoplasm and of the outer layer of wall with its epiphytes	72
Total protein‡ in cytoplasm	565
Total protein in typical vesicle, free of epiphytes	637

* This protein contained 6 per cent of its weight as proline and 19 per cent as glycine but no detectable hydroxyproline.

† This protein contained 2 per cent of its weight as proline and 3·5 per cent as glycine but no detectable hydroxyproline.

‡ This protein contained 9 per cent of its weight as proline and 5 per cent glycine but no detectable hydroxyproline.

became standard after earlier work[13,14], and their content of combined amino-acids (especially of proline and hydroxyproline) was measured.

Table 1 shows that (i) the combined proline in the natural *V. ventricosa* cell wall is in its outermost contaminated layer; (ii) less than a 5 per cent residual contamination of the inner by the outer layer would have accounted for its proline content, even without regard to the known presence of minute hapteron cells at the base of the vesicle which, by "Uhrglaswande"[4], enclose some protoplasm; (iii) the small amount of proline recorded in the "clean" *Valonia* wall was not heavily accompanied by hydroxyproline, and (iv) virtually all of the combined proline of the intact, cleaned vesicle was present in its protoplasm. From this work an upper limit (< 2 per cent of its dry weight) can be placed on the content of protein in the mature *Valonia* wall in its habitat.

Intact living vesicles of *V. ventricosa* were induced to form aplanospores by making controlled minute punctures[5]. The green sporelings so produced survive and grow, either in the original vesicle (Fig. 3) or when isolated into seawater (Figs. 4 and 5). At their formation within the vesicle the aplanospores were free from other organisms and they were maintained in artificial or natural seawater in a unialgal, virtually bacteria-free, state for the duration of these experiments. During this time the entire growth of the sporelings was in the presence of ^{14}C-proline (10 µCi/ml.). Some conversion of ^{14}C-proline to ^{14}C-hydroxyproline by the sporelings could be detected but only in very limited amounts. Washed sporelings were also prepared for transmission electron microscopy by standard methods and then their thin sections so treated[11] (using the procedure there designated SP–1) that the distribution of radioactivity from proline became evident (Figs. 6–8). These figures are deliberately reproduced at low magnifications to show extensive fields.

The fine structure of the cytoplasm of *Valonia* presents problems to be taken up elsewhere. Nevertheless, Figs. 6–7 reveal the newly developed cell walls, the peripheral cytoplasm which contains abundant chloroplasts (up to 60–70 per cent of its volume)[18] with relatively large starch grains and, of course, very numerous nuclei. Fig. 8 shows a substantial area of wall which had developed on an otherwise naked surface of protoplasm and in contact with ^{14}C-proline.

The results are self evident. The radioactivity absorbed during the growth of these sporelings was not significantly present in the cell wall. Any radioactivity there detected did not exceed the expected background, or cross scatter, or the possibility of casual contaminations during cutting. Virtually all the radioactivity from ^{14}C-proline was present in the protoplasm; and, as in the case of cultured carrot cells, it was heavily concentrated in the nuclei.

A predominantly cellulose wall (up to 12 µm thick) comprising as many as twenty composite laminated layers may therefore develop during 19 days on an otherwise naked protoplasmic surface. Although this wall had direct access throughout to exogenous ^{14}C-proline, its content of combined proline was negligible. Thus because all proteins surely contain proline, these newly developed walls did not contain appreciable protein moiety. These data confirm the analysis of the walls of mature vesicles.

The choice of *V. ventricosa* to test the relationship between proteins in cytoplasm and wall was motivated by the relative ease with which cytologically recognizable cytoplasm and wall could be separated in the large vesicles, even without any grinding or killing. By contrast, the extensive work[7–9] referred to on various other algae, which purports to show that proline-containing proteins commonly exist in their walls, was based on the analysis of hydrolysates of fractions of homogenized preparations. The sharp distinction between wall and cytoplasm, as they exist *in situ*, has not been critically demonstrated in such homogenized preparations. In fact, the difficulties in so doing have been acknowledged[8,10,19]. Similarly, the analysis of plant materials for combined hydroxyproline, which is then considered to be the measure of cell wall protein[6,20], does not, in our view, yield meaningful results. Furthermore, those who earlier located the combined hydroxyproline in the wall of angiosperm cells[20] now recognize that it is also to be found extensively in protoplasm[21]. It is in this context that work on the protein content of a mature *Valonia* vesicle on the one hand, and on the incorporation of ^{14}C-proline into the vesicles growing from aplanospores on the other, as here demonstrated, has particular significance.

The concept that there is a major cell wall protein is clearly inapplicable to *Valonia* vesicles, even as it is inapplicable to cultured carrot cells. The protein moieties in cells which do contain combined proline and the hydroxyproline which arises from it *in situ*, and their relations to rapidly growing cells, undoubtedly retain their interest. Nevertheless, this interest is accentuated, not minimized, because these events seem to occur first in protoplasm, especially in nuclei (Figs. 6 and 7) and even more so in nucleoli[11]. These observations are reiterated in spite of a recent report[22] which, in our view, is quite inadequate in its microscopy and autoradiography.

The walls of different senescent, or even dead, plant cells frequently incorporate many non-cellulosic substances (from lignin to tannins and even some special nitrogenous compounds), but such events, interesting as they may often be, should not be allowed to confuse the relative roles of cytoplasm and walls in still viable growing cells. Furthermore, the extrusion by a sort of "reversed pinocytosis" of isolated, membrane-bound proteinaceous "pockets" into the walls of such highly specialized, often protein-secreting cells as pollen, should be recognized as highly interesting but very special cases[23]. Such examples do not require that protein is a necessary integral part of unspecialized plant cellulose walls, nor that the normal mechanism of wall growth requires a protein moiety such as that which has been called extensin[6,24].

This work was supported by a grant, to F. C. S., from the National Institute of General Medical Sciences, Bethesda, Maryland. R. L. M. held a predoctoral fellowship of the US National Institutes of Health. We thank the members of Dr M. M. Salpeter's laboratory at Cornell for localizing radioactivity in the sections.

F. C. STEWARD
R. L. MOTT
H. W. ISRAEL
P. M. LUDFORD

Laboratory for Cell Physiology,
Growth and Development,
Cornell University,
Ithaca, New York 14850.

Received September 2; revised December 11, 1969.

[1] Sponsler, O. L., *Protoplasma*, **12**, 241 (1931).
[2] Astbury, W. T., Marwick, T. C., and Bernal, J. D., *Proc. Roy. Soc.*, B, **109**, 443 (1932).
[3] Preston, R. D., and Astbury, W. T., *Proc. Roy. Soc.*, B, **122**, 76 (1937).
[4] Steward, F. C., and Mühlethaler, K., *Ann. Bot.*, N.S., **17**, 295 (1953).

[5] Steward, F. C., *Papers from Tortugas Lab.*, **32**, Carnegie Inst. Washington, Publ. No. 517, 87 (1939).
[6] Lamport, D. T. A., *Adv. Bot. Res.*, **2**, 151 (1965).
[7] Thompson, E. W., and Preston, R. D., *Nature*, **213**, 684 (1967).
[8] Gotelli, I. B., and Cleland, R., *Amer. J. Bot.*, **55**, 907 (1968).
[9] Thompson, E. W., and Preston, R. D., *J. Exp. Bot.*, **19**, 690 (1968).
[10] Punnet, T., and Derrenbacker, E. C., *J. Gen. Microbiol.*, **44**, 105 (1966).
[11] Israel, H. W., Salpeter, M. M., and Steward, F. C., *J. Cell Biol.*, **39**, 698 (1968).
[12] *Nature*, **221**, 123 (1969).
[13] Steward, F. C., Thompson, J. F., and Pollard, J. K., *J. Exp. Bot.*, **9**, 1 (1958).
[14] Pollard, J. K., and Steward, F. C., *J. Exp. Bot.*, **10**, 17 (1959).
[15] Steward, F. C., Kent, A. E., and Mapes, M. O., in *Current Topics in Developmental Biology* (edit. by Monroy, M. A., and Moscona, A. A.), **1**, 113 (Academic Press, New York, 1966).
[16] Steward, F. C., and Mott, R. L., *Intern. Rev. Cytol.*, **28**, 275 (1970).
[17] Lyndon, R. F., and Steward, F. C., *J. Exp. Bot.*, **14**, 42 (1963).
[18] Doyle, W. L., *Papers from Tortugas Lab.*, **29**, Carnegie Inst. Washington, Publ. No. 452, 13 (1935).
[19] Cronshaw, J., Myers, A., and Preston, R. D., *Biochim. Biophys. Acta*, **27**, 89 (1958).
[20] Cleland, R., *Plant Physiol.*, **42**, 669 (1967).
[21] Cleland, R., *Plant Physiol.*, **43**, 865 (1968).
[22] Savada, D., and Chrispeels, M. J., *Science*, **165**, 299 (1969).
[23] Knox, R. B., and Heslop-Harrison, J., *Nature*, **223**, 92 (1969).
[24] Lamport, D. T. A., *Biochemistry*, **8**, 1155 (1969).

Lack of Synergy of EDTA with Antimicrobials in Resistant Enterobacteriaceae

ETHYLENEDIAMINETETRAACETIC acid (EDTA) is known to increase the permeability of Enterobacteriaceae to certain compounds such as actinomycin[1] and to make *Escherichia coli* susceptible to lysozyme lysis[2]. Presumably, this is related to the disruption of the cell wall characterized by loss of lipopolysaccharide[3]. Weiser, Asscher and Wimpenny[4] reported that EDTA increases the sensitivity of certain organisms resistant to penicillin, ampicillin, chloramphenicol and tetracycline presumably by overcoming their permeability barrier to these antimicrobials. Resistance to ampicillin, penicillin and chloramphenicol has been demonstrated to be the result of an inactivation of the drugs by penicillinase[5,6] and chloramphenicol acetyl transferase[7]. The mechanism of resistance to tetracycline is unclear although Franklin[8] suggested a change in permeability in *E. coli* with transferable resistance.

We sought to determine whether EDTA is synergistic with ampicillin, chloramphenicol and tetracycline in strains in which resistance is either chromosomally or episomally determined.

Bacteria were grown overnight in 'Penassay' broth (Difco). Tubes containing serial dilutions of the drug and EDTA were inoculated with approximately 10^5 cells/ml. The tubes were incubated at 37° C for 16 h. The lowest concentration which prevented visible turbidity was considered the minimal inhibitory concentration (MIC). Each experiment included a determination of the MIC of EDTA and drug alone.

Tables 1, 2 and 3 give the MIC of several test organisms with and without EDTA. As can be seen, the maximum increase in sensitivity found with the concentration below the MIC for EDTA was four-fold. This does not represent a synergistic effect of EDTA but can be attributed to an additive effect as well as variation inherent in the method.

Table 1. ABSENCE OF SYNERGY BETWEEN EDTA AND AMPICILLIN

	MIC ampicillin (μg/ml.)				
EDTA (mM)	FW/ R126	*P. mirabilis*	*E. coli* DB100	*S. typhimurium* 100	*A. aerogenes*
0	4,000	16,000	500	2,000	64
4×10^{-2}	—	—	500	—	—
8×10^{-2}	—	—	500	—	—
$1{\cdot}0 \times 10^{-1}$	4,000	8,000	—	—	—
$1{\cdot}6 \times 10^{-1}$	—	—	500	2,000	64
2×10^{-1}	4,000	8,000	—	—	—
$3{\cdot}2 \times 10^{-1}$	—	—	250	1,000	64
4×10^{-1}	2,000	8,000	—	—	—
$6{\cdot}4 \times 10^{-1}$	—	—	—	1,000	64
8×10^{-1}	2,000	8,000	—	—	—
1·25	—	—	—	1,000	32

MIC of EDTA for each organism (mM): FW/R126, 1·6; *P. mirabilis*, 1·6; DB100, 8×10^{-1}; *S. typhimurium* 100, 2·5; *A. aerogenes*, 2·5. Strains FW/R126 and *S. typhimurium* 100 are resistance transfer factor strains.

Table 2

	MIC tetracycline (μg/ml.)			
EDTA (mM)	*E. coli* DB100	*S. typhimurium* 100	*E. coli* K12/R6	*P. mirabilis*
0	400	400	200	80
5×10^{-2}	400	—	—	—
1×10^{-1}	200	—	200	40
2×10^{-1}	100	—	200	40
4×10^{-1}	—	400	—	—
8×10^{-1}	—	400	—	—
1·6	—	200	—	—
3·2	—	100	—	—

MIC of EDTA (mM): DB100, 4×10^{-1}; *S. typhimurium* 100, 6·4; K12/R6, 4×10^{-1}; *P. mirabilis*, 4×10^{-1}.

Table 3

	MIC chloramphenicol (μg/ml.)		
EDTA (mM)	*E. coli* DB100	*E. coli* K12/R6	*E. coli* DB34
0	200	200	400
5×10^{-2}	100	200	400
1×10^{-1}	100	200	200
2×10^{-1}	50	200	200
4×10^{-1}	50	50	100

MIC of EDTA (mM): DB100, 8×10^{-1}; K12/R6, 8×10^{-1}; DB34, 8×10^{-1}. K12/R6 carries a resistance transfer factor.

Further experiments were performed with strains in which resistance was to less than 30 μg and not episomally determined. Both a Tris-based medium and a phosphate medium low in divalent cations were tested. The MIC for ampicillin and tetracycline was not lowered in *E. coli*, *Aerobacter aerogenes* or *Proteus mirabilis* strains by the use of EDTA.

The inability of EDTA to reverse tetracycline resistance of *Salmonella* was demonstrated in the following experiment. A culture of *S. typhimurium* 131 containing an episomal resistance factor for tetracycline was treated with 0·1 mM EDTA in 0·5 M sucrose for 10 min. The bacteria were then returned to 'Penassay' broth, and half of the culture received tetracycline (30 μg/ml.). Growth was followed spectrophotometrically at 660 nm for 2 h. The treated culture was able to grow in the presence of the drug in these conditions at a rate comparable with the control.

EDTA cannot be thought of as synergist with ampicillin, chloramphenicol or tetracycline in these Enterobacteriaceae. It is possible that selected isolates of Enterobacteriaceae contain permeability barriers, and that in these organisms EDTA would be efficacious in altering resistance patterns, but so far we have not found such laboratory or clinical isolates. Furthermore, the fact that the penicillinases of Enterobacteriaceae are surface enzymes[6] would mean that EDTA treatment of cells would make the destructive enzymes more accessible to penicillins. It is extremely doubtful that a clinical situation would arise in which an EDTA-penicillin, ampicillin or tetracycline combination would prove of value in treating infections caused by Enterobacteriaceae.

H. C. N. is a career scientist of the New York Health Research Council. This work was supported by a grant from the US Public Health Service.

HAROLD C. NEU
ELAINE B. WINSHELL

Department of Medicine,
College of Physicians and Surgeons,
Columbia University,
New York, NY 10032.

Received July 21; revised August 27, 1969.

[1] Leive, L., *Proc. US Nat. Acad. Sci.*, **53**, 745 (1965).
[2] Repaske, R., *Biochim. Biophys. Acta*, **30**, 225 (1958).
[3] Leive, L., *Biochem. Biophys. Res. Commun.*, **21**, 290 (1965).
[4] Weiser, R., Asscher, A. W., and Wimpenny, J., *Nature*, **219**, 1365 (1968).
[5] Anderson, E. S., and Datta, N., *Lancet*, i, 405 (1965).
[6] Neu, H. C., *Biochem. Biophys. Res. Commun.*, **32**, 258 (1968).
[7] Shaw, W. V., *Antimicrob. Agents Chemother.*, *1966*, 221 (1967).
[8] Franklin, T. J., *Biochem. J.*, **105**, 371 (1967).

Complete Mutagenesis in a Bacterial Population induced by Thymine Starvation on Solid Media

BACTERIA unable to synthesize thymidylic acid (*thy*⁻) undergo thymineless death if cultivated in a liquid medium deprived of thymine[1,2]. We have found, however, that if *thy*⁻ bacteria are plated on solid nutrient devoid of thymine at a moderate density (not more than 10^5 cells in a Petri dish) thymineless death does not occur. Cells of *Bacillus subtilis* remain viable in these conditions for more than 70 h (Fig. 1); cells of *Escherichia coli* decrease slowly in number: for instance, the cell count of strain *B_3thy*⁻ manifests a drop of 50–60 per cent in the course of 60–70 h. We conclude that the effect is related in some way to the characteristics of growth on a solid surface as distinct from a liquid medium.

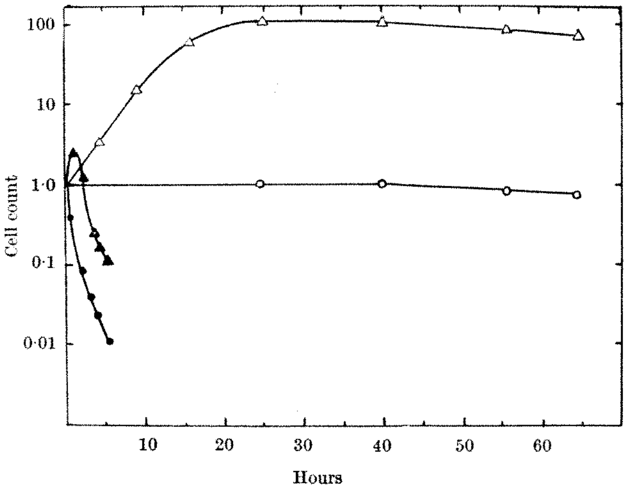


Fig. 1. Survival of *B. subtilis* cells (strain *168thy*⁻) in liquid and solid media at 32° C. ●, Incubation in Spizizen's growth medium I without thymine; ▲, incubation in Spizizen's growth medium I supplemented with 0·3 μg/ml. of thymine. Initial cell concentration, about 10^4 cells/ml.; ○, agar or silica gel solid medium supplemented with SGM without thymine; △, agar or silica gel solid medium supplemented with SGM plus 0·3 μg/ml. of thymine. About 10^5 *thy*⁻ cells were plated on every Petri dish and afterwards washed off with a standard volume (3·0 ml.) of SGM supplemented with an optimal concentration of thymine. Survival was determined by titration of the cell suspension on maximal agar plates.

The number of viable cells was measured by washing them from the plate and plating them subsequently on thymine-containing agar, or by transferring the agar block with the thymine starved cells to another plate supplemented with thymine. The constancy of the number of viable cells during thymine starvation proves that thymineless death is not occurring. If the solid medium is supplemented with a concentration of thymine (0·2–0·3 μg/ml.) which is far too low for the conventional cultivation of thymine deficient bacteria, the cells grow slowly and divide 5–10 times on the surface of agar. The bacterial population thus increases 50–100 times (depending on the density of seed) and thereafter stops growing. We have found that precisely at this time complete mutagenesis in the cell population develops: within 60–80 h it has affected more than 50 per cent of all cells present. During this time, moreover, the percentage of lethal events remains insignificant. Mutations are induced in all regions of the genome. The number of mutants increases in these conditions 10^3–10^4 times compared with the usual level of spontaneous mutations. Many of the cells become multiple mutants in different loci. It is well known that the thymine starvation which develops when thymine deficient bacteria are suspended in a liquid medium and are subject to thymineless death leads to a small mutational effect[3-6]. That the origin of the mutagenesis which we have observed is different is clear because it does not occur if thymine is entirely absent.

Two strains of *B. subtilis* were used. One (*168thy*⁻*ind*⁻) was obtained from F. Rothman (compare ref. 2). This strain originated from the well known Marburg strain and was deficient in thymine and indole. The second was obtained from *168thy*⁻*ind*⁻ by means of transformation to prototrophy for indole and marked as *168thy*⁻. Two well known strains of *E. coli* were used. One was *B_3thy*⁻, the other *15T*⁻. For normal growth these bacteria require 5 μg/ml. of thymine in the medium. Spizizen's growth medium I was used for cultivation[7]. An overnight culture was washed from the thymine containing medium with Spizizen's glucose-mineral medium (SGM) on a 'Millipore' filter, resuspended and plated on a series of Petri dishes at about 10^5 cells per plate. The agar contained SGM supplemented with a low concentration of thymine (0·3 μg/ml.). The Petri dishes were stored at 32° C for 3 days and some were taken at 10–15 h intervals for investigation. The cells were sampled by washing them off with 3 ml. of SGM containing an optimal (5·0 μg/ml.) amount of thymine. The cell suspension was titrated on maximal agar plates. Scoring for mutants was performed by transferring the colonies grown on maximal agar to plates with SGM supplemented with 20·0 μg/ml. of thymine. From the second day of incubation, when the growth of the culture stops, a massive accumulation of mutants was observed. These are wholly or partially auxotrophic, with different requirements for nutrient supplements. Either they are unable to grow on minimal

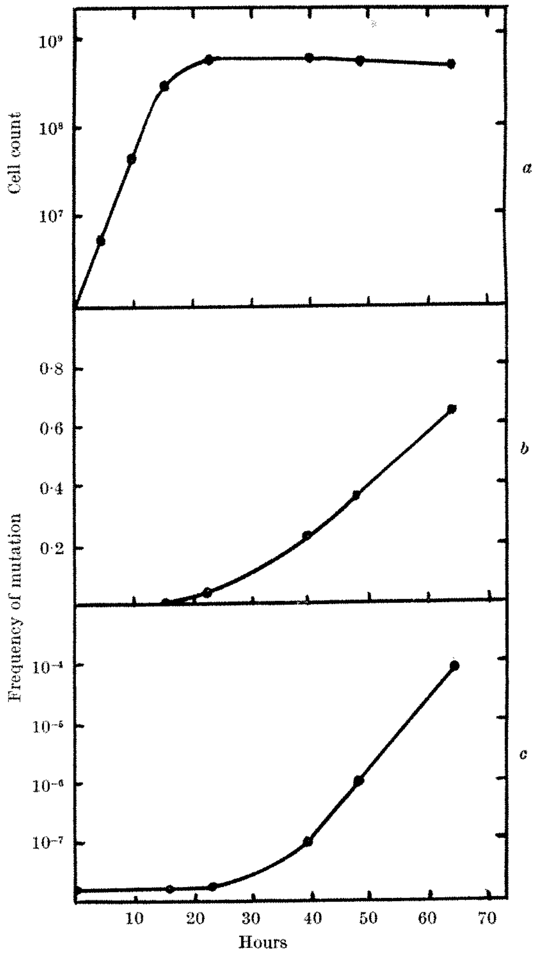
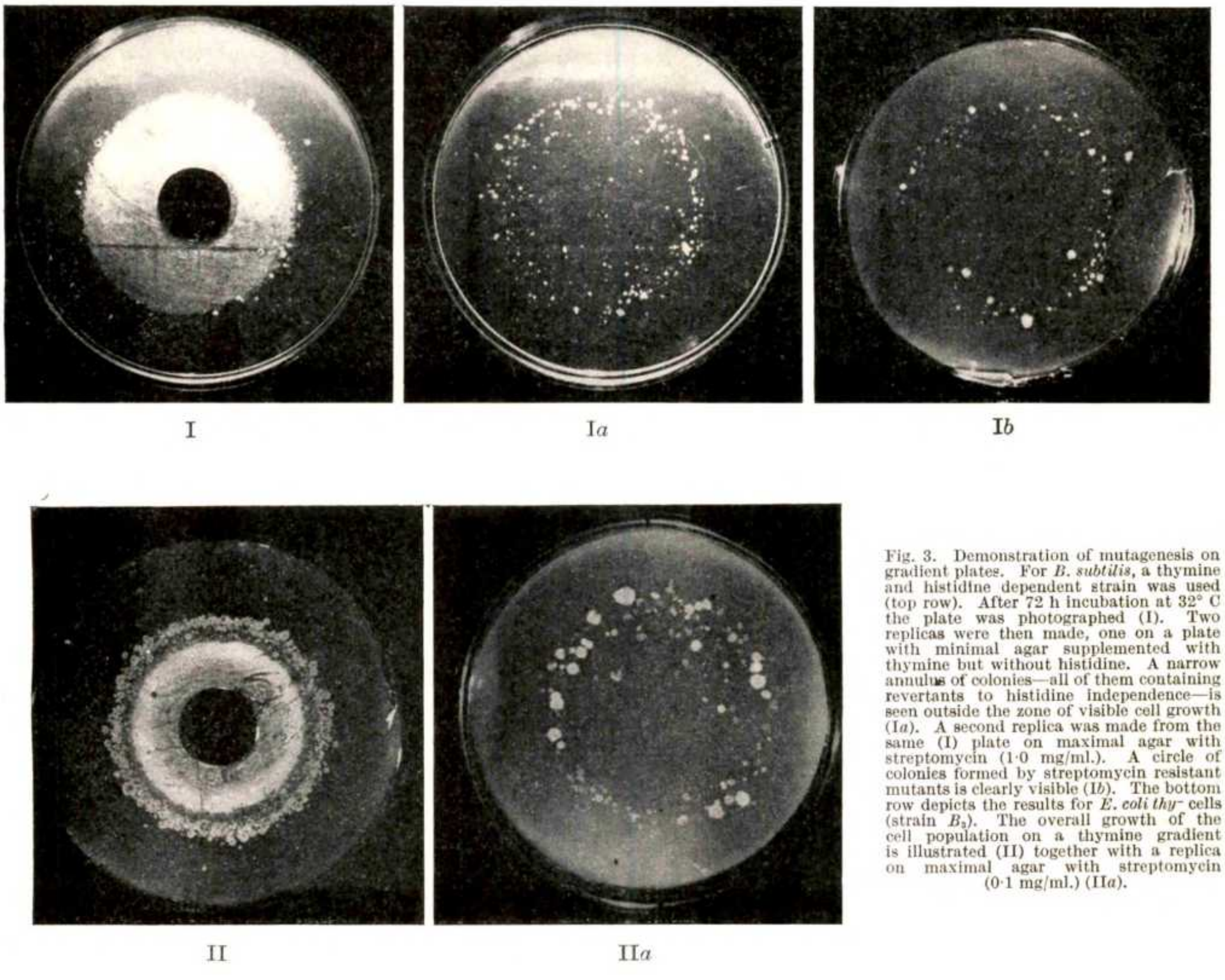


Fig. 2. Kinetics of overall mutagenesis and of the induction of specific (*Sm*ʳ) mutants in *B. subtilis* (strain *168thy*⁻) during incubation at 32° C on solid medium containing 0·3 μg/ml. of thymine. *a*, Kinetics of growth of the cell population on Spizizen plates with 0·3 g/ml. of thymine; *b*, overall yield of auxotrophic mutations; *c*, kinetics of induction of *Sm*ʳ mutants (resistant to 1·0 mg/ml. of streptomycin).

agar or their growth rate is much lower than that of normal cells. The number of mutants increased gradually with time and finally attained the level of 50–70 per cent of all cells present. The mutants induced in these conditions contained genetic lesions in different cistrons coding for the synthesis of amino-acids, nucleotides and vitamins, for drug resistance and so on.


I Ia Ib

II IIa

Fig. 3. Demonstration of mutagenesis on gradient plates. For *B. subtilis*, a thymine and histidine dependent strain was used (top row). After 72 h incubation at 32° C the plate was photographed (I). Two replicas were then made, one on a plate with minimal agar supplemented with thymine but without histidine. A narrow annulus of colonies—all of them containing revertants to histidine independence—is seen outside the zone of visible cell growth (I*a*). A second replica was made from the same (I) plate on maximal agar with streptomycin (1·0 mg/ml.). A circle of colonies formed by streptomycin resistant mutants is clearly visible (I*b*). The bottom row depicts the results for *E. coli thy*⁻ cells (strain B_3). The overall growth of the cell population on a thymine gradient is illustrated (II) together with a replica on maximal agar with streptomycin (0·1 mg/ml.) (II*a*).

We performed controls to be sure that we were dealing with real mutations. The cells from a mutant colony were cultivated alternately in liquid maximal medium and on maximal agar. This procedure was repeated three or four times. No change of mutant phenotype resulted. In 14 cases the mutational locus was identified by systematic trial of nutrient requirements. The particular mutations obtained in the case of *B. subtilis* $168thy^-$ were: dependence on histidine, arginine, biotin, nicotinamide, uracil, and a double mutant for serine and threonine and streptomycin resistance. For the strain $168thy^-ind^-$ they were: reversions from ind^- to ind^+. In the case of *E. coli*, the mutants identified were a riboflavine constitutive strain derived from 15*T*-strain, and lac^- and Sm^r derived from the B_3thy^- strain.

Fig. 2 depicts the kinetics of the increase in the overall auxotrophic mutations in a *B. subtilis* population; we plot the sum of full and partial auxotrophs. The latter are leaky mutants growing on minimal medium supplemented with 20·0 μg/ml. of thymine at a reduced rate. We emphasize that a substantial number of mutants, varying from experiment to experiment, was found in mixed clones because of segregation. To take them into account a slight modification of the experimental procedure was adopted. The colonies originally identified as non-mutated were taken from maximal agar, suspended in SGM solution, and replated on maximal agar (100–200 cells per Petri dish). Then, however, we replicated them on minimal agar to score for mutants. This improvement allowed us to detect all the auxotrophic mutants formed even if they were initially in mixed clones.

We studied, kinetically, not only the overall mutation rate but also certain special cases of identified mutations. Fig. 2 shows the kinetics of induction of streptomycin resistance in the conditions of our experiment. We see that the rate of induction of Sm^r mutants in *B. subtilis* amounts to 10^{-4}. In the case of reversions from indole deficiency to independence in the strain $168thy^-ind^-$ of *B. subtilis* the mutation rate is about 10^3 times higher than the background of spontaneous revertants which is found when an amount of thymine optimal for growth is supplied.

Of particular importance is the choice of a thymine concentration optimal for mutagenesis. The existence of such an optimum is demonstrated in the following experiment. A radial gradient of thymine concentration is generated in an agar plate initially devoid of thymine but with a round hole filled with 0·2 ml. of thymine solution (0·2 mg/ml.). Fig. 3 presents photographs of such plates on which 10^5 thymine deficient cells were spread uniformly. The mutants become concentrated in a narrow annulus in a distinct region of the plate, outside the zone of overall growth. Zero concentration of thymine, as already stated, is unfavourable for mutagenesis; some intermediate concentration is the most suitable.

We believe that the unusual phenomenon of complete mutagenesis is the result of replication mistakes induced by the slow replication of DNA or by an imbalance of DNA precursors. Observations on the origin of thymineless death and its dependence on growth conditions will be published elsewhere.

S. BRESLER
M. MOSEVITSKY
L. VYACHESLAVOV

Institute of High Molecular Weight Compounds,
2d Birjevoy, 6,
Leningrad.

Received July 14; revised October 9, 1969.

[1] Cohen, S. S., and Barner, H. D., *Proc. US Nat. Acad. Sci.*, **40**, 885 (1954).
[2] Farmer, J. L., and Rothman, F., *J. Bacteriol.*, **89**, 262 (1965).
[3] Coughlin, C. A., and Adelberg, E. A., *Nature*, **118**, 529 (1956).
[4] Weinberg, R., and Latham, A. B., *J. Bacteriol.*, **72**, 570 (1956).
[5] Kanazir, D., *Biochim. Biophys. Acta*, **30**, 20 (1958).
[6] Holmes, A. J., and Eisenstark, A., *Mut. Res.*, **5**, 15 (1968).
[7] Spizizen, J., *Fed. Proc.*, **18**, 957 (1959).

Population Growth and the Multi-type Galton–Watson Process

THE general Galton–Watson (GW) process with n types, T_i ($i = 1, 2 \ldots n$), is specified[1] by the type of the individual starting the process at time (generation) zero, and the vector-valued probability generating function (PGF)

$$f(\mathbf{s}) = [\mathrm{f}^1(\mathbf{s}), \mathrm{f}^2(\mathbf{s}) \ldots \mathrm{f}^n(\mathbf{s})]$$

where the scalar quantity $\mathrm{f}^i(\mathbf{s})$ is the multi-type PGF of the numbers of individuals of the various types produced by an individual of type T_i ($i = 1 \ldots n$).

I first consider a special case of the general process in which

$$\mathrm{f}^i(\mathbf{s}) = \sum_{j=1}^{n} p_{ij}\mathrm{f}(j;\ s_j),\ i = 1 \ldots n$$

where

$$p_{ij} \geq 0,\ \sum_j p_{ij} = 1,\ i, j = 1 \ldots n$$

$\mathrm{f}(j;\ s)$ being for each j a univariate PGF. The process so specified may be given the following general interpretation. Suppose that there is basically only one type of individual present in a population, but that the offspring distribution is not fixed once and for all, and may be any one of the n generated by the $\mathrm{f}(j;\ s)$, $j = 1 \ldots n$. Then, at each generation succeeding the initial ancestor, the population may be thought of as consisting of n types $T_1 \ldots T_n$, an individual being of type T_r if he arose as a result of reproduction according to the PGF $\mathrm{f}(r;\ s)$. He then chooses the distribution generated by $\mathrm{f}(j;\ s)$ for reproduction with probability p_{rj}, $j = 1 \ldots n$. The specialized GW process that I have presented then describes the evolution of such a population (with the usual "independence of reproduction" assumptions).

One biological implication is as follows: if the population under consideration consists of males only, the choice of offspring distribution may be interpreted as corresponding to the choice of type of wife out of n possible types, this choice being influenced via the Markovian probabilities $\{p_{ij}\}$ by the type of the male individual's mother. In a genetical context, the model may be used to describe the evolutionary progress of mutants whose fitness fluctuates in a stochastic manner, according to a finite Markov chain with transition matrix $P = \{p_{ij}\}$, along any line of direct descent (. . . grandfather, father, son . . .). If the Markov matrix P is stochastically degenerate in that it describes purely deterministic movement (for example, purely cyclic), all individuals at a particular time are of a single type only, and reproduce in the same manner, though the manner changes through time in a deterministic way; one may then interpret the process as describing the effect of a deterministically fluctuating environment, special cases of which have been studied by Koutský[2], Pollak[3] and Ewens[4]. (A stochastically fluctuating environment is not amenable to treatment by the technique, though an appropriate theory exists.)

Going on to another model, I now consider the n types of the general multi-type GW process as falling into two classes, the K-types and the R-types, so that the types can be written as $K_1, K_2 \ldots K_p$, $R_1, R_2 \ldots R_q$, where $p + q = n$. The special case of the process in which I am interested arises from the following assumptions: the process is initiated by a K-type, and is otherwise specified by the transition laws

Prob [1 individual of type R_j produces a vector $\mathbf{i}$ of R-types] = $\mathrm{f}_j(\mathbf{i})$

Prob [1 individual of type K_h produces a vector $\mathbf{e}_r$ of K-types and a vector $\mathbf{i}$ of R-types] = $p_{hr}\mathrm{b}_h(\mathbf{i})$

all other reproductive transitions having zero probability. Here $\mathbf{e}_r$ is a vector with p entries, all of which are zero save the rth, which is unity, and $\{\mathrm{f}_j(\mathbf{i})\}$ for each $j = 1 \ldots q$ denotes a multi-type probability distribution on the non-negative integer q-tuples $\mathbf{i}$, as does $\{\mathrm{b}_h(\mathbf{i})\}$ for each $h = 1 \ldots p$, the set of quantities p_{hr} defining a stochastic matrix, P, of dimension p.

This $(p+q)$-type process may be interpreted in terms of a population of q types, evolving according to GW reproduction rules, and whose offspring distributions are given by the $\{\mathrm{f}_j(\mathbf{i})\}$, the population at each time after the zeroth (when there are assumed to be no individuals) being subject to immigration according to one of the p immigration distributions $\{\mathrm{b}_h(\mathbf{i})\}$, fixed at time one, and the immigration then fluctuating from one distribution to another at successive times according to a Markov chain with transition matrix P. The progress of such a population is the progress of the R-types alone in the $(p+q)$-type GW process. (The introduction of the "fictitious" K-types in a simpler context to give the effect of immigration is due to Sevastyanov[5].)

Both the models described may thus be further investigated by using known results on the multi-type GW process. The second (immigration) model always gives rise to a reducible process, but may still be treated in the manner of Kesten and Stigum[6]. It also seems possible to consider to some extent the effect of an infinite matrix P in both cases.

I acknowledge support by a Nuffield Foundation travelling fellowship during my absence from the Australian National University.

E. SENETA*

Statistical Laboratory,
16 Mill Lane,
Cambridge.

Received October 22, 1969.

* Present address: Department of Mathematics, Imperial College, London SW7.

[1] Harris, T. E., *The Theory of Branching Processes* (Springer, Berlin, 1963).
[2] Koutský, Z., *Trans. Third Prague Conf. Inf. Th., Stat. Dec. Fns., Random Proc.*, 429 (1964).
[3] Pollak, E., *Biometrika*, **53**, 391 (1966).
[4] Ewens, W. J., *Population Genetics* (Methuen, London, 1969).
[5] Sevastyanov, B. A., *Teor. Veroyat. Primenen.*, **2**, 339 (1957).
[6] Kesten, H., and Stigum, B. P., *J. Math. Anal. Appl.*, **17**, 309 (1967).

Book Reviews

NATURE OF METAPHYSICS

Metaphysics and Common Sense

By A. J. Ayer. Pp. xi + 267. (Macmillan: London, November 1969.) 58*s*.

IT is disappointing to discover that this is not a new book by Ayer on metaphysics. It is, in fact, a substantial collection of his essays, from various sources, on a variety of topics. The nature of metaphysics is a recurring theme, however, and the essay which, like the book, is called "Metaphysics and Common Sense" is an extremely interesting discussion of it. The essay shows common sense, but the "Common Sense" of the title is G. E. Moore's. Moore's answer to those who sought to establish the unreality of time was that its reality was far more obvious to him—perhaps in such facts as that some of the events of his life lay in the past—than any premises and inferences which might be used to disprove it. Ayer is sympathetic to Moore's attitude, but rejects it because it implies that philosophy, being forced always to defer to common sense, is confined to the analysis and elucidation of opinions. Philosophical theories do not, Ayer argues, conflict with common sense in the direct way Moore assumed. They conflict only with the theories in which common sense views are embedded and against these theories they may prevail. Ayer makes generous amends to metaphysicians for his strictures in *Language, Truth and Logic*. Speaking of the paradoxical conclusions of Zeno about motion and McTaggart about time, he says: "But the problem for our metaphysicians was that they did not see how these consequences were to be avoided; they did not see what other account of time or motion could be given. And in this they were not so greatly to blame". The problem of the nature of philosophy arises again in the essay, "What Must There Be ?". Ayer maintains, characteristically, that such questions as "Do electrons exist ?" raise no one problem, but may give expression to a variety of problems about the nature of electrons. His approach here is an eminently successful one.

Ayer's great interest in the philosophy of science is evident in many of the essays, although most of them are general expositions rather than original contributions. In "Man as a Subject for Science" the reader longs for him to slow down and dwell for a while on one of the many problems he raises. The essay called "Chance" is marred, in my opinion, by a failure to distinguish two contentions about the calculus of probabilities: one that its propositions are true *a priori*, the other that they concern probabilities which hold *a priori*. It is possible that the propositions of the calculus of probabilities are *a priori* because they concern relations of implication between probability propositions which are not *a priori*.

Among several critical essays on other philosophers, including Russell, Moore and the existentialists, an especial interest attaches to his assessment of J. L. Austin's book, *Sense and Sensibilia*. For many years Austin gave, both at Oxford and in the United States, a course of lectures on perception in which he brought his very considerable powers of wit and irony to bear on phenomenalism and particularly on Ayer's presentation of it. Ayer had little opportunity to reply, because the lectures were not published until after Austin's death. Whether this essay vindicates sense-data is a question beyond the scope of this review. Philosophers should not too readily assume that the task is impossible.

JOHN WATLING

WASTE WATER PROBLEMS

Urban Planning Aspects of Water Pollution Control

By Sigurd Grava. Pp. xi + 223. (Columbia University Press: New York and London, November 1969.) **$7.50**; 67*s* 6*d*.

ONE can hardly pick up a newspaper nowadays without finding an article dealing with some aspect of pollution of the environment, for, as the blurb to this book says, the 1960s have seen "a general public awareness" of urban pollution and "the popular press has joined the battle". We can therefore expect that many more authors will be producing books such as this, which, again quoting the blurb, "is intended as a guide and a source of information for urban planners, community decision makers and active citizens".

The book is sponsored by the Institute of Urban Environment at Columbia University, at which the author is assistant professor of urban planning. It is essentially non-technical but contains some technical material. The principal sections of the book deal with the general nature of the problem, with the technology of waste water treatment, with legal and financial implications, with local planning aspects and, finally, with special problems of the developing countries.

The lengthy chapter on technological aspects is a readable and generally correct account of the present situation in regard to sewerage, sewage treatment and reclamation of water from sewage effluent. The only major error, repeated in the glossary, is the reference to the Zimmerman process (now promoted as wet-air oxidation) as a method of sewage treatment—it is, in fact, a method of sludge treatment. Some of the definitions in the glossary itself are not particularly helpful, for example: "Ion exchange: the separation of materials in solution by electrical charge". The chapter on sources and types of pollutants contains an adequate description of the nature and amounts of domestic sewage discharged by a community, but is much less adequate in discussing the problems of industrial wastes. It also contains the following incredible statement: " . . . any value of BOD above zero indicates pollution, i.e. that state where no free dissolved oxygen is found in the water".

The chapters on administrative, legal and financial aspects refer almost exclusively to the situation in the United States. The difficulties in achieving pollution control by cooperation between municipal authorities are pointed out, as is the desirability of extending control over a wider region—preferably a watershed.

It would be presumptuous to assert that the Old World has all the answers to the pollutional problems of the New; nevertheless, pollution of surface waters became a problem in Britain and in some parts of Europe at least as early as the appearance of similar problems in the more heavily industrialized areas of the United States. Presumably, the author, in proposing such measures as river basin management, regional control, and charging for reception of industrial effluents into municipal sewers, has had regard to the relevant experience of other countries in pollution control. The only reference to European experience, however, is a brief description of the operation of the Ruhrverband, the highly successful water supply and effluent disposal organization in Western Germany.

This book will no doubt serve a useful purpose in its country of origin, but its value to the non-American reader is less certain. Most of the technical matter contained within it is available in Britain in a number of specialized publications, while those parts of the book dealing with administration and finance would seem to have value chiefly to the student of American affairs. The last chapter on problems in developing countries has some sensible things to say and is apparently based on the author's own experience as a planning consultant.

One must agree with the author that the major questions nowadays are concerned not with the technical solutions to pollution problems—these are in most cases available—but with the amount which the community is prepared to pay to maintain a clean environment. There will be much discussion of such problems in Britain, but one hopes that the "decision makers and active citizens" involved will be able to supplement the material contained within this book by drawing on the ever-increasing volume of information now available from other sources.

G. E. EDEN

SERMON FOR THE UNCONVERTED

The Last of Lands

Edited by L. J. Webb, D. Whitelock and J. Le Gay Brereton. Pp. xvi + 203. (Jacaranda Press: Milton, Queensland, 1969.) $A6.95.

To the biologist, at least to the biologist who lifts his eyes above the laboratory bench, Australia is the most exciting of all continents. Admittedly, as the late Jock Marshall so graphically described in *The Great Extermination*, its wildlife has been raped as thoroughly as North America's in the nineteenth century and India's in the twentieth, but what remains is an assemblage at least as worth preserving as any other fauna in the world. Living fossils are thicker on the ground than almost anywhere else, and adaptive radiation is nowhere better illustrated than by the way the marsupials have exploded to fill so many of the niches occupied elsewhere by the carnivores, ungulates and other orders—could perhaps a marsupial primate have evolved if Captain Cook had stayed away for a few more thousand years ?

One might expect therefore that the Australian in the street would be prouder than he is of the natural endowment of his native land. But all too many Australians—and they are not alone in this—think of wildlife only at the end of a gun, so that the tiny handful of Australian naturalists have until very recently been very much a voice crying in the Great Sandy Desert. This book is part of the awakening of the Australian people to the realization that they and their ancestors have very nearly destroyed a pearl of great price, and, what is more, one that might even make them some money, if wildlife tourism is skilfully developed.

Stemming from a symposium held at the University of New England in 1964, the editors have produced a handsomely illustrated volume, with twenty-two contributions from twenty-six authors and the Australian Conservation Foundation, which incidentally benefits from the royalties. Such a composite work inevitably lacks the fire in its belly it would have had if Jock Marshall had survived to write even a couple of pages for it, but it is a sound and sober assessment of the problems of conservation in Australia: what has been done already and what needs to be done. Australia has a fine complement of national parks and nature reserves—New South Wales had a national park as early as 1870, eighty years before the United Kingdom—but so fast is the pace of development in this century that immensely more remains to be done. The manifold threats to the Great Barrier Reef, not least the natural one constituted by the decimation of the living coral by the crown of thorns, are a good example.

Prince Philip puts the dilemma clearly in his foreword: "Even today there are many Australians who find it hard to believe that the problem of conservation exists at all. Unfortunately these people are not likely to read this book". It was an Irish naturalist who pointed out recently that wildlife conservationists spend far too much time preaching to the converted in the comfort of the lecture hall and not nearly enough bellowing to the unconverted at street corners. If there is a criticism of this book, it is that it is one more sermon directed to the devout. Will somebody else now please take a deep breath, remember Jock Marshall and use the facts in this book to make a noise that all the unconverted will be bound to hear.

RICHARD FITTER

DESERT ENCYCLOPAEDIA

Deserts of the World

An Appraisal of Research into their Physical and Biological Environments. Edited by William G. McGinnies, Bram J. Goldman and Patricia Paylore. Pp. xxviii + 788. (University of Arizona Press: Tucson, 1968.) $15.

THIS encyclopaedic compendium, edited and written by faculty members of the University of Arizona, is a United States contribution to the International Hydrological Decade. Intended for those "seriously interested in planning, managing and executing research or development efforts in the arid parts of the world", it contains evaluative comments on the work that has been accomplished in these areas, summaries of what is known about them and recommendations for further research. The contents include: location maps of extremely arid, arid and semiarid regions by continents (after Meigs); appraisals of research on the weather and climate of desert environments by Clayton M. Reitan and Christine R. Green; geomorphology and surface hydrology by Lawrence K. Lustig; surface materials by Harold E. Dregne; vegetation by William G. McGinnies; fauna by Charles H. Lowe; desert coastal zones by Joseph F. Scheiber, jun.; and an appendix on ground-water hydrology by Eugene S. Simpson.

Each chapter is organized in the same way: a discussion of existing knowledge, followed by evaluations, recommendations and comprehensive bibliographies. The approach throughout is anthropocentric. For example, plants are assessed according to their qualities and uses by man, and whether they are undesirable and should be controlled. The fauna is classified as poisonous (potentially lethal)—"amphibians are considered to be potentially dangerous and they should be skinned before eating"—or suitable for use as human food, and so on. Pests, "unwanted animals", are divided into poisonous forms—centipedes, millipedes, tarantulas, urticating moths and vesicant beetles—and non-poisonous pests which include Reduviidae, sand-flies, mosquitoes, ticks, Solifugae, woodlice, ants, termites, locusts and other insects. In spite of this unbiological and somewhat unpromising emphasis, no doubt dictated by editorial policy, the various chapters contain a large amount of useful information. Nevertheless, each author decries the lack of basic knowledge in his own and related subjects; the geomorphologist of climatic data, the botanist of information on soils, the climatologist of satisfactory systems for determining local requirements of water. At the present rate of progress, however, it may take centuries before the information already available is properly applied.

The solutions advocated for disseminating further knowledge are not unfamiliar. They emphasize the importance of international programmes, electronic bibliographical control of translation services which are now scattered throughout the world, and so on. All this is highly commendable and could sometimes be very useful although it is possibly somewhat over-rated: there can be no substitute for hard and prolonged research work in the field. The deserts of the world are largely man-made. Particularly in developing countries, they are rapidly being extended through overgrazing, the felling of trees for firewood and charcoal, and by bad agricultural practices. Progress in the development or reclamation of deserts cannot be achieved without local governmental support, a genuine understanding of the inhabitants, their traditions and religions, and a real appreciation of the actual problems of their existence.

This volume will probably be of greatest use to research workers on account of its excellent summaries of existing knowledge and extensive bibliographies, rather than for its recommendations and perhaps unrealistic ideals.

J. L. Cloudsley-Thompson

GLOBAL CLIMATOLOGY

Climate of the Free Atmosphere

Edited by D. F. Rex. (World Survey of Climatology, Vol. 4.) Pp. x+450. (Elsevier: Amsterdam, London and New York, 1969.) 335*s*.

The free atmosphere comprises the whole of the atmosphere apart from the lowest layer, about 700 to 900 m deep, known as the friction layer, within which turbulence induced by the passage of the air over the Earth's surface is confined. In the words of the editor of this book, the reason for eliminating the friction layer from consideration is so that "we may deal with the atmosphere on a global basis without resorting to extensive discussions of point to point surface differences. Such pronounced regional and/or local features as may produce climatological anomalies in the free atmosphere will be discussed in the appropriate regional volumes of the series". In fact, some of the contributors to the seven sections of the book find this restriction inconvenient and deal with the atmosphere from the surface. Also, because routine climatological measurements have been directly made only up to a height of 50–60 km, the data presented in the multifarious tables and graphs refer only to the troposphere and stratosphere.

The book begins with an account and tabulation of recent standard and supplemental atmospheres. The succeeding four chapters deal with the characteristics of the troposphere and its upper boundary, the tropopause. In the first of these, median tropopause heights, average temperature profiles, meridional temperature cross-sections and global distributions of temperature and humidity are given for the different months of the year or for the seasons. The next, the longest chapter in the book, provides an excellent account, largely theoretical, of the dynamics of the tropospheric circulation including a detailed analysis of the structure and generation of the jet streams as well as shorter descriptions of tropical cyclones, monsoons and föhn and katabatic winds and concluding with an examination of the climatological consequences of the mean tropospheric flow. There follow a discussion of the major tropical and extratropical cloud systems, examined in the light of the synoptic pictures provided by meteorological satellites, and a further chapter presenting seasonal distributions of cloudiness, albedo and outgoing long wave radiation, also derived from satellites. Although it has not yet been possible to obtain absolute values of the radiation budget from TIROS data because of difficulties of calibration and the degradation of the radiation sensors, it is useful to have these preliminary findings.

Conditions in the stratosphere in so far as they are known are examined in the last two chapters. It was only with the development and use of meteorological rockets that reasonably accurate observations of temperature and wind in the stratosphere became available, and a descriptive account is given here of the data obtained by the American Meteorological Rocket Network during the early years of its operation from about 1960. The Stratospheric Circulation Index is defined as the mean zonal and meridional wind speed in a 10 km thick layer centred at an altitude of 50 km and from it is derived a set of stratospheric seasonal definitions. Seasonal mean wind (zonal and meridional) and temperature profiles are drawn for each of the rocket-sonde launching sites. Finally, there is a comprehensive account of the theory, measurement and significance of atmospheric ozone, beginning with the photochemical theory and then comparing theoretical ozone distributions with those observed. Below a height of about 25 km, photochemical reactions are so slow that the ozone concentration is largely determined by the redistribution of the gas by air currents. Thus in the lower stratosphere, the ozone–air mixing ratio is a quasi-conservative property of an air parcel and so ozone can be used as a tracer in studies of the circulation. It is pointed out that the three dimensional distribution of ozone and its variation with time are now sufficiently well known to make it possible to examine ozone flux in the atmosphere and the resulting ozone budget.

Even allowing for the cost of reproduction of more than 300 graphs, maps and line diagrams and nearly 60 tables, and for the fine book production, the price of this volume seems excessive.

James Paton

SIMPLE MAP MAKING

Simple Photogrammetry

Plan-Making from Small-Camera Photographs taken in the Air, on the Ground or Under Water. By J. C. C. Williams. Pp. x+211. (Academic Press: London and New York, December 1969.) 50*s*; $8·50.

Photogrammetry is the science and art of deriving the dimensions of objects by measuring the size and shape of their photographic images. The precision with which this can be done is satisfactory for many mapping, engineering and scientific projects. Much of the equipment that is used to achieve this precision is expensive although the overall cost of survey by photogrammetric means is often less than would otherwise be incurred. Where sophisticated equipment is not available it is possible to use simple graphical methods to construct plans of the areas or objects photographed. The accuracy of this work is inferior to that which can be achieved with better equipment but, none the less, it may be satisfactory for certain rudimentary surveys. This book deals with the simple graphical methods that can be used to derive the dimensions and relative positions of objects and areas using small-camera photographs taken in the air, on the ground or under water. One chapter introduces elementary astronomy and the uses of the Sun's shadows for measuring heights and directions. Many of the diagrams are intended as working drawings so that the processes discussed in the text can be followed by taking measurements on illustrations. Theoretically this is an asset but it makes for tiring reading. A good diagram should be self-explanatory but much of the text is given to describing the derivation of the illustrations.

The versatility of photogrammetry and its adaptability to a large range of problems are very considerable. The student should, however, bear in mind that the techniques advocated are often not the most appropriate. A false sense of accuracy is implied in much of the text and one must remember that graphical methods require good draughtsmanship and much patience. One must dispute the claim that the practical work discussed forms a good introduction to the study of map making by stereophotogrammetry—this book will not help the serious student of the subject. It is, however, a valuable reminder of what can be done with simple equipment, and those with sufficient patience to stick with this book will not be unrewarded.

P. F. Dale

MAGNIFICENT DESOLATION

The Times Atlas of the Moon

Edited by H. A. G. Lewis. Pp. xxxvii + 110. (Times Newspapers Limited: London, December 1969.) 95*s*.

For your money you get thirty pages of articles on the Moon, on mapping the Moon and the techniques of getting there, and of course the maps—110 pages of them, based on the charts produced by the US Air Force Aeronautical Chart and Information Centre. (Strangely, not the most complete set. Several tantalizingly blank areas of *luna incognita* appear on the far side maps.) The charts are, of course, a tribute to painstaking work in the United States beginning with mapping from photographs taken at the Pic du Midi, Yerkes, Lick and Mt Wilson observatories, and continuing with the Lunar Orbiter missions and the Soviet investigation of the far side, chiefly with Zond 3. Most people would rather the Orbiters had spent less time scanning the equatorial maria for allotment-sized crater-free areas and more time looking at more fascinating parts of the Moon, but that was the demand of the Apollo project. Without it there would have been none of the later Orbiters in polar orbits which made the photographic coverage of the Moon better than that of the Earth, providing the new breed of lunar geologists with enough material for a lifetime.

The 110 maps, then, which cover the best part of the front of the Moon, are at a scale of about 20 miles to the inch. The excepted parts are the polar regions beyond about 65° of latitude, and the limbs beyond 70° from the zero meridian (less at higher latitudes). The only coverage of these awkward areas is on the front and back endpapers, which are small scale maps of the northern and southern hemispheres. Then the far side, rather oddly, is elsewhere in the atlas, buried between a general article on the Moon and one on mapping. The area between 48° N and 48° S is given about 17 inches by 9·5 inches, with two separate projections for the polar regions. They are without the contribution from Orbiter 5, however, and hence the white patches. The accompanying text is a fair enough résumé of what is known about the Moon, although in places one feels that the stress might have been misplaced, and it is not without its errors. It seems to have been written at the time when the first news about the Apollo 11 specimens was leaking out of Houston, and enshrines the view that titanium is common in the maria.

It seems to me that the reproduction of the otherwise excellent illustrations in the text is not as good as it might have been. Green has been used lavishly. Many of the Apollo 8 pictures came out green of course, but in the atlas they look positively verdant, including one which I remember as blue when it first appeared. Somebody seems to have been inspired even to give the Orbiter photograph of the Alpine Valley a green caste—surely stunning enough in its original black and white. The Apollo 8 view of Goclenius gets the same treatment, and Copernicus from Orbiter 2 is battleship blue. One would like to have seen more use of the incredibly detailed Orbiter photographs, but not this way.

People will enjoy using the atlas, more I suspect for seeing how the published photographs from lunar flights fit in than for following the journeyings of the astronauts on the surface, which for the time being at least are going to be on too small a scale to register on these maps. It ought to be bought by libraries, but one wonders whether the non-astronomer, the extremely devoted excepted, would get enough out of it to make the outlay worthwile.

Edward Phillips

GROUNDING IN STATISTICS

Statistics and Experimental Design

By Geoffrey M. Clarke. (Contemporary Biology.) Pp. xi + 161. (Arnold: London, November 1969.) 42*s* boards; 21*s* paper.

This book is largely intended for undergraduate students of biology as basic guidance in the applications of elementary statistics to practical problems in their subject. Mathematical manipulation is therefore almost entirely absent, although there are clear explanations of the practical meaning of any statistical procedure discussed and worked examples are used to illustrate not merely the use of the procedure but also its appropriateness and limitations in different situations. The examples, and in one or two respects the subject matter, are conditioned by biological applications—reference to sample surveys and non-parametric tests is rather limited; however, as the author says, the book should also prove useful to non-biologists. Non-mathematicians will be helped by the author's consistent use of notation as listed at the beginning of the book.

Statistics as the common sense summarization of numerical data is developed from simple ideas of random variation, discrete and continuous variates and frequency-probability. The basic distributions (binomial, poisson and normal) are discussed with reference to practical examples, and the importance of the central limiting function of the normal distribution is emphasized. Significance tests and confidence theory involving the normal, t, χ^2 and F distributions are dealt with, but there is no graph of an F distribution and the expected value (not elsewhere defined) of F is wrongly stated to be 1 (page 67). There is also no mention of paired comparisons, and for Fisher's z-test of correlation the reader is referred elsewhere. There are good introductions to regression analysis (based on $y=a+bx$), and experimental design: there are careful discussions of the planning and analysis of completely randomized, randomized block and Latin square designs, together with simple factorial experiments. Unusually, interactions are introduced in factorial experiments rather than as a refinement of additive analysis of variance models. The author considers the problems of missing observations and non-normality and he describes the non-parametric sign and U-tests—but he gives no rank test of independence. Finally, the inclusion of comments and answers to exercises should help many students.

The book is usually clear and well written and may be strongly recommended to non-mathematical students requiring a common sense grounding in statistical methods.

K. L. Q. Read

VARIABLE RELATIONSHIPS

Models in Regression and Related Topics

By Peter Sprent. (Methuen's Monographs on Applied Probability and Statistics.) Pp. x + 173. (Methuen: London, October 1969.) 35*s*.

The subject of regression has changed a lot since Galton used the term to describe the phenomenon whereby the heights of adult males in successive generations regress toward the population average; tall fathers having, on the average, sons who are shorter than themselves. Nowadays, it is the method of describing asymmetrically the relation between one variable and one or more other variables, through the mean value of the first, conditional on specified values of the others. The most common form for this relationship is a linear one where, if y is the single variable and $x_1, x_2, \ldots x_n$ values of the others, it is supposed that the mean $E(y|x_1, x_2, \ldots x_n)$ is linear, say $\beta_0+\Sigma\beta_1x_1$. There is hardly a computer whose software does not include a program for performing linear regression, estimating the β's, carrying out significance tests and deciding which of the x's have an important influence on y. The procedure is undoubtedly popular because there are many research problems that call for an understanding of how one quantity is influenced by others, and the machine technique is easily applied to yield apparently meaningful results.

Unfortunately, the field of regression is not quite so simple as it often seems to the research worker. There are subtleties involving variates and variables, structural and functional relationships, and confusions between the joint and separate effects of factors. Dr Sprent has therefore written a monograph designed to give guidance in regression techniques to scientists. Topics include least-squares, law-like relationships, the effects of inhomogeneous and correlated errors, multiple regression, canonical analysis and non-linear models. There is a useful chapter on applications which occurs rather late in the book.

The author says that the prerequisite is about fifty lectures on statistics plus a knowledge of calculus and matrix notation. I feel that more than this is needed to understand a book which tries to get to grips with the difficulties but may have suffered from being written too hastily. I think research workers will find it hard going. This is a pity, because the material is there.

D. V. LINDLEY

HELIUM RESEARCH

Helium-3 and Helium-4

By William E. Keller. (The International Cryogenics Monograph Series.) Pp. xi + 431. (Plenum: New York, 1969.) $18.50.

W. H. KEESOM's classic book *Helium*, published in 1942, was a monumental and exhaustive work containing almost all that was then known about the helium-4 atom and its behaviour in the gaseous and condensed states (the helium-3 atom had not been identified until 1939, and then only as a minute fraction in naturally occurring helium-4). To a large extent, "Keesom" was a collection and summary of published papers, and it became and remained the reference bible of helium researchers until well into the 1950s—and is indeed still used. The mass of information accumulated since 1942 has been immense, and a proposal a few years ago to up-date Keesom, keeping its essential character, seems, not surprisingly, to have come to nothing. Such a book is now no longer possible, and some strict selection of material has to be made. Most of the later authors have chosen to select by limiting the subjects treated, usually to superfluid helium (for example, F. London, Atkins, Lane, Khalatnikov, Pines and Nozières, Donnelly), but the two most recent (Wilks and the present author) have attempted to cover both liquid and solid helium-3, helium-4 and helium-3–helium-4 mixtures (and, in the present case, gaseous helium) by selecting material within each topic. One advantage of this approach is that, although the physics of the various topics is really quite different, it encourages the tendency to enlarge one's point of view beyond the immediate problem. A disadvantage is that, because of the vast range of material and of physics which it spans, it may become a glorified review, which is not perhaps what this sort of book is for (Atkins, incidentally, seems to me to have combined most successfully the didactic and the factual approach—though he was writing ten years ago when there was a less severe problem).

As Dr Keller says in his preface, he has indeed deliberately constructed his book with each chapter like a short review article. This statement does not quite do justice to the book, which hangs together better than it implies. Two consequences of it are perhaps apparent though: namely, that the cross-referencing is not always as good as it could be, and that there is some imbalance reflecting the author's own special interests (which, he himself points out, is inevitable). For example, while the gas–atom properties of helium and superfluid flow are dealt with extensively (and very usefully), the nuclear magnetic properties of condensed helium and the properties of "finite" helium (thin films) and of isotopic mixtures are comparatively summarily treated. On the other hand, the treatment is up to date, although I should have liked to have seen more on the theories of phase transitions in the chapter on critical phenomena.

It is always easy to find deficiencies in a book, real or apparent, and much more difficult to assess its value until one has had the opportunity of using it for some time. I have greatly enjoyed this book. I am sure that it will be very much used, and that there is much to be learned from it. It will certainly form an essential part of the helium researcher's library, and will be a most useful introduction for new students.

D. F. BREWER

SEMICONDUCTOR ENGINEERING

Applied Solid State Science

Advances in Materials and Device Research, Vol. 1. Edited by Raymond Wolfe. Pp. x + 404. (Academic Press: New York and London, September 1969.) 140*s*.

A NOTABLE result of recent applied research on electronics materials has been the confirmation of silicon as the basic material of the semiconductor industry. It is now clear that the development of the science and technology of new semiconductors to a corresponding level of understanding and reliability requires very long-term effort and is only justified for specific applications for which silicon cannot be used. The advent of this present series, which aims to provide authoritative reviews of current work of industrial importance, is therefore timely.

All four articles in this first volume are well chosen. Each one covers an area of electronics materials science which is active and important, and each is written by authors of high professional standing. Without doubt, however, this first volume is distinguished by its first article on junction electroluminescence by P. J. Dean of Bell Telephone Laboratories. This leads the reader through a brief explanation of energy bands in semiconductors to an account of injection mechanisms in homojunctions and in heterojunctions. The bulk of the article is concerned with a detailed and extensive survey of energy transport processes and radiative recombination mechanisms in semiconductors with reference to both incoherent and coherent modes of emission. The relevant literature is thoroughly and critically explored and a great deal of information on energy levels and spectra is presented. There are more than 400 references for further reading.

The second article, by A. Goetzberger and S. M. Sze, also of Bell Telephone Laboratories, is concerned with the metal–insulator–semiconductor materials system which is a basic component of many devices and of monolithic integrated circuits. There is a detailed account of surface charges, and of accumulation, inversion and depletion layers in terms of standard semiconductor theory. The article continues at some length to describe, with reference to the silicon–silicon oxide system, the understanding and interpretation of the capacitance and conductance measurements which have been and still are useful in evaluating the physics of surfaces and interfaces.

The third article, by J. W. Mayer of the California Institute of Technology and O. J. Marsh of Hughes Research Laboratories, surveys the use of ion implantation for semiconductor device fabrication. The practical value and economic level of this technique are still uncertain and this article gives an authoritative and informative account of its capabilities and difficulties. The article begins with an account of range–energy relations in amorphous solids and of channelling in crystalline solids and then continues with descriptions of lattice disorder, annealing and diffusion. The measurement and interpretation of the electrical characteristics of implanted layers are then discussed and the article finishes with an outline of device structures which have been made wholly or partly by implantation technology.

The fourth article, by S. R. Pollack of the University of Pennsylvania and J. A. Seitchik of the Sperry Rand Corporation, deals with electron transport through thin films. This is a short review which summarizes the various physical mechanisms of current. Barrier-limited mechanisms, such as tunnelling and field emission, are first outlined and bulk mechanisms such as tunnelling between and emission from impurities and defects are then described. The article finishes with a very brief presentation of some typical experimental results on oxide films.

The volume ends with an extensive and detailed author index and a shorter but adequate subject index. The text, mathematics and diagrams are clear, typesetting and proof-reading errors are virtually absent, and the result is a book which is a pleasure to read.

This is a book primarily for the semiconductor engineer. Essentially, it is a treatment of selected areas of solid state physics which are being investigated and exploited by the semiconductor industry. Physical mechanisms which are relevant to the fabrication and operation of devices are described and explained sufficiently for the reader who has a good physics background to achieve a working understanding. Within its selected areas, this book will certainly provide a valuable source of fundamental information for many years to come. A surprising omission in a book of applied science is the almost complete lack of description of experimental techniques or practical processes. This is deliberate editorial policy, for the aim of the series is "to emphasize the basic physics and materials technology on which devices are based". The result is, however, in this first volume at least, to give a feeling of remoteness from the real world of the industry which the book is about.

G. T. Wright

MAMMALIAN CELLS IN CULTURE

Axenic Mammalian Cell Reactions

Edited by George L. Tritsch. Pp. xii+413. (Dekker: New York and London, August 1969.) $18.75; 178*s*.

The inclusion of the word "axenic" in the title of this book is potentially misleading; it is concerned neither with pure cultures of microorganisms nor with germ-free animals. It is composed, in fact, of eleven essays by American authors on various aspects of the establishment and use of long-term cultures of "pure" strains of mammalian cells. They are uneven in clarity and interest, but several of them may be instructive, depending on the particular interests of the reader.

Collectively, the essays reflect the increasing tendency of tissue culture workers to apply basic physiological principles to the design of culture systems, with closer control of factors such as pH and oxygen tension, so that the technique becomes less empirical and the environment provided for the cells approaches a little more closely to the *in vivo* situation. This approach is exemplified in the chapter by McLimans on the physiology of the cultured mammalian cell.

The other topics that are discussed include the cytogenetics of haematopoietic cells, transformation of human amnion cells by simian virus 40, the effects of exogenous nucleic acids on cultured cells, study of enzyme regulation and inhibition by antimetabolites, the use of isolated thyroid cells in studies of thyrotropin and the role of peptides in growth control.

Typographical errors are sufficiently numerous to detract from an otherwise well produced volume; some of the half-tone illustrations are too small, and Fig 2 on page 160 has a mistake in the legend.

The book is intended to give both tissue culture specialists and those having a passing interest in the technique some indication of the difficulties and potential value of "axenic" cultures of mammalian cells; it does so quite successfully.

J. A. Sharp

Obituaries

Dr Anna Cosslett

Anna Joanna Cosslett (née Wischin) died in Cambridge on December 6, 1969, at the age of 57, after a long illness. Born in Vienna and educated at Baden, she took her doctorate in physical chemistry at the University of Vienna in 1937. Her work with Professor Philipp Gross was interrupted by a period in prison, as a result of her activity in the Titoist wing of the underground Yugoslav Communist Party. When Hitler annexed Austria in 1938 she fled to London, where she worked for a time as a domestic servant before being rescued by the Society for the Protection of Science and Learning. She found a research position at Bristol University, working with Professor W. E. Garner on microscopical studies of the crystallization of sugar and later the explosive decomposition of azides, in the interpretation of which she was associated with Professor N. F. Mott. After her marriage in 1940 she spent the war years in Oxford as a demonstrator to the elementary classes in the electrical laboratory of the university, and also as instructress in air raid precautions to the women undergraduates.

Migrating to Cambridge with her husband after the War, she became a part-time research assistant in the Cavendish Laboratory, building up a unit for optical microscopy and microphotometry in the electron microscopy section. She was particularly insistent on the need for the coordinated use of optical and electron microscopy in biological research. As there was no suitable instrument on the market, she built a versatile ultraviolet microscope by marrying refractive optics to the Beck-Burch reflecting stand, with which several comparative studies were made in collaboration with microbiologists. Out of an early interest in evaporated carbon films, used for replicas and specimen supports, came a critical investigation of their optical density as a measure of thickness which led to a study of their structure by electron diffraction.

When methods of ultra-thin sectioning were developed she set about improving the technique as well as making accurate measurements of section thickness by two-beam interferometry. She devised a simple way of making glass knives with reliable cutting properties for the ultramicrotome, long before a commercial gadget became available. Always a stickler for accuracy, she was at pains at meetings and in print to deflate the wilder estimates of section thinness made by some electron microscopists. In the same vein she was interested in what happened to a section during microscopy, and she made one of the first quantitative investigations of the loss of embedding material in the electron beam. Her last research was to devise a method of reflexion interference microscopy for comparative measurement of the size of scales on hair of differing origins, in collaboration with Dr R. T. Sims of the anatomy department at Cambridge.

Her work became so well known that her advice was much sought not only by other departments in Cambridge, but by laboratories in Britain and abroad. She did not publish a great deal, but acknowledgments of her help appear in numerous papers by others. Her knowledge of German was also made freely available; in particular she collaborated in the translation of *Equidensitometry*, by Lau and Krug. She took a full part in the social activities of the Cavendish, whether of the assistant or the academic staff, and she had a warm-hearted sympathy for the personal (often the family) problems of successive generations of research students. Perhaps she will be most remembered for the annual party, to which all ranks were invited, in the garden in Long Road which was her chief leisure interest.

Correspondence

Science and Government

SIR,—A group of the Nobel Prize winners published in *Nature* (October 4, 1969) an appeal to the scientific community concerning measures which ought to be taken by scientists against those countries the governments of which interfere with international scientific communications by preventing their scientists from travelling freely and from enjoying other civil rights which are essential to scientific communication.

Certainly, manifestations of solidarity with the scientists afflicted by persecution are touching, and those who are emotionally induced may even see in this a certain kind of consolation. However, a good scientist is guided by reason rather than by emotions, and efficient help means more for him than touching sympathy. There are doubts about the effectiveness of the measures suggested by the Nobel Prize winners who signed the appeal. A government which would encroach by brutal restrictions on civil rights, and whose scientists would be prevented from participating in conferences in their fields of interest held abroad, or who would be persecuted in some other manner, would only be too glad if scientists from other countries were to boycott conferences held at home. The ones to suffer from these measures would be the persecuted scientists themselves. In short the retaliatory measures suggested would only have one positive though questionable effect—they would appease the consciences of the scientists who are incapable of inventing a more ingenious way of helping their persecuted colleagues.

An example of the absurd consequences of such well intended actions can be seen in the communication published in "Miscellaneous Intelligence" (*Nature*, January 10, 1970). It appeals to the colleagues who have been invited by the Institute of Macromolecular Chemistry to attend symposia to be held in Prague and Marienbad to think twice about doing so, on the grounds that I am politically persecuted in my country and that I would not be allowed to reciprocate their visit because of the ban imposed on my travelling abroad.

What has really happened represents a rather mild persecution compared with what happens in other authoritarian systems. Because of differences of opinion, I have only been dismissed from the post of the Director of the Institute and from some other functions, which is a measure stipulated by offended bosses all over the world. As far as my travelling is concerned, I daresay that so far I have much less been afflicted by the restrictions than other colleagues in this country. And even if both my friends and myself were to become victims of whatever evil terror there may arise, non-participation in the conferences organized in this country would not help us but only those who wish to put restrictions on our work and communications with the other countries.

Yours faithfully,
O. WICHTERLE

Institute of Macromolecular Chemistry,
Prague.

Attitudes to Conservation

SIR,—I am sure that I am not alone among your readers in being dismayed by your optimism in envisaging the problems of conservation, and especially in the way in which you have taken sides, your derisive attitude to opponents, your disregard of interests unable to exercise political pressure and your acceptance of short-term expediency.

In your one-sidedness, you are unfair to opponents, as shown in the use of emotive words such as "hawks" and "doves", "doomsdaymen" and "jeremiahs"; and in misrepresenting your opponents as counselling "despair". You suggest (*Nature*, December 27, 1969) that the conservation lobby is threatening us with science fiction horrors ("unisexed morons", etc). Surely it is for theologians and not for scientists to accuse their opponents of heresies ? And surely it is an impropriety to stigmatize the statements of responsible persons with whom you disagree as "misguided" and "reprehensible" ?

In this series of leading articles, one is inclined to see a consistent campaign, honourably intended, to protect the advance of technology. But your view appears to be directed along a narrow perspective, without awareness of such important areas as ecology and ethology. Surely no biologist could have written (*Nature*, November 15, 1969) "human beings . . . may be more be more like ants and bees than laboratory rats". Your concern for man, mainly that he should continue to be fed, is for man, and not at all for the world which is his home. Let us assume that your optimism is justified, and that food production can keep pace with population growth for an indefinite period. Is not the future that would be upon us in another hundred years even more horrible than that of those unisexed morons ? Your faith that no acceleration of the rate of change will be beyond the power of self-correction or the power of our governors to adjust to will not be well taken by the cyberneticist or the social scientist. In making your forecasts do you think you should make room for contingency planning ? Suppose that the green-house effect of increasing carbon dioxide in the atmosphere does show up, and that the Antarctic ice-cap does begin to melt. Have you a plan ready for what we should do then ?

These are problems in which our own emotional responses are necessarily involved, and perhaps we should disclose our "interests", our convictions. I believe that mankind has a responsibility to more than himself; if the Earth is our space-ship, then we are only the officers and we must have a care for the crew. Of all the dangers, perhaps the worst are those of genocide. Once a species is extinct, its loss can never be made good. What is the present rate of genocide, in terms of species per century ? The destruction of non-human societies and local eco-systems must be proceeding at an accelerating pace. It is probably impossible, I would say, for any ordinary man to make an equivalent positive contribution over the course of his lifetime for all the damage he does just by living. Right now, every new child born is an entry on the debit page, acceptable only if required for replacement. I conceive it our first duty to try to contain this destructive process; and to turn ourselves from exploiters to guardians, curators, trustees. It is not too soon, now, to be thinking of what the Earth will be a million years from now.

Yours faithfully,
ELIOT SLATER

Institute of Psychiatry,
De Crespigny Park,
Denmark Hill, London SE5.

The Third London Airport

SIR,—One had become accustomed to the way in which the popular Press has long been campaigning for a decision to locate the Third London Airport at Foulness without waiting for the evidence presented to the Commission enquiring into the matter, leave alone its decision; but it is a surprise to see you join them (*Nature*, **225**, 486; 1970). This is one of the most interesting, difficult and portentous decisions that our generation is ever likely to have to make in Britain, and your decision that "there is little to choose between one site and another", so that "in short, the airport will be best placed where it will be least nuisance. Foulness, it would seem, is the best place" seems so superficial that a protest is surely called for, if only in the hope that more attention will be paid to some of the implications of the evidence now becoming available in time to permit a better-informed discussion at Stage V of the enquiry in two months' time.

First, permit me to point out that the "extra" cost of an airport at Foulness compared with the cheapest site, Cublington (discounted to 1975) is £120 million, which is surely no small matter when the greatest difference in cost of the inland sites is only £9·3 million. While this may be only 5 per cent of the total cost, personally I doubt whether it can be glossed over so easily, because it is my impression that the Commission has in many cases taken conservative estimates of the likely cost of factors difficult to estimate, such as difficulties with transport, preparation of the site, meteorology, and air safety; and with the exception of meteorology, on present showing most of these unknowns are likely to be most important at Foulness, so that the most serious risk of underestimated costs occurs there. There is already ample experience of construction and operational problems inland, but it is quite possible that our hopeful entrepreneurs will encounter a whole new dimension of unforeseen expense as they start to construct transport facilities out through the East End of London to build runways among the shifting sands and sea fogs of the North Sea. And it will not be they, but the taxpayer, who receives the bill.

Second, while you abuse the devoted and (I would have thought) generally rather efficient civil servants, who have made our existing international airports the busiest and most successful ones outside North America, with none of the major transport problems that beset the larger ones there, for ignoring wider aspects of the decision where to site the Third London Airport in favour of the choice which they know to be safe, may I point out some other wider implications which you in your turn do not mention ? The question of the nuisance value of the airport to which you attach prime importance is of only local, not even regional, interest, but the establishment of a major, well appointed, conveniently sited airport in the near future to take overflow traffic from our existing airports is a matter of national importance if we are to compete for the transatlantic tourist trade with foreign centres such as Paris, and facilitate the smooth growth of air freight services in the interests of the need to increase exports. If there is any question of undue delay or difficulty of access to the new airport as a result of a decision to minimize its local nuisance value in the way you suggest, the cost to the country could be very serious in terms not just of a little local noise but as an influence on our total balance of trade.

Third, while national considerations surely ought to take precedence over local ones, there are also some local considerations that have received curiously little comment. It would appear from the commission's calculations that they anticipate that if the Third London Airport is built inland it will be ready fairly soon and will result in the closure of a whole series of other nuisances such as Luton Airport and various military airfields, and will attract business away from more distant sites such as Birmingham and Manchester airports, so that while a major new nuisance will be created in a tract of countryside apparently specially chosen because its remoteness will minimize the effect, other nuisances are liable to be reduced over a wide area round about. Judging by recent protests in the Press, the closure of Luton airport alone will cause more alleviation of local discontent than will ever be provoked by the utmost development of any of the other inland airport sites currently under consideration for development. On the other hand, the Commission appears to anticipate that if the new airport is sited at Foulness, both Luton and Gatwick airports may have to be developed to take overflow traffic while it is being constructed, while much traffic from the Midlands, which might have gone to an inland Third London Airport, will be diverted to Birmingham and Manchester instead. If so, Foulness will miss much of the business that might have been expected inland, which will be diverted to not just one but four or more other proliferating sources of local nuisance instead, leaving an expensive white elephant stranded in idleness on the Thames mudflats.

There are several other matters which one could raise, such as what will happen to Southend and north Kent if it turns out that Noise and Number Indices are among the variables that have been underestimated; but I have added too much to the verbiage being lavished on this issue already. Basically, I would like to suggest that whereas it is possibly arguable that the original decision to select Stansted was perhaps based on too narrow grounds of operational convenience, even now the level at which you argue the decision should be taken, local nuisance value on a regional basis, is still too narrow. The moment that it is enlarged further to a national scale, in terms of the facilitation of trade, and the alternatives of the dispersal of traffic to many sites, each of which becomes a growing local nuisance itself, or concentration at a single central site, deliberately placed where it will cause least nuisance, with the minimum difficulties of construction, and close to existing transport facilities, then an inland site must win.

Yours faithfully,

W. R. P. Bourne

62 Vicarage Road,
Watford,
Hertfordshire.

Goldfinger no Longer

Sir,—I wish to associate myself with the views of R. S. Glover (*Nature*, **225**, 570; February 7, 1970) and in particular his comments on the relationship between field data, the analysis of natural fluctuations and the study of marine pollution. To the number of points which, as he says, are self-evident, I would like to add another that cannot be over-emphasized but does not yet appear to have been appreciated: the quantitative data relating to complex ecosystems will be obtained only by the simultaneous deployment of experienced personnel on a scale and for a duration that would be new to environmental research in this country. The one man/one species approach, forced on so many biologists by circumstances beyond their control and which has nonetheless contributed so much to our knowledge of marine biology, must now be supplemented by the multi-disciplinary, large-team approach if the dynamics of natural populations are to be recorded, analysed and understood. As one who has tried with limited means to move into this more complex sphere, I am obliged to concede that the majority of marine biologists, working as we do in coastal universities as small units with or without a few research students, can achieve neither the continuity nor the breadth essential for this type of study. Collaboration between people with complementary or similar interests who also happen to be close neighbours affords a partial remedy, but the nature of the subject makes it pre-eminently one for research institutes or other major units.

Your doubts about routine data collection ranking as research must surely stem not from failure to see the relevance to population dynamics, but perhaps from a belief that natural fluctuations are so well documented and understood already that we need routine observations solely to set against established base-lines. Nothing could be further from the truth. For a variety of reasons, not least the scale of effort required and the pursuit in recent decades of more fashionable fields or quick results, we know very little about the extent and causes of natural fluctuations. Nor are we within sight of being able to predict the probable biological repercussions of the elimination or weakening (through pollutants) of one or two "key" species in marine communities. A massive new programme of fundamental research is required to establish the base-lines of natural variation, and its essential basal requirement is routine, long-term field observation and measurement. The point where such activity becomes "routine" in the perjorative sense and contributes little to research is far distant.

If, however, base-line studies are seen as merely the collection of numerical data at particular sites in anticipation of a possible pollution incident, and if such data are not used to further our understanding of natural variation, you are correct to feel that this activity does not qualify as research. If it is considered necessary, and this I doubt in most cases, the problems of who does it and finances it have yet to be faced; but it seems most unlikely that universities will accept it as a just demand on their limited research time and facilities.

Yours faithfully,

J. R. LEWIS

Wellcome Marine Laboratory,
Robin Hood's Bay,
Yorkshire.

Sir William Grove

SIR,—In your issue of January 24, 1970, there appeared an extract from *Nature* of 100 years ago consisting of a letter from Sir W. R. Grove concerning the correlation of physical forces. The following footnote to the letter was appended: *From a letter to the Editor*, Nature, **1**, 335, *January* 27, 1870. *Grove was the first to demonstrate the electrolytic decomposition of water.*

The *Dictionary of National Biography* gives the dates of birth and death of Sir W. R. Grove as July 11, 1811, and August 1, 1896, respectively. If these particulars are correct, Sir W. R. Grove could hardly have been the first to demonstrate the electrolyte decomposition of water, as the first mention of this having been accomplished was in *Nicholson's Journal*, **4**, 183 (1800), which was an account of experiments done by W. Nicholson and Sir A. Carlisle.

Yours faithfully,

SYDNEY GILL

University of Manchester
Institute of Science and Technology.

The Social Sciences

SIR,—In "Miscellaneous Intelligence" for January 31 (*Nature*, **224**, 405; 1970) you state: "if any of the social sciences is a science, social anthropology is, . . .". A more preposterous statement is difficult to imagine. Surely that must have been written by one of the visionary prophets of the sect. It is not rigour that is exhibited by the long period of introspection between field work and publication, but an inability to apply anything like scientific method to the materials. I think you owe an apology to anthropological linguists, physical anthropologists, and archaeologists, who exhibit some measure of the rigour of science and its methods.

Yours faithfully,

JOHN BUETTNER-JANUSCH

Duke University Medical Center,
Durham,
North Carolina.

British Diary

Monday, February 23

Genius in Science (5 p.m.) Professor Michael Polanyi, London School of Economics and Political Science, at Houghton Street, Aldwych, London WC2.

Modern Mixing Systems (6.45 p.m.) Mr P. Whitaker, Institution of the Rubber Industry, at the Waldorf Hotel, Cooper Street, Manchester 2.

Terminal Units and Transmission in Electronic Telephone Exchanges (5.30 p.m.) Mr T. H. Flowers, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Mechanical Equipment for the Construction of the Victoria Line (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Thermoplastics in the Cable Industry (7.30 p.m.) Mr R. H. Greenhalgh, Plastics Institute, at the Stork Hotel, Queen Square, Liverpool.

Tuesday, February 24

Design of Pressure Vessel Components (all-day discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Electrolyte Movements within the Colon (5.30 p.m.) Professor O. M. Wrong, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1. (Fourteenth of fifteen lectures on "The Scientific Basis of Medicine".)

Excavations at Swanscombe 1968–69 (5.45 p.m.) Dr J. Waechter, University of London, at the Institute of Archaeology, 31–34 Gordon Square, London WC1.

Some New Concepts in Polymer Stabilization (6 p.m.) Professor G. Scott, Society of Chemical Industry, Plastics and Polymer Group, at 14 Belgrave Square, London SW1.

The Content of Measurements Courses for Electrical Engineers (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Yorkshire's Pink Gold—Its Discovery, Exploitation and Evaluation, Mr L. S. Phillips; **The Analysis of Crude Potash Materials,** Dr J. M. Skinner (8 p.m.) Society for Analytical Chemistry; and the Royal Institute of Chemistry, at Constantine College of Technology, Borough Road, Middlesbrough.

Wednesday, February 25

Hi-Fi (6.30 p.m.) Mr J. Moir, Institution of Electrical Engineers, at King Edward VI Grammar School, Chelmsford.

Investigation into the Permeametry and Crystal Structure of Fats and Fat Blends, Mr G. C. Evans; **The Use of Low Resolution NMR for the Rapid Determination of Solids Content of Fat Blends,** Mr P. Mansfield, Society of Chemical Industry, Oils and Fats Group, at 14 Belgrave Square, London SW1.

Kinetics and Regulation of Erythropoiesis (2 p.m.) Dr L. G. Lajtha, University of London, at the Royal Postgraduate Medical School, Du Cane Road, London W12.

Low Light Television Tubes (6 p.m.) Dr P. L. Batey, Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Man, Mind and Machine (1.10 p.m.) Professor Philip M'Pherson, City University, at the Graduate Business Centre, Gresham College, Basinghall Street, London EC2.

Maps as a Medium of Scientific Information (1 p.m.) Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Newer Techniques of Thermal Analysis with special reference to Calorimetry (2.30 p.m.) Society for Analytical Chemistry; and the Chemical Society, in the Department of Chemistry, University of Keele, Staffs.

Stereophonic Transmission (7.30 p.m.) Dr G. J. Phillips, Institution of Electrical Engineers, at the Star and Garter Hotel, Dorking, Surrey.

Sterilizable Plastics Packaging for Foodstuffs (10.30 a.m. symposium) Society of Chemical Industry, in the Meeting Room of the Zoological Society of London, Regent's Park, London NW1.

The Application of Test Results to the Calculation of Short-circuit Levels in Large Industrial Systems with Concentrated Induction Motor Loads (5.30 p.m.) Mr C. B. Cooper, Dr D. M. McLean and Mr K. G. Williams, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Direct Determination of Oxygen in Organic Compounds (6.30 p.m.) Mr P. Gouverneur, Society for Analytical Chemistry, at the Leicester Lounge, Glasshouse Street, London W1.

The Interaction of Power Supply Systems and Digital Equipment in Aircraft (6 p.m. discussion) IEE/RAeS, London Joint Group, at the Royal Aeronautical Society, 4 Hamilton Place, London W1.

The Neuropharmacology of Sleep (5.30 p.m.) Professor M. Jouvet (Lyon), University of London, at Charing Cross Hospital Medical School, Chandos Place, London WC2.

Trends in Diffusion (two-day conference) Institute of Physics and the Physical Society; The Institute of Metals; and the National Physical Laboratory, at the NPL, Teddington, Middlesex.

Thursday, February 26

Ammonium Nitrate Production (6.30 p.m.) Mr R. Carter, Institution of Chemical Engineers, at I.C.I., Ltd, Billingham.

Architectural Aerodynamics (9.30 a.m., two-day meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

Medical Problems of Cold Immersion (5.30 p.m.) Dr W. R. Keatinge, University of London, at the Institute of Child Health, 30 Guilford Street, London WC1.

Man's Place in Nature (1.15 p.m.) Dr J. R. Napier (Smithsonian Institution), University of London, at Queen Elizabeth College, Campden Hill Road, London W8.

Numerical Machine Tool Control (6.30 p.m.) Mr D. F. Walker, Institution of Electrical Engineers, at the University Engineering Laboratories, Trumpington Street, Cambridge.

Precoated Plates and Films for Thin-layer Chromatography (6.15 p.m.) Mr E. J. Shellard and Mr G. Whitechurch, Society for Analytical Chemistry, at the Leicester Lounge, Glasshouse Street, London W1.

The Binding Mechanism of Agglomeration and the Hardness of Agglomerates (5.30 p.m.) Professor Hans Rumpf (Karlsruhe), University of London, at the School of Pharmacy, 29/39 Brunswick Square, London WC1. (Further lecture on February 27.)

The Development of Agricultural Research in the United Kingdom (8.15 p.m.) Sir Frederick Bawden, at Wye College, near Ashford, Kent.

The Lubrication of Moving Elements in Present and Future Machines (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Zinc Production Expansion at Avonmouth (6.30 p.m.) Mr R. M. Sellwood, Institution of Chemical Engineers, at the School of Chemistry, University of Bristol.

Friday, February 27

Abortion Problems (9 p.m.) Dr C. B. Goodhart, Royal Institution, at 21 Albemarle Street, London W1.

Lithographic Printing Inks (7.15 p.m.) Mr A. Stone, Oil and Colour Chemists' Association, at the Royal Hotel, Bristol.

Numerical Machine Tool Control (8 p.m.) Mr D. F. Walker, Institution of Electronic and Radio Engineers; and the Institution of Electrical Engineers, at Cambridge University Engineering Laboratories, Trumpington Street, Cambridge.

Saturday, February 28

Recent Excavations at Swanscombe (3.30 p.m.) Dr John Waechter, Inner London Education Authority, at the Horniman Museum, London Road, Forest Hill, London SE23.

Monday, March 2

Developments in the Use of Plastics in Railway Rolling Stock (6 p.m.) Mr B. J. Hawthorne, Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Physics Exhibition (four days) Institute of Physics and the Physical Society, at Alexandra Palace, London.

Stereophonic Transmission (7 p.m.) Mr G. J. Phillips, Institution of Electrical Engineers, at the Royal Star Hotel, Maidstone, Kent.

Structural Enzymology (1): Conformations of Enzyme Molecules (5 p.m.) Professor D. C. Phillips, University of London, at the Middlesex Hospital Medical School, Mortimer Street, London W1.

The Competition for Cash between Innovation and Extension (6.30 p.m.) Dr D. S. Davies, Society of Chemical Industry, at 14 Belgrave Square, London SW1.

The First Steps Into Space (8 p.m.) Professor R. A. Lyttleton, FRS, University of Hull, in the Middleton Hall. (The St John's College, Cambridge, lecture.)

The Value of Polymer Blends (7.30 p.m.) Mr P. W. Milner, Institution of the Rubber Industry, at the Midland Hotel, Burton-on-Trent.

Reports and Publications

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

Cotton Research Corporation. Progress Reports from Experiment Stations. Season 1966–67. Swaziland. Pp. 22. Season 1967–68. Republic of the Sudan. Pp. 58. Tanzania—Western Cotton Growing Area. Pp. 36. Swaziland. Pp. 10. Western State, Nigeria. Pp. 19. (London: Cotton Research Corporation, 1969.) [241]

British Engine, Boiler and Electrical Insurance Co., Ltd. Technical Report, 1969, Vol. 9, New Series. Pp. 100. (Manchester: British Engine, 1969.) 20*s*. [2511

Northumberland. (National Park Guide No. 7.) Edited by John Philipson. Issued for the Countryside Commission. Pp. viii + 130 + 26 plates. (London: HM Stationery Office, 1969.) 7*s* 6*d* net. [2511

Key to Contentment—The 54th Annual Report of St Dunstans 1968/69. Pp. 23. (London: St Dunstans, 1969.) [2511

Marine Biological Station, University of Liverpool. Annual Report No. 81 for 1968. Pp. 80. (Liverpool: Liverpool University Press, 1969.) [2511

Proceedings of the Royal Irish Academy. Vol. 68, Section B, No. 6: Type Material of Fishes from the "Challenger" Expedition in the National Museum of Ireland. By A. Wheeler and C. E. O'Riordan. Pp. 89–100. 2*s*. Vol. 68, Section B, No. 7: Comparative Electrophoretic Studies of the Water-Soluble Muscle Proteins of some Irish Freshwater Fishes. By P. J. Haen and F. J. O'Rourke. Pp. 101–108 + plates 3–5. 3*s*. (Dublin: Royal Irish Academy, 1969.) [2611

Students—Why Some Fail—an Interim Report on Enquiries into Failure and Withdrawal of Students. Compiled and written by J. A. Wankowski. Statistical Section Cohort Method by R. W. Prince. Pp. 35. (Birmingham: University of Birmingham, Education Survey, 1969.) 10*s* 6*d*. [2611

Ministry of Transport, Road Research Laboratory. RRL Report LR 283: Road Accident and Casualty Rates based on the 1300 Point Census. By H. D. Johnson. Pp. 19. (Crowthorne, Berkshire: Road Research Laboratory, 1969.) *Gratis*. [2611

Proceedings of the Symposium on Laboratory Safety presented as ISTEX 68 at Paddington Technical College, London, December 1968. Pp. 66. (London: Institute of Science Technology, c/o Geology Department, King's College, Strand, 1969.) 7*s* 6*d*. [2711

Proceedings of the National Conference on the Change to the International System (SI) Units for Energy, held at Church House, Westminster, London, 31st October and 1st November, 1968. (Sponsored by the Ministry of Technology.) (St Mary Cray, Orpington, Kent: Ministry of Technology, 1969.) 22*s* 6*d* (plus 2*s* 6*d* postage). [2711

British Council. Annual Report 1968/1969. Pp. 104. (London: British Council, 1969.) [2811

Other Countries

US Department of Commerce, Bureau of the Census. Government Finances/GF68 No. 6: Local Government Finances in Selected Metropolitan Areas in 1967–68. Pp. iii + 38. (Washington, DC: Government Printing Office, 1969.) $0.50. [1811

Annals of the New York Academy of Sciences, Vol. 160, Article 2: The Pharmacological and Chemotherapeutic Properties of Niridazole and other Antischistosomal Compounds. By F. C. Goble and 95 other authors. Pp. 423–946. (New York: New York Academy of Sciences, 1969.) [1911

ICRU Report No. 14: Radiation Dosimetry—X-rays and Gamma Rays with Maximum Photon Energies between 0·6 and 50 MeV. Pp. vii + 30. (Washington, DC: International Commission on Radiation Units and Measurements, 1969.) $2.50. [1911

Republic of South Africa, Department of Industries. Division of Sea Fisheries Investigational Reports. No. 74: Distribution of the Abalone (*Haliotis midae*) and the Effect of Temperature on Productivity. By G. G. Newman. Pp. 7. No. 75: Analysis of Atlantic Waters off the West Coast of Southern Africa. By G. A. Visser. Pp. 26. (Sea Point, Cape Town: Division of Sea Fisheries, 1969.) [1911

US Department of the Interior: Geological Survey. Water-Supply Paper 1535–0: Water Load of Uranium, Radium, and Gross Beta Activity at Selected Gaging Stations, Water Year 1960–61. By E. C. Mallory, Jr., J. O. Johnson and R. C. Scott. Pp. iii + 31 + 1 plate. $0.65. Water-Supply Paper 1879-H: Water Quality and Discharge of Streams in the Lehigh River Basin, Pennsylvania. By Edward F. McCarren and Walter B. Keighton. Pp. iv + 48. $0.35. (Washington, DC: Government Printing Office, 1969.) [1911

Geological Survey of New South Wales. Geological Survey Report No. 25. Taleeban Tin and Tungsten Prospect, New South Wales. By D. A. Nicholson and D. W. Suppel. Pp. 19. (Sydney: Department of Mines, 1967.) A$0.75. [1911

Annals of the South African Museum. Vol. 55, Part 2: *Apletodon pellegrini* (Chabanaud) and other Clingfishes (Pisces: Gobiescocidae) from South West Africa. By Marie-Louise Penrith. Pp. 123–134. (Cape Town: South African Museum, 1969.) R1.20. [1911

Population Reference Bureau, Inc. Annual Report 1968. Pp. 33. (Washington, DC: Population Reference Bureau, Inc., 1969.) [2011

US Department of the Interior: Geological Survey. Water-Supply Paper 1870-B: Floods of April 28, 1966, in the Northern Part of Dallas, Texas. By Willard B. Mills and Elmer E. Schroeder. Pp. iv + 37 + 2 plates. Professional Paper 604: On Batholiths and Volcanoes—Intrusion and Eruption of Late Cenozoic Magmas in the Glacier Peak Area, North Cascades, Washington. By R. W. Tabor and D. F. Crowder. Pp. vii + 67 + 1 plate. (Washington, DC: Government Printing Office, 1969.) [2011

Institut Royal des Sciences Naturelles de Belgique. Mémoires. No. 159: Végétaix Mésodévoniens Récoltés aux Confins du Massif du Brabant (Belgique). Par Francois Stockman. Pp. 49 + 14 planches. No. 160: Les Acritarches de l'Ordovicien et du Silurien Belges. Détermination et Valeur Stratigraphique. Par Francine Martin. Pp. 175 + 8 planches. (Bruxelles: Institut Royal des Sciences Naturelles de Belgique, 1968.) [2011

Science Council of Canada. Special Study No. 8: Scientific and Technical Information in Canada, Part 2. Chapter 4: International Organizations and Foreign Countries. Pp. x + 63. (Ottawa: Science Council of Canada, 1969. Available from Queen's Printer.) $1. [2411

HOW TO BUY NATURE

Volumes start in January, April, July and October, but subscriptions may begin with any issue.

The direct postal price per subscription for one copy of NATURE each week is:

12 MONTHS (52 issues and 4 indexes)

Great Britain and Eire.............................. **£14.0.0 each**
United States of America........(by air freight)............ **$48 each**
Canada..........................(by air freight).......... **$52 each**
Elsewhere overseas by surface mail.................. **£14.0.0 each**

(Charge for delivery by air mail on application)

•Shorter periods *pro rata*. (Minimum three months.)

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED
4 LITTLE ESSEX STREET, LONDON WC2
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2

Subscription Department

MACMILLAN (JOURNALS) LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 5431

Advertisements only should be addressed to

T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2
Telephones 01-242 6264/01-405 4743
Telegrams: Textualist London WC2

Registered as a newspaper at the Post Office

Copyright © Macmillan (Journals) Limited, February 21, 1970

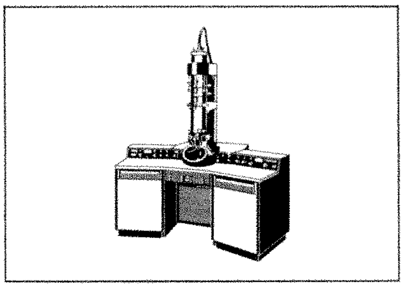
What are the membership privileges of the EM 300 Club?

MEMBER SIGNATURE OF THE PHILIPS EM 300 CLUB
EM 300 CLUB

The members of the EM 300 Club are the over 500 users, in 24 countries, of the EM 300 Electron Microscope.

They are a privileged class - we cannot deny it - by the mere ownership of the finest high-resolution electron microscope on the market today. And they enjoy other benefits as well. For example. At the recent Rome Conference on Electron Microscopy, four new EM 300 accessories were introduced: a new TV display system with image intensifier, a new X-ray spectrometer attachment, a new pre-pumped airlock, and a radically improved specimen chamber cold trap capable of reducing specimen contamination rate to 1 Å per minute or less.

The existing owners of the EM 300 have the first opportunity to benefit from these innovations because they can all be retrofit to existing equipment. But that's only the beginning. Philips' staff of research scientists and engineers are developing new improvements all the time, and these developments are available for incorporation onto instruments already in use. Ownership of an EM 300 is an investment against obsolescence! There are even more membership privileges. To learn the whole story, write for our EM 300 brochure.



N.V. Philips' Gloeilampenfabrieken
Analytical Equipment Department,
Eindhoven, the Netherlands.

ANALYTICAL EQUIPMENT

PHILIPS

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF CHEMISTRY

OIL POLLUTION AT SEA

Applications are invited from qualified colloid scientists for a **POST-DOCTORAL RESEARCH FELLOWSHIP** to commence at a date to be arranged in the near future to work with Professor D. D. Eley, F.R.S., on water-in-oil (" chocolate mousse ") and oil-in-water emulsions, their formation, structure and stability in relation to oil pollution. The salary will be in the range £1,240 by £115 to £1,585 according to qualifications and experience, reviewable yearly for a total of three years. (786)

UNIVERSITY OF IBADAN

NIGERIA

Applications are invited for **LECTURESHIPS in (i) GENETICS and (ii) COMPARATIVE AND CELLULAR PHYSIOLOGY IN DEPARTMENT OF ZOOLOGY.** Salary scale £N1,200 to £N2,175 per annum (£N1 = £1 3s. 4d. sterling). Salary supplemented in range £450 to £625 per annum (sterling) and education allowances and children's holiday visit passages payable in appropriate cases under British Expatriates Supplementation Scheme. F.S.S.U. Family passages ; biennial overseas leave.

Detailed applications (six copies), naming three referees, by March 13, 1970, to Inter-University Council, 90/91 Tottenham Court Road, London, WIP ODT, from whom particulars are available. (663)

UNIVERSITY COLLEGE OF RHODESIA

Applications are invited for two posts of (a) SENIOR LECTURER or (b) LECTURER IN DEPARTMENT OF GEOGRAPHY. Salary scales: (a) $R5,616 to $R7,536 per annum ; (b) $R3,624 to $R5,832 per annum ($R1 = 11s. 8d. sterling). Family passages and allowances for transport of effects on appointment. Installation loan of up to half of one year's salary if required. Assistance with accommodation for three years from persons recruited from outside Rhodesia. Sabbatical and triennial visits with travel allowances. Superannuation and medical aid schemes.

Detailed applications (six copies), naming three referees, by March 23, 1970, to Inter-University Council, 90/91 Tottenham Court Road, London, WIP ODT, from whom particulars are available. (665)

UNIVERSITY OF EAST AFRICA

MAKERERE UNIVERSITY COLLEGE UGANDA

Applications are invited for the post of **PROFESSOR IN PLANT SCIENCES IN DEPARTMENT OF CROP SCIENCE AND PRODUCTION.** Faculty of Agriculture, available in July, 1970. Salary £U3,150 per annum (£U1 = £1 3s. 4d. sterling). Salary supplemented by £1,068 per annum (sterling) and education allowances and children's holiday visit passages payable in appropriate case by British Government. F.S.S.U. Family passages ; biennial overseas leave.

Detailed applications (six copies), naming three referees by March 25, 1970, to Inter-University Council, 90/91 Tottenham Court, Road, London, WIP ODT, from whom particulars are available. (667)

VIROLOGY SENIOR TECHNICIAN OR Technician required to assist in the day to day work of the non-specific immunity section. Experience in Virology and tissue culture techniques essential. Salary in range £868 to £1,436 according to age, qualifications and experience. 37½ hour week, superannuation scheme and four weeks annual leave. Applications in writing to Departmental Superintendent, Department of Biochemistry, Imperial College, London, S.W.7. (765)

EAST MALLING RESEARCH STATION. Graduate or equivalent in horticulture, botany or forest botany, male, keenly interested both in scientific aspects of practical fruit culture and in people, required for Scientific Liaison Section. Appointment in Assistant Experimental Officer or Experimental Officer grade. Details and application forms from the Secretary, East Malling Research Station, Maidstone, Kent. (767)

TWO RESEARCH ASSISTANTS

are required to join an Electron Microscope Unit engaged in a study of chromosome structure.

ASSISTANT (I) is required to develop methods for the study of chromosomes and other biological materials in the Scanning Electron Microscope.

ASSISTANT (II) will be responsible for the establishment of tissue culture methods and processing techniques involved in the extraction of chromosomes.

Graduates or Technicians with an interest/experience of cell research and who seek an opportunity to work in new, well-equipped laboratories, should apply, giving full details, to Dr. B. V. Kingsley Smith at the above address. (792)

LAKEHEAD UNIVERSITY

THUNDER BAY, ONTARIO, CANADA

ASSISTANT PROFESSOR OF CHEMISTRY

Applications are invited for the post of Assistant Professor in the department of chemistry at Lakehead University. The successful applicant will be required to lecture in the area of physical-organic chemistry and should have applied research interests in, for example, some aspects of rubber or plastics.

The appointment will be initially for one year and the salary will be in the range $10,700 to $13,000 and be dependent on suitable experience. Applications, as soon as possible, should be sent to Professor S. Walker from whom further details of the department and its research interests are available. (791)

UNIVERSITY OF SIERRA LEONE

FOURAH BAY COLLEGE

Applications are invited for LECTURESHIP IN PALAEONTOLOGY in Department of Geology. Preference given to candidates with an interest in Micropalaeontology. Salary scale: Le2,400 to Le4,740 per annum (Le1 = 10s. sterling). Salary supplemented in range £425 to £650 per annum (sterling) and education allowances and children's holiday visit passages payable in appropriate case by British Government. F.S.S.U. Various allowances ; family passages ; regular overseas leave.

Detailed applications (six copies), naming three referees, by March 23, 1970, to Inter-University Council, 90/91 Tottenham Court Road, London, WIP ODT, from whom particulars are available. (666)

EDITOR WANTED FOR FUEL ABSTRACTS. Fuel Abstracts and Current Titles is a specialised international Journal. The Editor must be able to interlink detailed editorial shortening, classification and indexing of abstracts with supervision of small editorial staff, working to a monthly printing schedule; and also to produce and implement long range ideas and planning on every aspect of the Journal's production and circulation. Experience of abstracts classification, preferably in the field of fuel and energy, would be an advantage. Someone without abstracts experience but who likes collecting and sorting data and keeping several jobs running simultaneously should enjoy this job. Salary in range £1,500 to £1,800. Informal atmosphere in small professional organisation. Please apply to The Secretary, The Institute of Fuel, 18 Devonshire Street, London, WIN 2AU, marking envelope " Personal." (777)

GUY'S HOSPITAL—AN EXTENSION TO the laboratories of the Poisons Reference Service at New Cross Hospital, London, S.E.14, will be opened this summer. Vacancies will occur for graduates in Chemistry and Biochemistry. The work is concerned with the analysis of body fluids and other specimens for drugs and poisons, both to provide a service for clinicians and to advance research in pharmokinetics and the treatment of poisoning. Opportunities are afforded for original work in order to obtain a higher degree of the University of London. —Further details of these posts including salary and conditions of service may be obtained from the Director of the laboratory to whom applications should be sent. (776)

UNIVERSITY OF BRISTOL. APPLICATIONS are invited for the post of Technician in the Department of Physiology. The person appointed will be required to assist in tissue culture studies of nerve cells and, preferably, will hold a H.N.C. or be an A.I.M.L.T. Experience of tissue culture techniques would also be useful. Commencing salary within the range: £743 to £1,127, according to age, qualifications and experience.—Applications, giving full details, including qualifications, should be addressed to: Dr. T. Biscoe, Department of Physiology, The Medical School, Bristol BS8 1TD. (775)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(UNIVERSITY OF LONDON)

Applications are invited for the post of **LECTURER** in the Department of Medical Physics from October 1, 1970. Initial salary up to £1,585 on scale £1,240 by £115 to £2,045, then subject to review by £115 to £2,850, plus London allowance and superannuation benefits. Further particulars from the Secretary, 8 Hunter Street, London, W.C.1, to whom applications (5 copies) should be sent not later than March 6, 1970. (787)

SENIOR TECHNICIAN I OR II

Required in Department of Clinical Pharmacology to join a group studying the metabolism of new drugs in man, and be responsible for management and operation of new gas chromatograph and/or mass spectrometer. Considerable experience of analytical techniques essential. Experience with gas chromatography and/or mass spectrometry an advantage. Salary range: Senior I £1,265 to £1,635 ; Senior II, £1,725 to £2,120 per annum.

Applications to the Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, London, W.12, quoting ref.: 20/270. (711)

UNIVERSITY OF HULL

Applications are invited for a post as **TECHNICIAN** in the Department of Botany. Successful applicant will be required initially to assist a Senior Technician in the day to day running of the teaching laboratories and the training of Junior Technicians. Qualifications: City and Guilds Laboratory Technicians Advanced Certificate, or equivalent preferred. Salary on scale £773 to £1,127 depending on age and qualifications.

Applications to Technical Staff Officer, The University, Hull, quoting reference TB/1. (686)

UNIVERSITY OF MANCHESTER

LECTURER IN CHEMICAL BACTERIOLOGY

Applications invited for this post in the Department of Bacteriology and Virology. Duties include undergraduate teaching and supervision of research students. Salary range per annum: £1,585-£2,850. F.S.S.U. Duties commence as soon as possible after March 31.

Particulars and application forms (returnable by March 6) from the Registrar, The University, Manchester M13 9PL. Quote ref.: 20/70/Na. (687)

HEAD OR SENIOR TECHNICIAN

Required for new laboratories shortly to be opened. Applicants must have a wide experience in Pharmacological Techniques. The Position offers good opportunties with excellent conditions, salary and Pension Scheme.

Apply in writing to the Research Director, Biorex Laboratories Limited, Biorex House, Canonbury Villas, London, N.1. (672)

MASSEY UNIVERSITY

PALMERSTON NORTH, NEW ZEALAND

LECTURER IN AGRONOMY

Applications are invited from qualified persons for the above-mentioned position. The appointee will be largely concerned with undergraduate teaching in Agronomy with emphasis on Crop Science. Some postgraduate teaching may be required.

The particular requirement is for a graduate in agricultural science with some experience in applied research and extension in the field. Such a person would also be required to link with other staff members in extending and developing the basic principles taught in earlier stages of the course, to agronomic practice.

Opportunities and facilities for personal research in the general field of agronomy are available with particular encouragement being given to studies in crop agronomy.

Salary: $NZ3,100 to $NZ4,800 (by $200).

(As University salaries are at present under review, this figure could be changed in the near future.)

Further details of the position and of the University, together with the general conditions of appointment, may be obtained from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London, W.C.1, or from the Registrar of the University.

Applications close on May 1, 1970. (724)

For further classified advertisements see page viia

UNIVERSITY OF EDINBURGH

Department of Forestry and Natural Resources

Applications are invited for the post of DEMONSTRATOR (Ecology) in the above Department. The successful applicant will be expected to assist in the teaching of second-year ecology. He or she will also be expected to pursue research studies in the field of ecology.

Salary Scale: £1,060 to £1,445 per annum.

The successful candidate will be expected to take up duty not later than October 1, 1970.

Applications, giving full details of age, qualifications and experience, should be sent to the Secretary to the University, Old College, South Bridge, Edinburgh, EH8 9YL, from whom further particulars may be obtained. The closing date for applications is April 1, 1970. Please quote reference 1008. (669)

THE GAME CONSERVANCY

FORDINGBRIDGE, HAMPSHIRE

Applications are invited for the post of ASSISTANT SCIENTIFIC OFFICER to help carry out the diagnosis of disease in gamebirds and to assist in various field projects. Some previous experience in laboratory techniques in the diagnosis of disease in poultry would be desirable.

Further particulars from the Director, The Game Conservancy, Fordingbridge, Hampshire. (730)

UNIVERSITY OF STRATHCLYDE

SCHOOL OF MATHEMATICS AND PHYSICS

DEPARTMENT OF APPLIED PHYSICS

SENIOR LECTURESHIP OR LECTURESHIP

Applications are invited from physicists preferably with research interests in the biological applications of physics. A knowledge of either solid state physics, or of some branch of biology (e.g., physiology), or of cybernetics, is particularly desirable.

The successful candidate will be expected to take part in undergraduate and postgraduate teaching and to help to establish research with emphasis on long-term relevance to social and industrial problems, particularly in their biological aspects.

Salary Scales: Senior Lecturer £2,995–£3,670; Lecturer £1,240-£2,850 with F.S.S.U. Placing according to qualifications and experience.

Application forms and further particulars (quoting 1/70) can be obtained from the Registrar, University of Strathclyde, George Street, Glasgow, C.1. with whom applications should be lodged. (732)

DIRECTOR

Fishing Industry Research Institute South Africa

The post of Director of the Fishing Industry Research Institute, University of Cape Town, Rondebosch, will fall vacant on 1st June, 1970, on the retirement of Prof. G. M. Dreosti. Applications are invited for this post, which carries a salary not less than R10,000 (approx. £5,800).

Full details of the Institute, duties and privileges of the post may be secured on application to:

The Office of the Scientific Counsellor (Ref. N.),
Chichester House,
278 High Holborn,
London, W.C.1.

Closing date for completed applications 7th March, 1970.

(719)

AHMADU BELLO UNIVERSITY

ZARIA, NIGERIA

Applications are invited for the following appointments:

DEPARTMENT OF ELECTRICAL ENGINEERING

Chair of Electrical Engineering. Appointee will be expected to take a particular interest in the development of the teaching programme and he should be prepared to carry a large share of the administration of the undergraduate courses and the running of the department as well as leading research in his own field. There are, in addition, two other Chairs, both filled, in Power Engineering and Telecommunications, but specialisation in either of these fields would not necessarily disqualify a suitable candidate.

DEPARTMENT OF GEOGRAPHY

(a) **Professor and Head of Department.**

(b) **2 Lecturers** in either (i) Geomorphology and biogeography, (ii) economic and/or agricultural geography, or (iii) settlement geography. Preference given to candidates who in addition to these qualifications have experience and interest in resource analysis and regional planning.

DEPARTMENT OF GEOLOGY

Lectureship in Geology. Good honours degree with research interests in mineralogy and/or structural/metamorphic geology desirable.

DEPARTMENT OF MATHEMATICS/COMPUTER SCIENCES

(a) **Senior Lecturer and Lecturer.** Good honours degree in mathematics and considerable research experience desirable.

(b) **Computer Programmer and Systems Analyst** (both Lecturer grade). Candidates must be graduates with computer experience.

Salary scales: Professor and Head of Department, £N3,200 per annum; Professor, £N3,000 per annum; Senior Lecturer, £N2,275 to £N2,575 per annum; Lecturer, £N1,200 to £N2,175 per annum (£N1=£1 3s. 4d. sterling). Salary supplemented in range £450 to £925 per annum (sterling) and education allowances and children's holiday visit passages payable in appropriate cases by British Government. Family passages; various allowances; superannuation or gratuity; regular overseas leave. Detailed applications (6 copies), naming 3 referees, by April 6, 1970, to Inter-University Council, 90/91 Tottenham Court Road, London, WIP ODT, from whom particulars are available. (743)

LABORATORY SUPERVISOR

Applications for the post of Supervisor of a small veterinary field laboratory in the Cheltenham area are invited from those with not less than 5 years' experience in microbiology or pathology and who are Associates of the Institute of Medical Laboratory Technicians or hold equivalent qualifications.

The applicant should be male (25–30 years of age), physically fit and capable of acquiring and performing new techniques.

The post demands versatility, adaptability and the ability to accept sole responsibility for provision of laboratory services in support of field trials and investigations.

Starting salary will depend on age and experience but will not be less than £1,200; fringe benefits include contributory pension and free life assurance schemes.

Please write giving details of age, qualifications, experience and present salary to:

The Company Personnel
Manager, Cyanamid of
Great Britain Ltd.,
Bush House, Aldwych,
London W.C.2

CYANAMID

(794A)

LIVERPOOL EDUCATION COMMITTEE

LIVERPOOL REGIONAL COLLEGE OF TECHNOLOGY
(Polytechnic designate)
BYROM STREET, LIVERPOOL, L3 3AF

Applications are invited for the following post:

SENIOR LECTURER/ LECTURER GRADE II IN PHARMACOLOGY

SCHOOL OF PHARMACY

The successful candidate will be expected to assist in the expansion of research work and in the development of undergraduate and postgraduate courses.

SALARY: Senior Lecturer, £2,417 to £2,752; Lecturer Grade II, £1,827 to £2,417.

The commencing salary will be assessed according to experience with additions for graduate qualifications and training.

Application forms and further particulars can be obtained from the Director of Education, 14 Sir Thomas Street, Liverpool, L1 6BJ, on receipt of an addressed foolscap envelope.

Return forms to the Acting Principal of the College not later than March 4, 1970.

STANLEY HOLMES,
Town Clerk.

(759)

UNIVERSITY OF ADELAIDE

Applications are invited for the following appointments, the closing date for applications being stated in parentheses after each post.

LECTURER IN BIOCHEMISTRY. Present interests of the Department lie in the area of protein biosynthesis, phage and virus biochemistry, regulation of enzyme activity and metabolism, structural studies on proteins. (15.3.70.)

LECTURER IN MATHEMATICAL PHYSICS. Preference will be given to a candidate with several years' postgraduate experience who has specialised in the field of general relativity or elementary particle physics and is capable of supervising M.Sc. and Ph.D. students. (28.2.70.)

SALARY SCALES: Lecturer $A5,400 by $A280 (1) by $A270 (6) to $A7,300, with superannuation on the F.S.S.U. basis.

FURTHER PARTICULARS about each post and the conditions of appointment and other information sought will be supplied on request to the Registrar of the University, or to the Secretary-General, Association of Commonwealth Universities (Appts.), 36 Gordon Square, London, W.C.1.

Applications should be sent in duplicate and giving the information listed in the statement that will be supplied to the Registrar, The University of Adelaide, North Terrace, Adelaide, South Australia 5001. (752)

RESEARCH COUNCIL OF ALBERTA

A position is available within the Geology Division of the Research Council of Alberta, a research institute located on the campus of the University of Alberta, for investigations concerning the distribution, stratigraphy, and origin of the Quaternary glacial deposits of Alberta. Applicants should have an M.Sc. or Ph.D. degree in geology or physical geography with a strong background in Pleistocene geology, geomorphology, air photo interpretation, and sedimentology. Duties will involve mapping of surficial deposits at various scales and studies of specific problems related to urban development, land utilization, industrial minerals, and hydrogeology. Salary commensurate with qualifications and experience.

For further details write the Administrative Assistant to the Director, Research Council of Alberta, Edmonton, Alberta.

(704)

UNIVERSITY OF ADELAIDE

PROFESSOR OF PHYSICS

Applications are invited for the above-mentioned appointment.

There are two established Chairs within the Department of Physics and the present appointment relates to one that has recently been vacated. The major research interests of the Department are in the fields of atmospheric and space physics, cosmic rays, laboratory astrophysics, solid state physics and seismology. It is hoped that the research interests of the new Professor will be within one of these fields and that he will develop research activities in experimental physics which will strengthen and expand the present research work of the Department.

Further information about the work of the Department of Physics may be obtained from Professor J. H. Carver.

SALARY: at present $A12,000 a year with superannuation on the F.S.S.U. basis.

FURTHER INFORMATION: Potential candidates are invited to obtain from the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London, W.C.1, or from the Registrar of the University, a more detailed Statement about the Chair and formal terms of appointment. Any further information desired may be had on request to the Registrar.

APPLICATIONS (in duplicate) giving the particulars listed in paragraph 6 of the Statement should reach the Registrar of the University at North Terrace, Adelaide, S.A. 5001, not later than April 15, 1970. (722)

UNIVERSITY OF IBADAN

NIGERIA

Applications are invited for the following appointments in the FACULTY OF MEDICINE:

(i) SENIOR LECTURESHIP IN DEPARTMENT OF BIOCHEMISTRY from candidates with research experience in Human Nutrition.

(ii) LECTURESHIP IN DEPARTMENT OF BIOCHEMISTRY from candidates with research experience in Biochemical Toxicology.

(iii) LECTURESHIP IN DEPARTMENT OF PHYSIOLOGY.

Salary scales: (i) £N2,275 to £N2,775 per annum (ii and iii) £N1,200 to £N2,175 per annum (£N1 = £1 3s. 4d. sterling). Salary supplemented in range £450 to £825 per annum (sterling) and education allowances and children's holiday visit passages payable in appropriate case by British Government. F.S.S.U. Family passages; various allowances; regular overseas leave.

Detailed applications (six copies), naming three referees by March 26, 1970, to Inter-University Council, 90/91 Tottenham Court Road, London WIP ODT, from whom particulars are available. (744)

UNIVERSITY OF CAMBRIDGE

Applications are invited for the office of

UNIVERSITY DEMONSTRATOR IN THE DEPARTMENT OF PHARMACOLOGY

The initial appointment will be for three years from October 1, 1970 or earlier. The pensionable stipend is on a scale £1,355 to £1,815 a year with initial placing above the minimum where appropriate. There is a grant towards removal expenses.

Candidates should send twelve copies of their applications, together with the names of not more than three referees, to Mr. G. R. Anderson, The Old Schools, Cambridge, CB2 1TT, to arrive not later than March 16, 1970. (680)

NATURE

MACMILLAN (JOURNALS) LTD

No. 5235 SATURDAY FEBRUARY 28 1970 Vol. 225

CONTENTS

NON-EXISTENT MACHINERY DOES NOT FUNCTION 777

Old World—France to Double Research Budget—Plant Breeding—Television—Academic Cooperation—Environment—Auctions—SRC Fellowships—Oil—Moon—Soviet Union—Further Education—World Wildlife Fund—Research Stations—Parliament in Britain—Miscellaneous Intelligence 778

New World—TRENDY RESEARCH PROGRAMMES WIN FRIENDS—NAS—General Biology—Research Finance—Telecommunications—Space Research . . 785

News and Views—PROTEIN-LOVER'S BAEDEKER—Documentation—Superconductivity—Sedimentology—Geology—Amphibia—Tumour Virology—Genetics—Antibiotics—Health 791

BRAIN MECHANISMS AND MOTIVATION by Keith Oatley 797

AMINO-ACID SEQUENCE OF PORCINE PANCREATIC ELASTASE AND ITS HOMOLOGIES WITH OTHER SERINE PROTEINASES by D. M. Shotton and B. S. Hartley . . 802

THREE-DIMENSIONAL FOURIER SYNTHESIS OF TOSYL-ELASTASE AT 3·5 Å RESOLUTION by H. C. Watson, D. M. Shotton, Joyce M. Cox and Hilary Muirhead . 806

THREE-DIMENSIONAL STRUCTURE OF TOSYL-ELASTASE by D. M. Shotton and H. C. Watson 811

MULTIPLE FORMS OF HUMAN BRAIN MITOCHONDRIAL MONOAMINE OXIDASE by G. G. S. Collins, M. Sandler, E. D. Williams and M. B. H. Youdim . . . 817

RE-EVALUATION OF THE SYNAPTIC ACTIVATION OF AN ELECTROGENIC SODIUM PUMP by JacSue Kehoe and P. Ascher 820

NEUROTROPHIC CONTROL OF PROTEIN SYNTHESIS IN THE REGENERATING LIMB OF THE NEWT, *Triturus*, by Paul Lebowitz and Marcus Singer 824

CHARACTERIZATION OF THE GERMINATION RESPONSE TO TEMPERATURE OF SPECIES AND ECOTYPES by P. A. Thompson 827

Letters to the Editor (see overleaf) 832

Book Reviews by Lucille Bitensky, J. B. Finean, E. J. Holborow, Peter Barlow, F. G. J. Hayhoe, Peter Kelvin, M. Ebert, D. A. Edwards and L. Rosenfeld . 877

Correspondence—DDT in Milk by Göran Löfroth; Enzymes in Crystals by Hans Neurath, Kenneth A. Walsh and Ralph A. Bradshaw; Sternglass's Assumptions by Philip L. Taylor; View that may Upset Scientists by Ian Martin . . . 881

University News—Appointments—Announcements—Sabbatical Itinerants . 882

RECENT SCIENTIFIC AND TECHNICAL BOOKS xvii

Nature in North America: The *Nature* office in Washington DC has opened and contributors in the United States and Canada are invited to submit manuscripts for publication either to the address given below or to the present London office. The intention is that each office will be able to answer inquiries about all manuscripts and other matters. This is one of several steps by which it is hoped to provide a better service for readers and contributors.

London Office
4 Little Essex Street,
London WC2.
01-836-6633

Washington Office
711 National Press
Building,
Washington DC 20004

(Continued overleaf)

Unicam SP1800 Ultraviolet Spectrophotometer
Power
Deuterium Lamp
Scale divisions
Absorbance or Concentration
Meter Range
0-2
0-1
0-0.5
0-0.2
Concentration
Zero
Unicam SP 8000 Ultraviolet Spectrophotometer

Unicam UV Spectrophotometers make light work

What does this mean to you ? In research, results you can trust and repeat. In routine applications, error-free operation by the least experienced member of your staff.

When you come to Pye Unicam, even the selection of your UV spectrophotometer is simple. There is a choice of instruments broad enough to meet your every requirement and to match your budget, with a staff of experienced application specialists to back them up.

Just look at what is available . . . **SP500 Series 2** and **SP600 Series 2** quantitative single beam spectrophotometers, **SP700** for research, **SP1800** for low-cost double-beam operation, **SP3000** automatic digital instrument, **SP8000** for the ultimate in flexibility, and the **SP1300 Series 2** low-cost colorimeter.

Write or 'phone for free information today. That's simple too, isn't it.

Pye Unicam Ltd
York Street Cambridge, CB1 2PX, England
Telephone (0223) 58866 Telex 81215

1966 1969

PYE UNICAM

LETTERS TO THE EDITOR

Page

PHYSICAL SCIENCES

Variable galaxy—PK 0048–09 a radio variable—STULL (Michigan) 832

Interstellar dust—The garnet hypothesis—HUFFMAN (Arizona) 833

NP 0532—Effect of interstellar grains on X-rays—NARANAN and SHAH (Tata Institute of Fundamental Research) 834

Pulsars—Are they the explanation of the X-ray background ?—APPARAO (Tata Institute of Fundamental Research) 836

—Search for second periodicities—HESSE (Cambridge) 837

NP 0532—More about odd behaviour—RUDERMAN (Columbia) 838

Pulsars—Search for pulsed gamma rays—CHATTERJEE, MURTHY, MURTHY, SREEKANTAN and TONWAR (Tata Institute of Fundamental Research) 839

Planets—Dynamical relaxation and Bode's law—HILLS (Michigan) 840

Moon—Were the maria formed by "sea-floor spreading" ?—ELDER (Cambridge) 842

Geophysics—Symmetry of crustal earthquakes above Benioff zones—HATHERTON (DSIR, Wellington) 844

Thermoluminescence—Molecular oxygen as an electron trap in the thermoluminescence of saturated hydrocarbons—BOUSTEAD (Royal Military College of Science) 846

Electrons in solids—Electron channelling patterns from small selected areas in the scanning electron microscope—VAN ESSEN, SCHULSON and DONAGHAY (Oxford) 847

Fluid mechanics—Particle migration in Poiseuille flow—GOTOH (Hokkaido) 848

BIOLOGICAL SCIENCES

Influenza—1918 pandemic attributed to swine virus rather than human AO group—STUART-HARRIS (Sheffield) 850

Transplantation—Where Ag-B histocompatibility exists, heart transplants survive longer than skin—BARKER and BILLINGHAM (Pennsylvania) 851

Histoincompatibility — ^{125}I-labelled antigen for immune assay — FOSCHI and MANSON (Wistar Institute) 853

Immunoglobulins—Pepsin digestion distinguishes the subtypes of IgG—TURNER (London), BENNICH (Uppsala) and NATVIG (Oslo) 853

Rheumatoid factors — Anti Gm reactivity with immunoglobulin fragments — NATVIG (Oslo) and TURNER (London) 855

Page

Cytology—Reversion of blastoid cells to small lymphocytes does not involve cell division—POLGAR and KIBRICK (Boston) 857

Cell movement—Rebuttal of "passive theory" rebutted—CARTER (ICI Pharmaceuticals) 858

DNA — Ionizing irradiation promotes binding of amino-acids—BYFIELD, LEE and BENNETT (California) 859

Haemoglobins—Normal and unstable variants synthesized at similar rates—WHITE and DACIE (London) 860

Growth hormone—Alteration of development of drug metabolizing enzymes of the liver—WILSON (Children's Hospital of San Francisco) 861

Hormones—A new polypeptide regulating blood flow—SAID and MUTT (Karolinska Hospital) 863

Renin—Activity after binding to agarose—SEKI, JENSSEN, LEVIN and ERDÖS (Oklahoma) 864

ATP—Net synthesis by reversal of Na$^+$ pump—LEW, GLYNN (Cambridge) and ELLORY (ARC) 865

Muscular contraction—What are the roles of the various types of fibres ?—EDGERTON, SIMPSON, BARNARD and PETER (California) 866

Monoamines—Lithium counters increased excretion of dopamine in mania—MESSIHA (Maryland Psychiatric Research Center), AGALLIANOS and CLOWER (Spring Grove State Hospital, Baltimore) 868

Neurochemistry—RNA metabolism in rat visual cortex—DEWAR and READING (Edinburgh) 869

Visual pigments—Nearly all the protein in photoreceptor membranes is rhodopsin—BOWNDS and GAIDE-HUGUENIN (Wisconsin) 870

Vision—Interocular transfer of suppressive effects of retinal image displacement—MacKAY (Keele) 872

Sleep learning—Not supported by new experiments—BRUCE (Cambridge), EVANS (NPL), FENWICK (London) and SPENCER (Watford College of Technology) 873

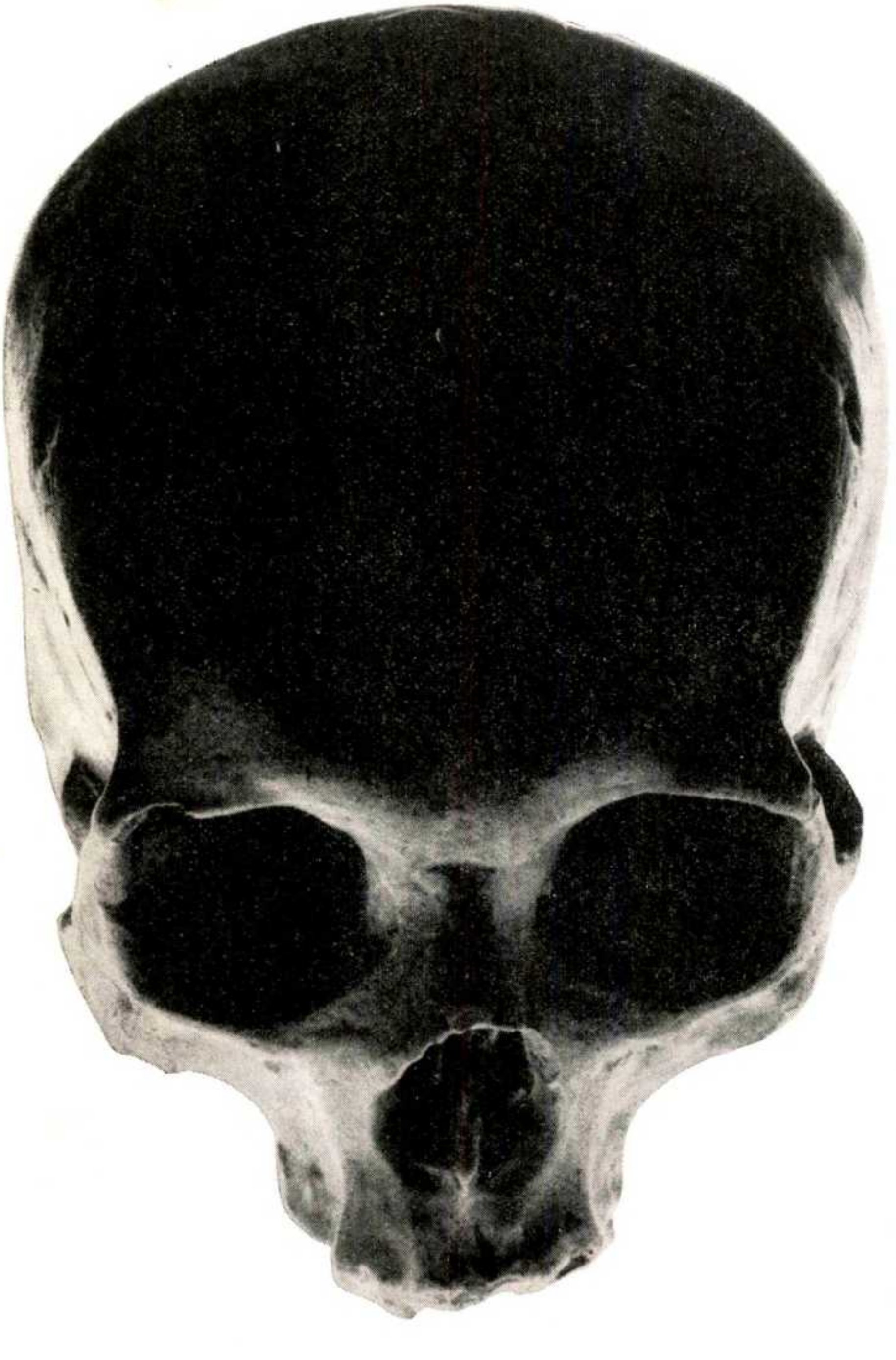
Haploids—High frequency production in barley—KASHA (Guelph) and KAO (National Research Council of Canada) 874

Martian life—Oxidation of carbon monoxides as a possible energy source—WOLFGANG (Colorado) 876

The titles on this page are prepared by the staff of *Nature* on the basis of the titles submitted by authors which appear in full on the pages indicated

YOU'VE JUST UNEARTHED BONE.

WITHIN WEEKS WE CAN TELL YOU ITS AGE.



Our radiocarbon laboratory and counting facilities can give you the fastest service available anywhere. A complete age determination report of your bone specimen will be in your hands within weeks.

And, the stress is on accuracy. Not only do we routinely date your bone specimens directly by the collagen method, at the same cost as normal radiocarbon dating, but our C^{12}/C^{13} analysis can compensate for natural isotopic fractionation.

Prices are based on the number of samples we date for you during any given 12 month period. Prices range from $170 for a single sample to $130 per sample for 50 or more samples. For C^{14} plus C^{12}/C^{13} analysis, the charge is $195. Again, less for multiple analyses.

For complete details, call or write ISOTOPES, 50 Van Buren Ave., Westwood, N.J., 07675. U.S.A.

ISOTOPES

A TELEDYNE COMPANY

NATURE Volume 225
FEBRUARY 28, 1970

Non-existent Machinery does not Function

THE examination of the budget of the National Science Foundation for 1971 which has been going on in Washington for the past ten days (see page 785) has only served to emphasize the long-standing deficiencies of the machinery for administering the money spent on university research in the United States. The latest trouble is that agencies such as the Department of Defense and the National Aeronautics and Space Administration are being forced not merely by the general shortage of money but also by a formal request from Congress—the Mansfield amendment to the defence budget for the present financial year—to apply with increasing stringency to proposals for research expenditure the tests of relevancy. The result is that a great many worthy projects are likely to be dropped by agencies which only a few years ago were trying hard to breathe life into them—in this spirit, for example, NASA will be spending literally nothing on long term research projects which have in the past cost $9 million a year, and will be trimming its sails in other fields as well. Unfortunately, however, there is no guarantee that these abandoned projects will find sponsors elsewhere. The National Science Foundation has $10 million in its budget for succouring the casualties, but this could easily fall short of the real need which will nevertheless not become apparent for several weeks. The biggest uncertainty lies in the spending of the Department of Defense. How much of the $370 million which has been requested for research as distinct from development will eventually fail to meet Congress's stern if somewhat arbitrary criteria of relevance? With a little bad luck, the National Science Foundation will have a crowd of orphans on its doorstep.

The first thing to be said is that the lack of coordination between the plans of the several government departments is excusable but nevertheless intolerable. The projects now at risk include schemes for training graduate students in noise engineering and schemes for operating particle accelerators—it seems, for example, that the accelerator shared by the University of Pennsylvania and Princeton University will be closed down. Although the sums of money involved are comparatively small, the careers of able people are bound up in them and the changes now in prospect, by their suddenness and the sense of arbitrariness which they convey, are bound to unsettle not merely those directly concerned but all those who depend on the knowledge that modern research must be sponsored by modern government. So why should not the shift of interest in the mission oriented agencies, inevitable as it has been, have been better coordinated? Now that the annual budget has been dumped more or less in the lap of Congress, the Administration can wring its hands and draw attention to the way in which scientific expenditure falls within the jurisdiction of a score of congressional committees. But should not something have been done to anticipate these problems and to arrange for a more orderly transfer of responsibility from one agency to another? After all, the National Science Foundation has power to take over research projects sponsored by other departments if both agencies are agreeable. Would it not have been better to have arranged for transfers of this kind instead of waiting for muddle to become overt? And is this not in any case what the Office of Science and Technology is for? Or is the coordination needed for the smooth administration of university research so far outside that office's power that some other instrument is necessary?

In one sense, of course, the gap between expectation and reality which has now emerged could have been predicted several years ago. In the fifties, when every agency of the federal government seemed a potential fairy godmother, the scientific community in the United States relished the opportunity which this diversity provided for playing off one benefactor against another. The result, unfortunately, was that most university research came to be sponsored by agencies whose real interests lay elsewhere. As things are, agencies other than the National Science Foundation provide more than 85 per cent of all the federal money that finds its way into academic science. Nobody can therefore be surprised that during a period when the agencies with other jobs to do have been under severe pressure to economize, the total expenditure on scientific research in the universities has been declining, both absolutely and in real terms. The increase of two per cent in the estimate of what will be spent in universities in the coming financial year will not outweigh the effect of inflation on the real cost of doing scientific work. In short, even if Congress agrees to what the Administration has asked of it, the coming year will be another bad one for university research. The shadow cast by the old plurality of research sponsors is long. The question now is how damaging it will be.

This is where the report of the National Science Board, published last week (see page 789), should be taken as a starting point. The decline of expenditure on university research which began in 1967 seems destined to continue. The implication is that the United States has decided, perhaps without acknowledging as much, that the time has come when university research should consume a smaller share of what the federal government has to spend. Dr Philip Handler was right last week to point to the consequences there must soon be for the output of graduate students from the universities and colleges which have in the past two decades striven successfully to meet what then seemed to be an urgent need. The report of the National Science Board itself makes vivid the way in which a prolonged halt to the growth of university research may damage not merely the pattern of university research and teaching but, in the long run, the capacity of industry in the United States to renew itself. At a time when only interdisciplinary proposals for dealing with the environment seem to have an easy passage, the appearance of the National Science Board's report on the physical sciences may serve as a challenge to the government and those who pay its taxes to make an explicit decision about the scale of university research in the sciences in the years ahead. It will be strange if the achievements of postwar research in the United States can be discarded as lightly and as passively as the budgets of the past few years imply.

But what is to be done ? The National Science Board, which manages the National Science Foundation as well as making public pronouncements, is in the invidious position of having to recommend that the best course is to channel more money through the National Science Foundation. This, however, has been beyond dispute for several years. Only when a substantial fraction of university research is supported in this way will it be possible for the merits of competing proposals to be objectively assessed and for the recipients of what money there is to spend to be assured of the kind of continuity on which good planning must depend. Those who doubt this proposition should be convinced by the quite scandalous way in which radioastronomy is being neglected in the United States—the best that the federal government has been able to secure for the radio-astronomers among its pensioners is $800,000 for resurfacing the instrument at Arecibo and the promise on behalf of the Jet Propulsion Laboratory that five per cent of the time on instruments in the Deep Space Tracking Network should be made available to academics. The way in which, by all accounts, a great deal of work in organic chemistry is being threatened by the tighter budget at the National Institutes of Health should be another proof of the need for the kind of continuity that can only be provided by an agency which has not merely the will to fight for what it believes in but the power to do so successfully. This is why it should now be a first objective, among scientists in the United States and by the National Science Foundation itself, to see more growth in the years ahead. The signs are that when all the chickens have come home to roost, the foundation will be as hard pressed in 1971 as it has been in the past. In the circumstances, it may well have been too soon to embark on some of the admittedly popular but possibly speculative projects which the foundation is about to embrace.

OLD WORLD

France to Double Research Budget

from our Paris Correspondent

For the past few days, members of the government have had on their desks the reports of the various commissions appointed to prepare the Sixth Plan. When these documents have been revised or approved by the Council of Ministers, they will be forwarded to parliament, where they will be discussed during the spring session. The commissions will then have to work out in detail ways of carrying out the projects that have been chosen. These will come into effect in 1971 and extend to the end of 1975.

It is probable that the report on the various possible research and development projects (the text of which runs to some twenty pages or so) will not be modified much in the course of the proceedings that have just begun. Thus the selections made by the Commission of Research give us quite a good idea already of what French scientific policy will be, *deo volente*, during the coming five years. This is provided, of course, that the execution of the Sixth Plan is not impaired along the way by economic difficulties like those which prevented the objectives of the Fifth Plan from being reached.

Expenditure on research and development now represents 2·2 per cent of gross home production (this criterion is now preferred to gross national product, which has been used in calculations until now). It should reach 3 per cent in 1975 if the annual rate of growth of the economy is only 5·4 per cent as had been originally forecast (in fact, the rate of growth chosen by the government will without doubt be greater than 6 per cent). In practical terms this means that expenditure on research and development will increase by an average of 13 per cent every year to reach 32,500 million francs in 1975, which is about double the present figure. The proportion of this total supported by the state will stay more or less at 70 per cent. On the other hand, it should be noted that the expected development will particularly favour civil expenditure; military research and development will certainly grow in volume but will remain stationary in relative terms.

Execution of the plan, however, does not only depend on financial resources but is strictly limited by the available manpower. The number of scientists cannot be increased at the drop of a hat and measures agreed on last week in the field of education will only bear fruit during the Seventh Plan (1975–80). Thus the existing manpower must be deployed in other ways than in the past. The commission believes that in the course of the next few years France will need a majority of young scientists (that is, 65 per cent instead of 35 per cent) to begin their careers in research, with a part of these then going into production work and teaching.

Another important change lies in the way in which money is used and this points up characteristically the distance between the politics of General de Gaulle and those of M. Pompidou. In fundamental research the accent will be on the biological and social sciences, where the annual rate of growth will be about 23 per cent. The study of the interaction between man and his environment and the struggle against pollution will be given preferential treatment. On the other hand, nuclear physics and high energy physics, both of which have for a long time been given priority, will be pegged at a growth rate of only 5 per cent. The fields of chemistry and physics will develop at the rate of 15 per cent a year.

Notable changes are in store for industrial research and development. The grand and spectacular projects favoured by the previous government (aeronautical programmes, nuclear complexes and the computer project Plan Calcul) will be severely restrained and France will be more circumspect before becoming involved in any more operations of the same kind. The purchase of foreign licences will be encouraged when this can obviate costly duplication of effort made in other countries. The execution of research and development programmes will, as far as possible, be the responsibility of industry rather than state organizations. The part devoted to development in the overall expenditure will increase. Among these sectors to which the commission attaches priority is that of housing, urban development and transport, which has admittedly been scandalously neglected until now. Other priority areas will be chemistry, metallurgy, mechanics and, of course, electronics. The writers of the report, obviously wary of lavish expenditure, declare forcefully that in the tactical choices stemming from this new policy, "the attribution of state aid should systematically take into account the probability of technical success and the possibility of profit from the proposed operations". It is, of course, surprising that these common sense rules should need to be solemnly declared in an official document after a decade of concerted scientific policy. But better late than never.

PLANT BREEDING

Fears of the Private Sector

The Agricultural Research Council (ARC) has decided that it cannot accede to the wishes of dissatisfied plant breeders who want the council's institutes to cease producing new varieties of plants. The present dissatisfaction has grown up since the passing of the Plant Varieties and Seeds Act in 1964, which made it possible for breeders to obtain royalties on the sale of new plant varieties. This was intended to encourage private companies to take more part in the breeding of new varieties. This task had for many years been largely the concern of state organizations, which were not obliged to make a profit on their new material. But many private breeders still feel that they are at an unfair disadvantage in trying to compete with institutes which have their overheads paid by the government. As a result, there has emerged a strong lobby which calls for the activities of the ARC to be restricted to fundamental research in plant breeding.

In response to this pressure the council has reviewed its policy with respect to the support of plant breeding at state aided research institutes. The decision to make no change was taken in the light of reports from the directors of the plant breeding institutes in Cambridge, Aberystwyth and Pentlandfield, Scotland, and from Mr G. C. Stevenson, the council's adviser on plant breeding. After visits to private breeders, Mr Stevenson has come to the conclusion that they are not ready to take over full national responsibility for producing new plant varieties. Apart from the fear that the private sector could not cope with all the breeding that must be done, there is concern for the morale of ARC workers who would have to hand over their work to private breeders once a new variety was in sight. This, the council thinks, would be a bad thing. Furthermore, the council sees its institutes as useful training grounds for plant breeders; a claim which must be supported by the companies that have recruited scientists from ARC staff.

The decision not to reduce ARC effort devoted to the breeding of new varieties does not mean that the worries of the private sector are being brushed aside completely. It is, after all, responsible for developing many of the new varieties of vegetables grown in Britain, although the state aided institutes dominate when it comes to cereals and grasses. There is already cooperation between state and private breeders, and the ARC is now looking into the question of extending this.

TELEVISION

Graph with a Slipped Slope

An unfortunate slip of the pen has been made on the drawing boards of Television House, London, the headquarters of Thames Television. Last month, in an issue of the "Today" programme, Professor Ernest Sternglass of Pittsburgh University exhibited a graph depicting the infant mortality in England and Wales since 1935. Many of those who watched the programme

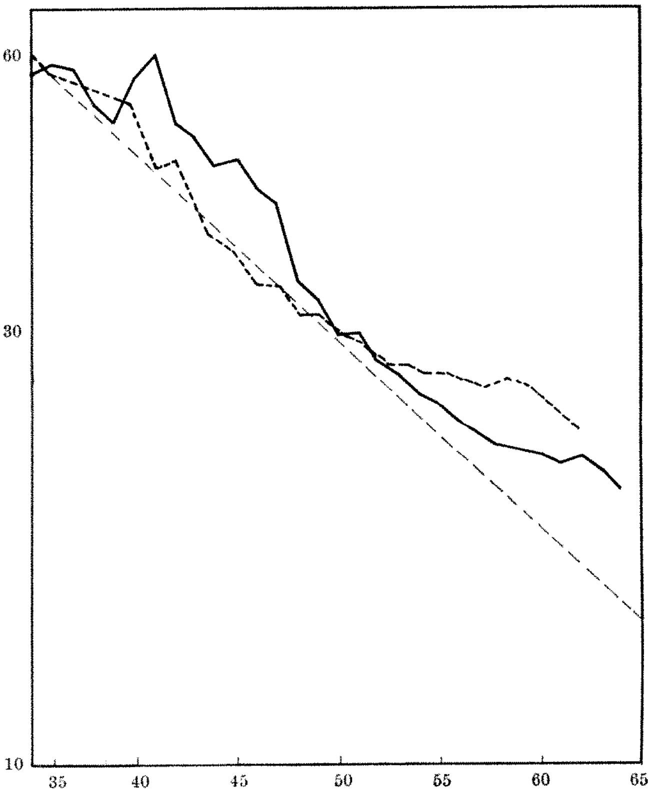
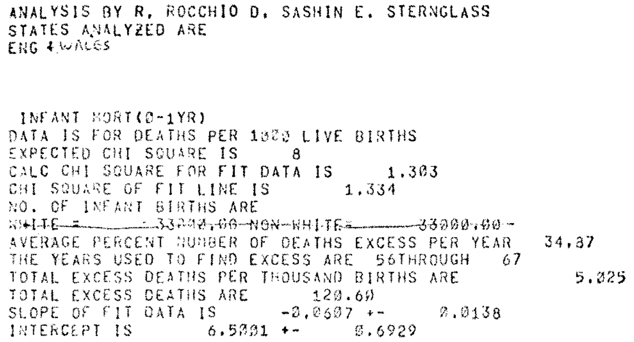


Fig. 1. Infant mortality in England and Wales (deaths per 1,000 of population). Solid line: graph based on statistics supplied by the Registrar General. Broken line: graph prepared by Thames Television for display on "Today" programme.



```
ANALYSIS BY R, ROCCHIO D, SASHIN E. STERNGLASS
STATES ANALYZED ARE
ENG & WALES


 INFANT MORT(0-1YR)
DATA IS FOR DEATHS PER 1000 LIVE BIRTHS
EXPECTED CHI SQUARE IS     8
CALC CHI SQUARE FOR FIT DATA IS      1.303
CHI SQUARE OF FIT LINE IS          1.334
NO. OF INFANT BIRTHS ARE
~~WHITE =          33000,00 NON-WHITE=          33000,00 -~~
AVERAGE PERCENT NUMBER OF DEATHS EXCESS PER YEAR   34,37
THE YEARS USED TO FIND EXCESS ARE  56THROUGH   67
TOTAL EXCESS DEATHS PER THOUSAND BIRTHS ARE              5.025
TOTAL EXCESS DEATHS ARE        120.60
SLOPE OF FIT DATA IS       -0,0607 +-      0.0138
INTERCEPT IS        6.5001 +-      0.6929
```

Fig. 2. From the print-out of a computer program designed to calculate "excess deaths" in various countries.

must have been alarmed by the graph, because Professor Sternglass used it to show that something like 100,000 British children may have died since 1950 because of nuclear fall-out.

Alarm of a different kind was felt by another of the participants in the programme, Professor Joseph Rotblat of St Bartholomew's Hospital, London, who had also prepared a graph of infant mortality in England and Wales, based on the statistics issued by the Registrar General. As can be seen from Fig. 1, the two graphs bear little relation to each other. A few days after the programme Professor Rotblat wrote to Thames Television to point out the discrepancy and suggested that a correction should be broadcast. Copies of the letter were sent to other participants in the "Today" programme, including Lord Kennet, who raised the matter with the Independent Television Authority. As the result of an inquiry by the ITA, Thames Television announced last week that the exhibited graph had been wrongly drawn but that the burden of Professor Sternglass's argument was not affected.

How can it have happened that the graph prepared by Thames Television was so totally at variance with the true data? From the reply sent by Professor Sternglass to Professor Rotblat and the other participants, it seems that the draughtsman at Thames Television was furnished not with exact figures but with a graph that had to be copied. But the figures used by Professor Sternglass to compute the tally of 100,000 "excess deaths"—the difference between the actual figures and a linear extrapolation of the apparent rate of decline in infant mortality between 1946 and 1955—are almost identical to those of the Registrar General. Professor Sternglass comments, "Any inaccuracies in the charts prepared by the draughtsman in the Thames Television studio without the benefit of actual numbers, although regrettable, do not appear to be so serious as to have given a misleading impression".

However, in the chart shown on the "Today" programme the infant mortality graph for England and Wales was displayed together with the corresponding graph for the United States. For both of these the slopes of the straight lines were drawn parallel, thus giving the striking impression of exactly the same behaviour in the two countries. With the true data for England and Wales, Professor Sternglass himself can only draw a straight line between 1946 and 1955 instead of 1935 to 1950 as on the chart displayed in the programme.

Another puzzling feature of the "Today" programme that has since come to light is the figure of 100,000 excess deaths calculated by Professor Sternglass for England and Wales. A copy of the computer print-out on which the figure was based has been supplied to *Nature* by Thames Television (Fig. 2), from which it appears that the "total excess deaths" are "120·60" or presumably 120,600. The computer presumably arrived at this figure by multiplying the "total excess deaths per thousand births" (5·025) by "years used to find excess" (12) and 1/1,000 × the average total of annual births in England and Wales. On this assumption the average total of annual births works out at a neat 2,000,000. If this were true Malthus would have leapt triumphantly from his grave long ago. Total live births in England and Wales between 1956 and 1967 (the period of Professor Sternglass's "excess deaths") have averaged about 800,000. On this basis the "excess deaths", according to Professor Sternglass's hypothesis, would be nearer to 48,000 than 120,000. That is still a lot of deaths, but it seems that something a bit more reliable than Professor Sternglass's computer or the draughtsmen of Thames Television is needed to prepare the certificates.

ACADEMIC COOPERATION

Another Name for Kew

by our Botany Correspondent

The special relationship between Kew and Reading has been made formal by the recognition of the Royal Botanic Gardens as an associated institute of the university. This sets the official seal on cooperation in teaching and research which has been steadily increasing during the past ten years. The director of Kew, Sir George Taylor, has been made a visiting professor of the university, and several of his colleagues have become members of the academic staff.

At present, cooperative ventures include an MSc course in pure and applied plant taxonomy, run jointly by staff at Reading and Kew. Botanists from Kew take part in graduate seminars at Reading, and also supervise PhD students from the university. Collaborative research includes the *Flora Europea* project which has been centred at Reading since one of the editors, Professor V. H. Heywood, became professor of botany there. The director of Kew is an advisory editor for this project.

These and similar joint activities are expected to increase in the future, although exactly what will happen depends to some extent on the attitude of the new director of Kew, due to be appointed soon on Sir George Taylor's impending retirement.

ENVIRONMENT

Appointment of Royal Commission

The Royal Commission on environmental pollution holds its first meeting this week. The commission is chaired by Sir Eric Ashby, Master of Clare College, Cambridge, and former professor of botany at Manchester University, and will be responsible in the first instance to Mr Anthony Crosland, Secretary of State for Local Government and Regional Planning. The commission is intended to be permanent, and has been given terms of reference that charge it "to advise on matters, both national and international, concerning the pollution of the environment; on the adequacy of research in this field; and future possibility of danger to the environment".

The interpretation of these terms depends largely on the members of the commission, who will also be free to decide when and how to report, what priority to give to international rather than British problems, and what sort of research is to be assessed. Advisory machinery on environmental matters already exists in the form of bodies such as the Natural Environment Research Council, but because the commission has no executive powers it is expected to provide a more general view by taking into account the arguments of both polluters and conservationists.

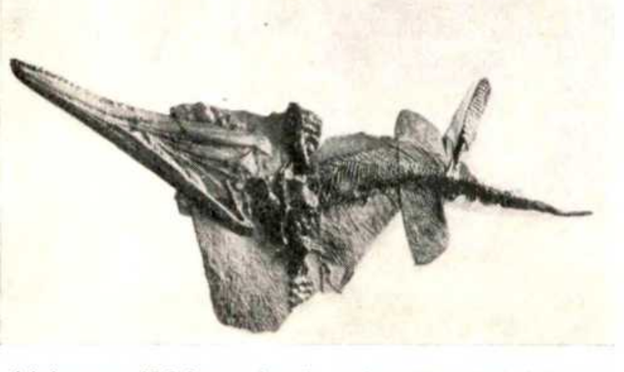
Other members of the commission are Dr W. Beckerman (professor of political economy at University College, London), Mr Aubrey Buxton (British Trustee of the World Wildlife Fund), Dr F. Fraser Darling (vice-president of the Conservation Foundation), Professor V. C. Wynne-Edwards (chairman of NERC), Dr W. L. S. Fleming (Bishop of Norwich), Mr N. A. Iliff (Shell Chemicals (UK), Ltd), Sir John Winnifrith (National Trust), and Sir Solly Zuckerman (the chief scientific adviser to the government).

AUCTIONS

Ichthyosaur under the Hammer

An ichthyosaur that paddled on Lower Jurassic Seas 150 million years ago and came to grief somewhere near the present site of Lyme Regis has now been washed up in the sales rooms of Sotheby's, the London auctioneers, and will fall to the highest bidder on March 5. For many years the ichthyosaur occupied a niche 17·5 feet long and 4 feet high on the walls of a corridor in the Grant Institute of Geology at Edinburgh. Because the corridor at that point had to be turned into an office, and there was no other place to house the ichthyosaur, Professor G. Y. Craig decided after consultation with the Royal Scotland Museum to put the creature up for sale. Market prices for small ichthyosaurs exceed £2,500 and Professor Craig will be "reasonably happy" if the Edinburgh ichthyosaur makes as much as £30,000.

The museum of the Grant Institute has no records of how the fossil came into its possession, but the presumed site is the Lower Lias of Lyme Regis, Dorset, where the first identified specimen of an ichthyosaur was discovered in 1810. The specimen, though sans its right hind paddle, is a fine example of *Ichthyosaurus platyodon* (Conybeare).



Ichthyosaur, 17·5 feet in length, to be sold at Sotheby's on March 5.

The ichthyosaur is joined in the sale by minerals, shells and other fossils including trilobites, ammonites and several fossil fish such as a Jurassic age *Leptolepsis sprattiformis*, 2·5 inches in length.

SRC FELLOWSHIPS

More for Fewer

From next October the Science Research Council is cutting the number of its postdoctoral fellowships from forty to twenty-five a year but is making them more valuable. The new fellowships will be worth between £1,450 and £1,800 a year, compared with the present value of £1,045 to £1,300. As a further bonus fellows who are working in Britain will be allowed for the first time to apply for SRC research grants on top of the basic award. The fellowships, which usually last for two years, are given to outstanding young researchers so that they can spend all their time on their investigations.

The changes imply that the SRC may be spending several thousand pounds less on the fellowships each year: with a mean figure for the value of a fellowship the expenditure would fall from about £47,000 to nearer £41,000. The SRC points out, however, that its total backing for the fellows is likely to increase because of their eligibility for grants. It seems that this increase will come simply from money that is earmarked for grants anyway, for the fellows who apply for grants will be judged on merit in the same way as anybody else.

In its report for 1968–69 the SRC said that it wanted to make its support of research more selective with respect to the quality of the people it finances and the practical relevance of their subjects. The new fellowships are accordingly designed to give better opportunities to an even more select few: the SRC is basing its choice entirely on ability and independent achievement. But it is not picking out any field for special benefit, and has not changed its industrial fellowships or its one-year awards to British scientists who want to come back from North America. Full details of the new scheme will be published shortly in an SRC booklet on *Studentships and Fellowships 1970*.

OIL

Search in Liberia

from our Geomagnetism Correspondent

Liberia, well known for allowing its flag to be flown on tankers carrying other people's oil, has begun to seek oil of its own. No oil has actually been found on Liberian territory, but scientists from the US Geological Survey and the Liberian Geological Survey who have been surveying the region report that "the potential for petroleum deposits beneath the continental shelf of Liberia is promising".

This discovery comes as a result of a joint US–Liberian programme of mineral resources investigations covering dry land as well as the continental shelf. The principal impediment in the search for oil on land has been the thick layer of residual soils, produced by tropical weathering, which masks most of the bedrock. Moreover, swamps, sandy savannas and bush have made ground-based studies of the underlying geology difficult if not impossible. But the resources and experience of the United States have now been used to provide airborne gravity and geomagnetic surveys. Mapping of the coastal areas has thus revealed the presence of at least two onshore structural basins filled with Cretaceous sediments. Preliminary surveys of the adjacent continental shelf and slope suggest the existence of similar basins there also. Such discoveries are guaranteed to make oilmen's (and governments') hearts jump for joy, for similar geological environments throughout the world are the sources of most of the oil produced today.

MOON

Moon Rocks Begin the Rounds

NASA has begun the distribution of 28·6 pounds of scrapings from the Ocean of Storms, and hard on its heels go the astronauts who did the digging on their obligatory exhausting itinerary of twenty countries in forty days. Other countries, the White House says archly, could well be added later. But the dissemination of the Apollo 12 lunar material to 139 scientists in the United States and 54 elsewhere will be less dignified than on the previous occasion, when Dr S. O. Agrell, of the University of Cambridge, made a much heralded arrival at the London offices of the Science Research Council with the British share in a canvas bag. This time most of the material will travel by registered mail, or by diplomatic courier, although some scientists will still be going to Houston to collect their samples personally.

NASA expects to get back 23·7 pounds of the Apollo 12 material—the rest will go in destructive experiments—and the analyses are to be presented at the January 1971 Lunar Science Conference at Houston.

The number of principal investigators involved shows an increase of 51 on the Apollo 11 list, when there was 18 pounds of material to share. One of the additions is Dr M. Vobecky, of the Nuclear Research Institute of the Czechoslovakian Academy of Sciences, who will be receiving a gram of fines and a gram of rock specimens for element abundance work by neutron activation. NASA is also to distribute more Apollo 11 samples to scientists in the United States and Japan for second generation experiments, and the bits of the Surveyor spacecraft have already been released to JPL and to the Hughes Aircraft Co.

The credo behind the examination of the Surveyor bits and pieces is to see how machinery might survive exposure to the lunar environment, but it seems clear from investigations at the Manned Spacecraft Center that damage to the Surveyor during the Apollo 12 touchdown far outweighed anything that happened previously. According to a report from NASA, the surfaces of the TV camera and of a length of tubing removed from the Surveyor show numerous recent shallow craters which seem to have come from the direction of the landing site of the lunar module 600 feet away. The fresh craters show up white against the brown colour of the painted camera, and it is clear that the particles originated from the dust cloud raised when the lunar module landed. So far nine craters which might be due to genuine meteoroid impacts have been identified on the pieces which were exposed on the Moon for 950 days.

SOVIET UNION

Another Academic Village

from our Soviet Correspondent

A SITE in Siberia has been chosen, a few miles from Novosibirsk, for yet another in the series of "Academic Villages". The latest site is to comprise the new Siberian Centre of Agricultural Science and its associated institutes and living accommodation.

The site has been carefully chosen in connexion with the proposed experimental work of the Centre, being on the bank of the Ob' and in an area of gentle relief. A further advantage of the site (following the Russian proverb "Don't look for a house, look for a neighbour") is the presence, a few miles across the river, of the Academic Village of the Siberian Branch of the Soviet Academy of Sciences. A close cooperation is envisaged between the two Villages, especially on problems of agricultural biology and chemistry.

The new Centre, which is being organized by the "V. I. Lenin" All-Union Academy of Agricultural Sciences, will comprise five research foundations. The Institute of Agricultural Economics and the Institute of Mechanization and Electrification of Agriculture are being organized on the basis of local branches of the All-Union Institutes. The existing Siberian Scientific Research Institute of Stock-breeding will be developed here into a full-scale research and development institute. New foundations will be the Institutes of Chemical Applications and Animal Foodstuffs.

The residential quarters of the Village will be provided with the usual cultural and recreational facilities including a landscape park. The designs for the Village have been produced by the same team of architects that worked on the Village of the Academy of Sciences, across the river.

FURTHER EDUCATION

Eighteen and Beyond

by our Education Correspondent

THE Association of Teachers in Technical Institutions, a body representing 27,000 teachers in further education, has fired the latest shot in the debate leading up to a new education act, which is almost certain to be the subject of a white paper later this year. In a policy document (*Education for the Future*, ATTI, 2*s*), the association calls for the school leaving age to be raised to eighteen, for industrial training to be removed from the control of the Department of Employment and Productivity and placed under a new Department of Education, Science and Training, and for a national council to plan and coordinate the whole of further and higher education.

The ATTI believes that when the school leaving age is raised to sixteen, pupils should be allowed to take "linked courses" at colleges of further education, while remaining firmly based in the schools. Such courses, the association argues, would help to give young people some idea of various jobs, and of the importance of further education when they enter employment. But the ATTI also believes that full-time education to the age of eighteen should become mandatory "as soon as is practicable". Education for this age-group should not be confined to one particular pattern, however: "the association considers that there is advantage in maintaining both sixth forms in schools and full-time education in colleges of further education alongside experiments in tertiary institutions".

Placing the responsibility for industrial training with the Department of Employment and Productivity, and not with the DES, has, in the opinion of the ATTI, created unnecessary confusion. Some courses of further education, the association claims, are designed to correspond to schemes of training rather than to the needs of the young person, with the result that education and training are becoming separated, and the student does not acquire the theoretical understanding of his training which would allow him to adapt to changes in technique. The ATTI argues that these problems could be alleviated if responsibility for training were vested in a new Department of Education, Science and Training.

As far as the binary system is concerned, the ATTI is very sceptical of proposals to bring all institutions of higher education under a unified administrative structure. Although it accepts the fact that "the present structure has an arbitrary division between the autonomous and the non-autonomous sectors of higher education which is hard to justify", the proposed alternative "would produce a far more arbitrary division between the parts of the further education system offering higher education and the remainder, and would be unworkable as well as unjustifiable". The ATTI argues that the best way to rationalize and standardize course structures would be to reduce the number of local authorities controlling higher and further education, and to set up a National Council for Further and Higher Education, composed of representatives of government, local government, staff, students, industry and commerce. This body should keep the needs of further and higher education under constant review, while similarly constituted regional councils would be responsible for approving plans of local authorities. Once approval is given, however, the ATTI suggests that local authorities should continue to administer their own institutions.

WORLD WILDLIFE FUND

Anonymous Bounty

THE British National Appeal of the World Wildlife Fund has suddenly become richer by £250,000 through an anonymous donation given to assist the appeal's target of £1 million in European Conservation Year. In making the donation, the donor paid tribute to the part Peter Scott has played in wildlife conservation throughout the world and asked for the donation to be used to establish a Peter Scott Wildlife Endowment Fund, but gave no stipulation as to how the money should be spent. The World Wildlife Fund does not only seek to protect wild animals from film stars with a yen for exotic furs, but also has many other worthy causes and successes to its credit both in Britain and internationally. Between January and May last year, for example, the British National Appeal provided £25,453 for international projects, which included the sum of £13,747 towards the cost of enlarging the Coto Donaña Nature Reserve through the purchase of the Guadiamar Marshes in southern Spain, £1,536 for a road building project for national parks in Botswana, and £7,125 to help with the establishment of the Hai-Bar Wildlife Reserve in Israel. Nearer home, the appeal is supporting research on the effects of oil pollution on sea birds and on ecological communities, and several nature reserves are benefiting. The fund has not yet found any other generous benefactors to match Mr X, but it has eleven more months to go before the deadline.

RESEARCH STATIONS

How to Help Industry

GOVERNMENT research stations should undertake more industrially sponsored projects, should concentrate on mission-oriented research, and should allow their staff more opportunities to transfer to industry. These proposals, which in effect call for extension of the government's stake in civil research and development, have been put forward by Mr John Lyons, deputy general secretary of the Institution of Professional Civil Servants, a body representing more than 80,000 scientists, engineers and associated specialists in government establishments (*The Role of Public R and D in Exploiting Technology*, available from the IPCS).

Mr Lyons argues that government research and development provides an important source of new ideas, and of long and medium term research which industry cannot provide for itself. In particular, the government sector can bring a wide range of disciplines and techniques to bear on particular projects, whether it be "back-up" research or research carried out directly under contract to industry. Mr Lyons criticizes the attitude of the Confederation of British Industry, which believes that government research establishments not engaged on defence or community work should be shut down as soon as possible. Such a policy, Mr Lyons argues, would cause a straightforward loss of the effective employment of qualified technological manpower.

To provide a better service to industry, Mr Lyons suggests that the policy management of government research stations should be shared with representatives of industry. This and other measures would remove the basis of the CBI's criticism that government research establishments have no feel for market economics. Mr Lyons says that it would be much more useful if the Confederation of British Industry, "instead of devoting its considerable energies solely to knocking the civil work done by the highly qualified scientists and technologists in the government stations, should bend its efforts together with the institution to securing their freedom to assist industry in the most helpful way".

Government policy, Mr Lyons says, is the chief obstacle to providing a useful service to industry. Arbitrary limits to staff imposed on the research establishments seriously inhibit their ability to do all the work that industry requires, and directors of the establishments should be given more freedom to determine how much work they can take on a repayment basis. Decisions about the nature, amount and time-scale of the payments for industrial research should also be left to the directors of research establishments.

Parliament in Britain

Concorde

The British and French governments have given the go-ahead for six production models of Concorde, and a further four will be authorized next summer. Materials have already been made available for the first seven to ten. Mr Wedgwood Benn, who made this announcement, also estimated in a written reply that about £500 million out of the total joint development estimate of £730 million will be spent by the end of 1970. In answer to an earlier question, Mr Neil Carmichael, Joint Parliamentary Secretary, Ministry of Technology, said that sixteen airlines have signed option agreements for seventy-four models of Concorde. These agreements do not specify delivery dates, and these will be a matter of negotiation between the manufacturers and the airlines when definite contracts are signed. (Oral and written answers, February 23, and written answers, February 19.)

Sea Bird Deaths

Mr Gerald Fowler, Minister of State, Department of Education and Science, said that the Natural Environment Research Council expects to publish a report in May on the deaths of sea birds in the North Irish Sea. The council has been carrying out bacteriological and virological tests on the dead birds, and has also been studying information about weather conditions, the availability of food, and traces of pollutants in seawater. (Written answers, February 19.)

Teacher Training

Students at present undergoing courses of initial or further training in colleges of education number 111,077, and existing plans provide for an increase in the number of places to 119,000 during the next three to four years. Mr Fowler, who gave these figures, also said that some colleges failed to fill all their places at the beginning of the current academic year. (Written answers, February 20.)

Gas Centrifuge Plant

Dr Ernest Davies, Joint Parliamentary Secretary, Ministry of Technology, said that, although no decision can yet be taken about the siting of a plant to manufacture gas centrifuges, it would be desirable for assembly of centrifuges to take place near the uranium enrichment plant at Capenhurst. No estimates of staff numbers or other details can be made for some time, he said. (Written answers, February 16.)

Beagle Aircraft Company

The government's handling of the Beagle Aircraft Company came under strong attack in the House of Lords. The Earl of Kinnoull, who asked whether the government is aware of the deep concern over the company's present position, charged the Minister of Technology with showing total disregard for ministerial responsibility during the whole affair, and the Earl of Bessborough called for an investigation into the situation. Lord Delacourt-Smith, Minister of State, Ministry of Technology, said that the government had become involved in Beagle Aircraft substantially as a rescue operation, and that, although there is deep regret and disappointment at the failure of the company, the government could not continue to finance it. Lord Delacourt-Smith also said that no concrete proposals for linking Beagle with an existing British aviation company were put to him. (Debate, Lords, February 19.)

Miscellaneous Intelligence

Two hundred scientists in the University of Cambridge who submitted to an intelligence test have revealed their corporate acumen to range from 110 points to 141, with a mean of only 126·5 (*Journal of Biosocial Science*, **2**, 1; 1970). Indeed, the 15 per cent of the savants who scored below 118 would have been expected (and some did) to fail the 11+ examination. The residents of other Academes should not be quick to scoff or assume that their groves are greener; the Cambridge graduates in the sample gained significantly better scores than the alumni of other alma maters. Nor is it clear whether the survey was designed to demonstrate the inadequate wit of scientists or of intelligence tests.

In a speech made to the Royal Society of Arts last week on the achievements of medicine, Sir Brian Windeyer, the vice-chancellor of London University, said that "in our increasingly permissive society [the pill] has taken away an important constraint and has contributed to a greater laxity in sexual morals and to greater promiscuity. The pill has played a part in the disturbing increase in venereal disease . . . particularly in the teenage groups". This week Sir Brian, who was formerly professor of radiotherapy at the Middlesex Hospital in London, declined to cite the evidence for his belief that the pill has helped to spread venereal disease, saying that he did not claim to be an expert on family planning.

An alarming trend is afoot in high energy physics, if the latest issue of *Physics Letters* is anything to go by. The account of an experiment performed at 50 MeV was signed by five authors, each of two papers on experiments at 5·7 GeV was compiled by seven authors, fifteen signatures were appended to an article describing experiments at 25 GeV, and two 60 GeV experiments conducted at the Serpukhov accelerator were described by thirty-nine and forty authors respectively. Extrapolation of this trend reveals that when the 200 GeV accelerator at Batavia, Illinois, comes on stream the editors of physics journals may expect communications signed by 130 to 140 authors at a time.

A portent unparalleled in the annals of biology has become the talk of Palermo. The Sicilian city is home to two million rats which have recently begun to dispatch the local cats by "tearing out their throats and nibbling at them". The rats of Palermo, which outnumber the humans by three to one, are thought to consume some 200,000 tons of foodstuffs a year, not counting the cats.

From the rats of Palermo to the dogs of Tokyo. The 100,000 stray canines that now roam the Japanese capital are being rounded up lest a rabies epidemic should start among them. Tokyo dogs have never had it so good since 1687 when the Shogun Tsunayoshi decreed that on pain of exile the citizens should not only protect dogs but address them honorifically as Mr or Mrs Dog (O Inu Sama) as the case might be. The city government resolved an intolerable situation by removing some 50,000 dogs to a specially built shelter in the suburbs where they were fed on rice and dried fish at the expense of the taxpayers.

NEW WORLD

Trendy Research Programmes Win Friends

Dr William D. McElroy, the new director of the National Science Foundation, has had an easy ride in the past ten days during his first application to Congress for funds. This is not just beginner's luck. Many people are anxious to see the National Science Foundation put firmly on its feet, but this year there is also the advantage of a trendy programme. Environment is the watchword, and there have been several occasions during the foundation's evidence to the Daddario subcommittee when Dr McElroy's own work with dinoflagellates in the ecosystem of Chesapeake Bay has been used to show that the foundation now has its heart in the right place.

To make assurance doubly sure, however, the proceedings before the subcommittee on science, research and development of the House of Representatives Committee on Science and Technology were begun by Dr Philip Handler, chairman of the board of trustees at the foundation, and, as president of the National Academy of Sciences, one of the foundation's most powerful champions.

This is only the second year in which the National Science Foundation has been dignified by authorization hearings. On the face of things, the prospects are brighter than for a long time. The United States budget for the coming financial year (which begins on July 1) includes $513 million for the National Science Foundation, an increase of $63 million, although much of this will be spent on projects abandoned by other agencies. Yet there have been several previous years in which the foundation has found riches running through its fingers at the last minute.

Mr Emilio Q. Daddario, the chairman of the subcommittee, has his own ambitions for the foundation, and its witnesses have done everything they could to jump through those hoops. First, the argument goes, "we have foremost in our mind the deteriorating environment". So people are inclined to put the blame on science and technology—the real trouble is a failure to anticipate their consequences. Mr Daddario, for one, will look to the National Science Foundation for making this kind of assessment, known by the name technology assessment, but will expect it in the process not to hinder the swift application of new techniques. But how can that be done without strong university research and teaching? This is why, Mr Daddario said, he for one is worried that in the new budget, agencies such as the Atomic Energy Commission, the National Aeronautics and Space Administration and the Departments of Defense and of Health, Education and Welfare have less to spend on research and development. This is a "shortsighted and dangerous policy", and even though many of those now short of funds will turn to the National Science Foundation, the foundation "has not thus far been given the resources".

When like-minded men listen to each other's speeches frequently, they are liable to say similar things, which is why, no doubt, Dr Handler's evidence to the subcommittee may have seemed an echo of the chairman's speech. Dr Handler has, however, a different gloss on the problem of the environment. "While it may be true that the environment is deteriorating, there is no quantitative evidence. And although Lake Erie may be in such a state that it will take 100 years to put it right, for the most part the environment is better now than several centuries ago . . . you can now drink the water in any American city without the risk of typhoid." "We're still ahead," even if increasing vigilance is needed to keep ahead.

Dr Handler was more concerned with young people. These are "troublous times", with scientists in a quandary and young people disturbed and insisting that science is not relevant. So is it not time that young people were reminded of tales like that of cytosine arabinoside—the potential means of treatment

Table 1. PAST AND FUTURE EXPENDITURE ON SCIENTIFIC RESEARCH (MILLIONS OF DOLLARS)

Support of scientific research:	1969 (actual)	1970 (est.)	1971 (est.)
Scientific research project support:			
Atmospheric sciences	8,205,695	8,100,000	9,800,000
Earth sciences	7,917,288	8,000,000	8,800,000
Oceanography	19,381,729	18,400,000	19,000,000
Biological sciences	38,307,362	40,400,000	42,000,000
Physics	30,346,738	27,700,000	31,400,000
Chemistry	17,848,276	18,000,000	19,300,000
Astronomy	6,816,879	6,000,000	6,600,000
Mathematics	12,696,597	12,900,000	13,500,000
Social sciences	15,238,808	15,900,000	17,300,000
Engineering	19,265,222	19,600,000	22,300,000
Subtotal	176,024,594	175,000,000	190,000,000
Specialized research facilities and equipment	7,215,817	6,000,000	6,200,000
National and special research programmes:			
International Biological Program (IBP)	1,215,800	4,000,000	7,000,000
Interdisciplinary research relevant to problems of our society	–0–	6,000,000	13,000,000
Global atmospheric research programme	536,490	1,500,000	2,000,000
Earthquake engineering	–0–	–0–	2,000,000
Weather modification	2,433,974	2,500,000	3,000,000
Subtotal for research	4,186,264	14,000,000	27,000,000
Total	187,426,675	195,000,000	223,200,000

for some forms of leukaemia which has emerged almost by accident from academic work on sponges and the organic chemistry of biogenesis ? It is not an acceptable solution to "retreat towards nature"—the truth lies in the other direction.

Dr Handler had a trade union point to make about the supposed surplus of PhDs in the past few years, based as he described it on "a few anecdotes about a few theoretical physicists". In the past three months the National Academy of Sciences has been able to trace the fate of 80 per cent of the PhDs graduating in 1968 and 1969. Of the earlier group, only 0·4 per cent are at work where their training is irrelevant and 0·7 per cent are unemployed. For those graduating in 1969, the corresponding figures are 0·7 per cent and 1·1 per cent. Allowing for the incidence of pregnancy, travel and the like, there is no "national tragedy", even if some PhDs may not now be doing what they "expected to at the beginning".

What Dr Handler does protest about is the way in which the mission oriented agencies are being frozen out of basic research. He said that the provision tagged on to the budget for the Department of Defense for the current fiscal year, which requires that it should spend money only on research that can be shown to be directly relevant to defence, may by implication apply to other agencies. To be sure, he said last week, the situation in the fifties when the military dominated research and development in universities was unhealthy, but he regrets the Mansfield amendment "only in the sense that it is absolute". For one thing, both universities and the military would profit if there were strong links between basic science and the Department of Defense. On the danger that the Mansfield amendment may affect policies of other agencies adversely, Dr Handler pointed out that the National Institutes of Health, which used to be an important source of funds for organic chemistry, will now hardly ever support a grant application with the name of a plant in the title, with the result that investigators in photosynthesis were under pressure.

Dr McElroy was anxious to emphasize that the National Science Foundation's new look is represented by activities which add to the existing programme and are not substitutes for it. Where the new programmes are concerned, the text is that "the American people are increasingly united in the sense of emergency under the three closely interrelated banners of environmental quality, social relevance and, most recently, technology assessment". For all purposes, Dr McElroy said, new research and interdisciplinary research are necessary. The foundation has decided to earmark a "modest though significant" part of its budget for activities such as hailstorm prevention, earthquake engineering and the mathematical modelling of environmental systems. One novel feature of this new work is that the foundation may have to take the initiative in seeking out new proposals. Dr McElroy emphasized that he would not expect the foundation to be able to see the pattern of its new activities clearly "within a single year", but that in approving proposals for socially relevant activities, the foundation would be guided by the potential benefits to other federal agencies and by the extent to which specific research projects would help universities to play a part in "finding solutions to local problems". So far as the foundation's familiar activities are concerned, it plans to devote more of its institutional support for science "in a direction that will strongly recognize the research needs in the social sciences". In the past few years the foundation has been spending roughly ten per cent of its budget on the general improvement of good university departments—centres of excellence as they were once known—and is asking for $55·5 million for the coming fiscal year. In practice, the foundation's interest in the support of science education is tailing off ($96·9 million in 1971 compared with $121·7 million in the coming fiscal year) and within this tighter budget plans to emphasize the development of science curricula among specialists, the support of research projects which are initiated and carried out by students and the development of curricula for people who are described as "highly trained technologists"—people "who can provide effective and much needed support, in industry as well as in the universities, to senior research and development personnel". The reduction in the foundation's education budget is, according to Dr McElroy, "due in large measure to the reduction of graduate traineeship support and for secondary school teacher institutes", and it is clear that the foundation is apprehensive of the consequences even though there will be no reduction of the scale of the foundation's support for graduate students in the academic year which begins next September.

Table 2. NSF SUPPORT FOR NATIONAL RESEARCH CENTRES

	1969 (actual)	1970 (est.)	1971 (est.)
Arecibo Observatory	$900,000	$1,400,000	$5,800,000
Cerro Tololo Inter-American Observatory	4,550,000	1,900,000	2,200,000
Kitt Peak National Observatory	5,699,510	6,465,000	7,220,000
National Radio Astronomy Observatory	7,278,214	5,855,000	6,460,000
National Centre for Atmospheric Research	11,036,737	11,705,000	15,530,000
Subtotal, obligations	$28,564,461	$27,325,000	$37,210,000

Table 3. RESEARCH SUPPORT IN BIOLOGY

	1969 (actual)	1970 (est.)	1971 (est.)
Cellular biology	$8,684,237	$8,700,000	$9,200,000
Ecology and systematic biology	6,887,157	7,900,000	8,000,000
Molecular biology	9,665,621	9,800,000	10,100,000
Physiological processes	9,295,115	9,800,000	10,100,000
Psychobiology	3,775,232	4,200,000	4,600,000
	$38,307,362	$40,400,000	$42,000,000

The emergence of the "technologist" seemed to take the subcommittee a little by surprise. Dr McElroy said that it would not be the foundation's intention to substitute technologists for PhDs and that, so far as the output from the graduate schools was concerned, "we're all right up to the 1980s", even though it might be necessary to produce more ecologists and oceanographers, for example. Dr Handler, however, has a slightly different gloss on the new development—"it is agreed that for some positions, the PhD is overtrained", and that less exacting educational courses might be more appropriate for many kinds of jobs. This exchange seemed only to deepen Mr Daddario's suspicions of the Bureau of the Budget, which, he said, has decided that the output of PhDs is excessive.

The response of the National Science Foundation to the research programmes being discarded by other agencies has been a recurrent theme in the past week. For one thing, it is clear that nobody yet knows how many university research projects will be looking for new sponsors in the coming fiscal year—that will only be clear after the mission oriented agencies have emerged from this year's congressional mill, by the end of March at the earliest. Even so, the foundation seems to have compiled what Dr Edward P. Todd, deputy associate director for research at the foundation, called a "pressure list" of projects in other agencies likely to be affected. He reckoned that the annual cost of these projects lies between $30 million and $35 million. In practice, the National Science Foundation's budget includes nearly $10 million for the support of projects like these which may have to be transferred from other agencies, which means that, come what may, the foundation will have very little extra elbow room for increasing its support for new research projects. The greatest confusion, however, seems to attend the problem of how best to handle the impending fall-out from the mission oriented agencies.

The National Science Foundation, in the person of Dr McElroy, promised last week that steps would be taken to make sure that younger investigators continued to receive a fair share of the funds available. He did not rise successfully to Mr Daddario's suggestion that there should be a formal mechanism for effecting transfer from one agency to another, although everybody seemed to agree that the Office of Science and Technology is inadequately equipped to do this job. Dr McElroy seems to be satisfied that "the President does not want funds to be cut from the academic community any more than they are at present". The current preoccupation with relevance and the environment seems likely to benefit a great many people.

Dr Todd explained, for example, that the National Science Foundation has decided to put $2 million into earthquake research, partly in the belief that a sufficiently farsighted programme which took account of engineering construction might help to diminish the cost of future earthquake disasters—the Alaskan earthquake in 1964 is reckoned to have cost $60 million in Anchorage alone. One part of the new programme consists of an earthquake simulator—a 20 foot square platform which can be moved in such a way as to simulate seismic movement—which is being built at the Richmond laboratories of the University of California, Berkeley. The hope is that it will somehow be possible to improve on the design of buildings now in use in the United States; 200,000 of them are thought to be potentially hazardous.

The concern for relevance has also justified the foundation's decision to take over from the Office of Naval Research financial support for the 500 foot superconducting electron linear accelerator at Stanford University. Dr Todd argued last week that apart from its fundamental interest, the pi-mesons which the accelerator could produce could "revolutionize" medical radiation therapy, while superconducting niobium cavities have potentially important applications in high intensity radar and the confinement of high temperature plasmas, and that the technology in general could stimulate new techniques for power generation and transmission.

The most startled Cinderella to benefit from the preoccupation with the environment must, however, be the US National Committee for the International Biological Program. The new budget includes $7 million for this purpose, more than twice what the foundation was able to spend on these activities in the current year. Even so, it seems likely that Dr W. Frank Blair, the chairman of the US National Committee, will still have to go around with his begging bowl. In the current year, he has been able to raise only $5 million towards the programme which could have cost $15·2 million. For the coming fiscal year, he and his colleagues are hoping to get their hands on $15·9 million, more than twice what the National Science Foundation will be able to spare, and in the present climate of short commons for most agencies, he is unlikely to be greatly fortified by the general expression of goodwill towards the International Biological Program (IBP) now passed by both the Senate and the House of Representatives.

Dr Blair said last week that although the IBP is not "in the environmental management business", he is hoping that it will provide "baseline data" for this purpose. He is hopeful that the request for a two year extension of the IBP will be agreed to later in the year by ICSU. The discovery of oil in Alaska has apparently given impetus to IBP's work in the Arctic tundra, but everybody, including the subcommittee, seems downcast that there has been very little support so far for the study of the Amazon forest—diplomacy and indifference in Latin America seem to be the causes. In general, Dr Blair last week was optimistic about what IBP might be able to accomplish—he agreed with one suggestion that "the decimal point might be moved over a notch".

The spearhead, if that is the word, of the National Science Foundation's march towards relevance is, however, the programme known by the name of "Interdisciplinary Research Relevant to Problems of Our Society". Dr Joel Snow, the acting head of the Office of Interdisciplinary Research, explained last week that "our society seems to be in very serious trouble", and that it was necessary to find ways of repairing the quality of the environment and making better use of human and economic resources without retreating to the eighteenth century. The National Science Foundation, he said, is the only one among the federal agencies with a charter broad enough to support fundamental research "on the whole environmental system". So far, he said, his programme has attracted enquiries from more than 300 university groups and the projects now being considered carefully include a plan by engineers and social scientists at a New England university to "distil from their previous experience the essential ingredients for successful attacks on environmental problems", a scheme to study the contamination of the environment by heavy metals and another to apply techniques of nuclear science to criminology.

Another cloud on the foundation's horizon is to be seen in the exchange between the sub-committee and Dr McElroy about the abandonment of the traineeship programme for graduate students. Although the foundation's sails may not be trimmed in the academic year beginning in September, Mr Daddario takes the view that decisions like this cast their shadow before them, spoiling plans and damaging morale. Is there not also a danger that stopping traineeships will further concentrate scientific research in a handful of universities? Dr McElroy took the line, on Tuesday this week, that many research investigators would respond by re-writing research applications so as to put more research assistants on the NSF payroll, while state universities would be able to obtain extra money for teaching posts. Even so, he thought, graduate enrolment at private universities might well be reduced by a half when the foundation's cutback came into effect.

According to Dr McElroy, the decision to reduce federal support for graduate students has been taken by the administration as a whole but the President's science adviser, Dr L. DuBridge, has agreed to "go along with it for a year" on the understanding that they may apply for the cut to be restored next year. At the same time, Dr McElroy said, both the foundation and the National Science Board considered that there is now a "good steady state" in PhD production and, in particular, no need to increase the rate of output. Whether this view will be accepted by the sub-committee remains to be seen—some members of the sub-committee are plainly bent on considering whether to add to the foundation's budget some part of the $9·5 million which will have been saved by cancelling graduate traineeships.

NAS

Environment and People

THE National Academy of Sciences, through its involvement with the International Biological Program, and the Public Affairs Council, which likes to think of itself as the conscience of American industry, put their heads together last week on the problems of the environment. The outcome of the proceedings seems to have been a recognition that there are serious but interesting problems to be tackled. The theme for the conference was provided by Mr Robert Finch, Secretary for Health, Education and Welfare, and the man widely credited with having made California conscious of the dangers of the automobile. He emphasized that for a better environment people should be "prepared to pay pollution taxes—put up with less powerful automobiles, for example", that international standards would at some stage be necessary and that the best guarantee for the environment would be to make sure that married couples had only two children.

GENERAL BIOLOGY

Salk Institute goes Public

THE implications of recent discoveries in biology are to be followed closely by an international organization called the Council for Biology in Human Affairs, set up last week by the Salk Institute. The intention is to carry out a series of specific studies of the public impact of the new biology. The programme, for which $400,000 a year is already available for each of the next five years, will be the responsibility of six separate commissions under the general coordination of Dr Jacob Bronowski, a resident fellow at the Salk Institute at La Jolla, California.

The new council differs from previous organizations formed to educate the general public in the implications of modern biology first by being provided with money and second by the distinction of the people who have so far agreed to serve on the council and to be chairmen of the commissions which will be responsible for the first group of studies. Dr Jacob Bronowski explained last week that the chairman of the commission on "biology and international affairs" will be Professor Paul Doty of Harvard University and that its first study will probably be an examination of the possible implications of intra-uterine genetic diagnosis.

The intention is that commission chairmen shall remain in office for a limited period and that each of the six commissions should include a lawyer and an educator among its members. A second commission on "biology, ethics and the law", under Professor Abram Chayes of Harvard University, will to begin with be concerned with the ways in which new pharmaceutical products are brought into service.

The remaining commissions are one on "biology, learning and behaviour" (Professor Eugene Galanter of Columbia University); "biology in contemporary culture" (Dr Bronowski); "ecology, environment and population" (Professor Cyrus Levinthal of Columbia University); and "biology, medicine and health care" (chairman not yet chosen).

The members of the council include Dr Daniel Bell (Harvard), Dr Robert W. Holley (Salk Institute), Mr Kiichiro Kitaura (Nomura Securities Company, Tokyo), Dr Antonie T. Knoppers (Merck and Company), Dr Daniel Lehrman (Rutgers), Dr Salvador E. Luria (MIT), Sir Peter Medawar (National Institute for Medical Research, London), Dr Robert K. Merton (Columbia), Professor Jacques Monod (Institut Pasteur, Paris), Dr Herbert Passin (Columbia), Professor David Pines (Illinois), Mr Oscard Ruebhausen (Russell Sage Foundation), Dr Jonas Salk (Salk), Mr Joseph E. Slater (Salk), Dr James D. Watson (Harvard) and Dr O. Meredith Wilson (Stanford).

By all accounts, the formation of the council is the outcome of the preoccupation of Mr Joseph Slater, the president of the Salk Institute, since he took over that job two years ago, with the implications of the contemporary research in biology. He says that he would like the council to be able to respond to the "problems that emerge from the biological revolution" more perceptively than was possible twenty years ago with the growth of nuclear power. Dr Bronowski says that the council will try to "form public opinion by informing it".

RESEARCH FINANCE

New Plea for More Money

A SOMBRE warning of the dangers of skimping on university research has been delivered by the National Science Board at what may be a telling moment—the point when the criterion of relevance seems to be all important. The warning appears in the second annual report of the National Science Board, which is now required by law to make regular assessments of the condition of various parts of science. In his introduction, Dr Philip Handler, the chairman of the National Science Board, says that the apparent remoteness of astronomy, physics and chemistry from the problems of environment, urban decay and foreign affairs should not tempt the United States to "surrender its leading position" in scientific research. He says that there is a danger that in the anxiety to use scientific resources to deal with social problems, the pace and scope of scientific research may be so reduced that it fails to provide "a platform for tomorrow's applied science". The board's report has been passed on to Congress by the President in what seems to be an ostentatiously nonchalant way, which is taken by Dr Handler to suggest that the physical sciences are not at present "at the center of the Administration's interest".

The report asks for a number of specific improvements of the present arrangements for support of university research. It asks that the federal government should increase its support for graduate education to the point at which universities can make forward commitments. It wants enough money for research in the physical sciences so that the threat of mediocrity can be avoided. It asks that "a substantial fraction of the necessary increase" should be channelled through the National Science Foundation, but that all agencies should keep an interest in the relevant physical research. The National Science Board is particularly anxious about the provision of new instruments for physical research, and draws attention to the plight of radio astronomers where, it says, $200 million should be spent in the next ten years.

In other fields, there is need for more national centres where expensive research facilities could be shared, but the cost of these could sometimes be offset by closing down central facilities no longer needed. The board is keen that the United States should collaborate with other countries wherever possible, that the National Science Foundation should be given funds to take over worthwhile research programmes which other agencies can no longer support and that "the present trend to decrease funding for the scientific aspects of the space program should be reversed". One anxiety here is that the fullest benefit will not be obtained from the space programme for lack of suitable supporting research in optical and radio astronomy, but the science board also asks for an urgent strengthening of the scientific plans for future visits to the Moon.

TELECOMMUNICATIONS

Intelsat's Woes

from a Special Correspondent

UNCOMFORTABLE is perhaps the best word to describe the conference on communications satellites now in progress in Washington. All of the 73 members of Intelsat are a bit embarrassed at their failure to agree on a permanent structure for their international satellite consortium. They do not want to go home without an agreement; an Australian delegate expressed a wish not to spend many more Australian summers wintering in Washington. But the fact is that a successful resolution to the conference, which began a year ago and has been chugging along, through working parties in the interim, is not in sight. There are two opposing camps —one led by the United States, and including Chile, Australia, Italy and numerous but uninfluential allies; and another, including Britain, most of Europe and Mexico. Each side is urging the other to compromise; each has less desire than ever to do so.

What divides them is the issue of the managership of Intelsat by Comsat, the American satellite corporation. The United States and cohorts want Comsat to continue as manager; failing that, they would like a long study of the problem of managing such an international corporation to be made by outside experts, during which time Comsat would continue as manager. Britain, Germany, Switzerland and others want Comsat kicked out—as formal manager. Instead they want an international team headed by a secretary general. (Mr James Gill, recently retired director of external telecommunications for the British Post Office, has been rumoured to be dreaming of this job.) Then it could buy from Comsat advice on what kinds of satellites and ground stations to buy, but it could make up its own mind. Under the present arrangements, which have prevailed since 1964, Comsat, as manager, has simply told members of the international steering committee, representing the biggest users of the satellite system, what was good for them. The foreigners had no way of questioning Comsat's advice even when they suspected that what was good for Comsat was not necessarily good for Intelsat. This came up particularly with the choice of design of the big Intelsat 4 satellite due to be launched next year. The Intelsat members from abroad suspected that Comsat was recommending a design that would be suitable for the American domestic satellite system that Comsat has been begging the Federal Communications Commission to build for several years.

The question of the American domestic system hangs heavy over the proceedings at the State Department. President Nixon's policy, just presented to the FCC, elbows Comsat to one side and opens the American skies to all comers; if the American Telephone and Telegraph Company and the big television networks want to put up their own satellites, Comsat would have no customers even if it got its domestic satellites into orbit—a nasty position, and one which the members of Intelsat appreciate for its awfulness. "I sometimes feel," said one delegate, "that seventy-three nations are here trying to deal with a domestic American problem." Needless to say, while haggling over the future of an American private company, the Intelsat people have little time to consider how their organization might be shaped so as to leave room for special services, like air navigation and meteorology, which their satellites might sell some day to all comers, whatever nationality.

It is possible, however, that some form of words may be found to shelve the managership issue and to allow delegates to agree on the very beginnings of an agreement before going home in a few weeks' time. There has been general admiration, even awe, for the report of the preparatory committee under Mr John Killick of the British Foreign Office. It has summarized all the opposing views so succinctly that extracts from it could be lifted bodily and put into a permanent agreement, once the conference decides which of the views to accept. The Foreign Office, like the State Department and other diplomats present, quite apart from telecommunications representatives, believe the Intelsat agreement to be important as a model for other international commercial agreements that may have to be reached in the future—on nuclear energy or exploitation of the seabed floor, for example. And that is why there is genuine anguish all round that the Intelsat treaty is proving so hard to draft. If this one fails, it will be a bad omen for future attempts.

So much for internationalism. The American view, as it is felt in Congress and in Comsat, if not in the State Department, is bitter. The United States, they believe, did not need to invite a lot of outsiders to participate in its satellite consortium. Having developed the technology through its space programme, the United States could have found a way simply to sell it. "'We gave it to them and now they're trying to take it away from us," is the complaint. "Why should we give it to them ? What did they ever contribute to it ?" These are questions which the British–European and other Comsat opponents believe put the problem in a nutshell. Now is the time to make sure that there is not over-dependence on American technology for decades to come. Some of them are even willing to come back to another conference to do so.

SPACE RESEARCH

Scientific Work for Space Stations

The scientific objectives of the piloted rockets on which the National Aeronautics and Space Administration is now working have been defined during the examination of the administration's scientific programme in the past week. After five more journeys to the Moon, the first so-called Saturn Workshop will be launched in the second half of 1972. The hardware for this device has already been tested on the ground and will be equipped with instruments and electronics in the next eighteen months or so. The first of the orbiting workshops, itself a part of the Apollo applications programme, will be used for the best part of a year by successive groups of men. Beyond that, however, the National Aeronautics and Space Administration is planning a more ambitious space station which will be supplied as necessary by means of the space shuttle rocket on which work is now beginning (see *Nature*, **225**, 685; 1970)—the best part of $110 million in the new budget will go on the design of the engine.

In his statement to the Committee on Science and Astronautics, House of Representatives, a week ago, Dr Dale D. Myers explained that it is now intended to launch the first Saturn Workshop as a completely equipped device using a Saturn V rocket, which will simply put the device into an orbit about the Earth 235 nautical miles up and at an inclination of 50° to the equator. The crew of three will be launched by means of a Saturn IB rocket one day later, and they will spend a month on largely astronomical work. It is also intended that a second crew will be launched after three months and will stay for two months and will in turn be replaced by a third crew.

The centrepiece of the first workshop will be a solar telescope consisting of five separate experiments for solar studies. The system can be pointed with an accuracy of 2·5 seconds of arc at any point on the surface of the Sun which is not too near the limb. The intention is that the signals received by the telescope will be monitored by the crew, although there is also an arrangement for sending a television signal to the ground. Data from the telescope experiments will be recorded photographically and eventually returned to the Earth.

The telescope instruments include two X-ray telescopes, one designed by the American Science and Engineering Company and the other by the Goddard Space Flight Center. The former is intended to have high geometrical and spectral resolution, while the second will provide a record of the X-ray emission over the entire disk and the near corona between 0·3 and 10 nm. The Naval Research Laboratory is providing a spectroheliograph operating between 15 and 65 nm and a spectrograph with high geometrical resolution operating between 90 and 390 nm. A complementary experiment (between 30 and 130 nm) has been designed by Harvard College Observatory—by making photoelectric scans of small parts of the disk, this spectrometer should provide precise information of the intensity of solar emission. The corona out to six solar radii will be recorded in white light by a coronagraph supplied by the High Altitude Observatory in Boulder, Colorado. One intriguing feature of the equipment is that the Sun's disk will be monitored by means of a display of the Sun obtained by recording extreme ultraviolet light and H alpha light in appropriate telescopes. Prototypes of this equipment have been carried on a variety of rockets in the past few months, so that final versions of the systems can be incorporated into the telescope used by midsummer.

The Saturn Workshop will also carry a great variety of scientific and technological experiments. For example, there will be a nuclear emulsion pack for recording cosmic rays, a Schmidt camera with calcium fluoride optics for obtaining stellar spectra in the ultraviolet, advice for photography of the Sun in the ultraviolet and a camera with a fused silica window for photographing the air glow in the ultraviolet. Similarly, the Saturn Workshop will be able to make photographs of the zodiacal light, while the enthusiasts for Earth resources satellites will be given a series of photographs of a number of ground locations taken through six synchronized shutters at different wavelengths. Micrometeorites will also be collected, while a number of cell cultures of human tissues will be carried and observed in flight in an attempt to determine the effects of weightlessness. Finally, there will be some experiments to determine the effect of space flight on circadian rhythm of *Drosophila*, mice and potato respiration.

NEWS AND VIEWS

Protein-lover's Baedeker

THREE articles in this issue of *Nature* (pages 802, 806 and 811) offer a brisk canter through the elastase molecule. The work, from the Cambridge Molecular Biology Laboratory, places this enzyme into the three-star category in the protein chemist's canon, as one of the still small number whose sequence and conformation have been fully determined, and the active site delineated in detail. It also marks the emergence of protein crystallography out of the primitive and into the classical phase of development. The sense of blood, sweat and broken research students which pervaded so many of the earlier effusions in the field is replaced by one of sureness of aim and mastery of technique. To most middle-aged research workers it will come no doubt as an infinitely demoralizing revelation that the determination of the sequence and of the three dimensional structure to 3·5 Å should form the substance of the PhD thesis of one of the authors.

To go so smoothly, the structure determination, and especially the chemistry which has to precede it, still of course requires a certain measure of luck. If, as is alleged, fortune favours the prepared mind, then the minds of the Cambridge group were immaculately prepared. It is clear that elastase was designed with the crystallographer in mind, even more perhaps than the pig (whose particular advantage in possessing the enzyme is uncertain): crystals 1–2 mm long, containing one molecule per asymmetric unit, grew overnight. They were well ordered, and gave reflexions to 2 Å, which is close to the theoretical limit. They were unusually stable in the X-ray beam, allowing a complete set of data to 3·5 Å to be collected from one crystal, and finally the inhibited (tosyl) and mercurial-modified molecules were wholly isomorphous, with a superbly clear electron density difference map.

Elastase is one of the serine proteases, and therefore related to the other pancreatic enzymes, chymotrypsin *A* and *B* and trypsin. The sequence, which is given in the first paper (Shotton and Hartley, page 802), shows extensive homologies with those of the related species, with some 40 per cent of corresponding positions filled by identical amino-acids. It is now an established rule that substitutions are much more likely in positions on the outside of the molecule, in contact with the solvent, than in the interior, where the close packing of the side chains restricts drastically the changes that can be accommodated by the conformation. Since the structure is known, it is possible to distinguish between external and internal residues in the chain, and it turns out indeed that in 71 per cent of the internal positions strict homology is preserved, and what substitutions do occur are highly conservative. Only 4 per cent of these positions show no homologies within the group of four enzymes. Practically all the charged residues are on the outside. The sequences bear out the supposition that all the pancreatic proteases evolved from a common precursor.

The structure determination, and comparison of the result with chymotrypsin, settle the issue of whether a pair of proteins with broadly homologous sequences should possess similar conformations. With the aid of sequence it was possible (Watson *et al.*, page 806) to follow the course of the chain, identifying successive residues, and to make out the disulphide bridges and the tosyl group in the inhibitor attached to the active centre. The chain conformation of elastase turns out to be almost identical with that of α-chymotrypsin (page 811), and Shotton and Watson predict that trypsin will also fit into the same mould.

Finally, a comparison of the active site geometries explains the relatively low specificity of the enzyme. In chymotrypsin, the peptide carbonyl group of the substrate abuts on the active site Ser-195 residues, and the aromatic side chain, which characterizes the specificity of the enzyme, hangs into a deep cleft, with another serine at the bottom. The cleft is present in elastase, but almost entirely occluded by a valine across the entrance. It is for this reason that elastase attacks at uncharged aliphatic residues, and an alanine peptide, its favoured food, will fit nicely on the active site, the methyl group in contact with the valine at the top of the cleft. Some reasonable speculations about the catalytic mechanism can be made. The crystals of elastase have enzyme activity, and it is interesting to note that in contrast with chymotrypsin the active centres front on to large lattice interstices, some 18 Å across. Shotton and Watson envisage the possibility of introducing various species of molecule into this space, and then using difference maps to determine the structure of the guest.

DOCUMENTATION

More Nuclear Information

from a Correspondent

IT was inevitable that the International Atomic Energy Agency's first symposium on the handling of nuclear information held in Vienna on February 16 to 20 should have included a full discussion of the International Nuclear Information System concept (INIS), although only a few speakers dealt directly with the topic. INIS is hardly a new idea (*Nature*, **213**, 4; 1967), but clearly progress is being made, as reported by Dr J. E. Woolston and others (International Atomic Energy Agency, Vienna), and indeed the system is expected to produce its first output to users next May. In outline, INIS will receive its input from cooperating countries, and from this will produce, by computerized techniques, a publication called *INIS Atomindex* supplemented by various other services. There should be no shortage of input—the system is supported by more than twenty countries which between them will provide coverage of more than 80 per cent of the sources of publications.

No doubt problems will arise in the setting up and day to day operation of what will be the world's largest computer-based documentation service relying on input prepared on an internationally decentralized basis. Much of the information to be included in *INIS Atomindex* is currently contained in *Nuclear Science Abstracts*. But it would perhaps be wrong to look at INIS from a narrow point of view—there is, after all, a wider community of scientists equally concerned about information problems and interested to know whether an international approach might effectively supplement the excellent endeavours made by the core abstracting journals. Indeed, Dr S. Adams (National Academy of Sciences) dealt with UNISIST, the feasibility study for a programme of increased voluntary cooperative networking of existing and future information services—in effect, a world scientific information system. If international cooperation can succeed in one scientific area this could be a happy augury for other areas potentially able to benefit from an international approach.

The symposium also gave nuclear librarians and documentalists a welcome opportunity to discuss the problems of handling the voluminous nuclear literature. Mr A. Mauperon (European Communities, Luxembourg) gave some idea of the size of the nuclear literature when he said that each year *Nuclear Science Abstracts* covers 40,000 journal articles, 10,000 reports from laboratories, institutes and so on, and 4,300 books, theses and patents.

SUPERCONDUCTIVITY

Promise in Thin Films

by our Solid State Physics Correspondent

HOPES that "high" temperature supercurrents may be induced in thin films have received a boost from a calculation of the effects of lattice orientation on the superconducting transition temperature. R. E. Allen and F. W. de Wette (*Phys. Rev.*, **187**, 883; 1969) have worked out the phonon frequencies for an ultrathin crystal specimen and have shown that the diminution from the values for the bulk material must cause an increase in the superconducting transition temperature. They also found that for aluminium the orientation of the film has a pronounced effect on T_c, which varies by a factor of three between films oriented with the (111) and (110) axes perpendicular to the boundary.

The incentive for this calculation sprang from the measurements of Strongin *et al.* in 1968, who observed a considerable increase in T_c when thin layers of two different metals were fabricated into a sandwich structure. The change in T_c was attributed to a drop in the phonon frequencies compared with the bulk material, although no direct measurement of the frequencies was made. Allen and de Wette decided to calculate the frequency distributions for oriented face-centred cubic films from a simple Lennard-Jones power law potential.

For thin films, of course, the static displacement of atoms cannot be assumed to vanish. Minimizing the static energy, therefore, they found that the vibration spectrum includes a low frequency surface mode which greatly reduces the mean frequency of the crystal. The transition temperature, which depends on the electron–phonon coupling constant and the mean square frequency, was then found to increase by up to twenty-two times for correctly oriented aluminium, although Allen and de Wette point out that it would be hard to attain this for the imperfect free films that normally exist. In contrast, lead was found to be quite isotropic, with a transition temperature about 25 per cent greater than that of the bulk material.

In practice, it is, of course, extremely hard to manufacture films only a few atoms thick, let alone to obtain them as single crystals and to orient them. But vacuum deposition and sputtering techniques can be used to produce films thinner than 100 Å quite reliably, and the promise of surprises in the superconducting properties must add impetus to refining these techniques.

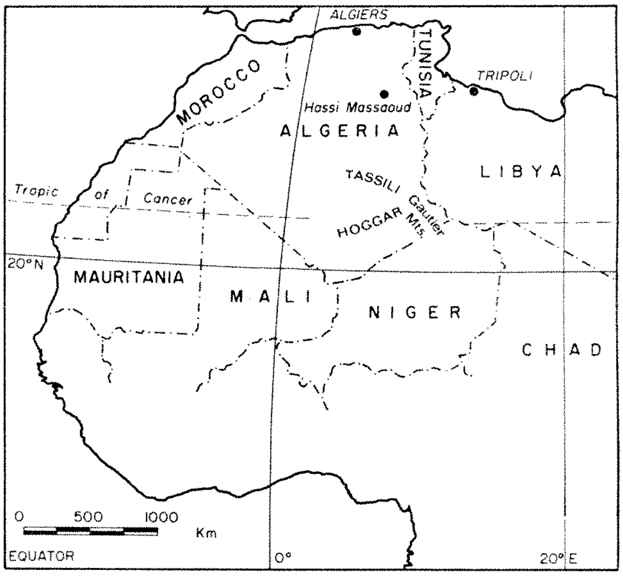
SEDIMENTOLOGY

Saharan Glaciation

from a Correspondent

THE hottest part of the Earth was once the coldest. Proof of this has come from Algerian and French geologists during the past decade, and between January 11 and 25 the Institut Algérien de Pétrole mounted an expedition to demonstrate the evidence on the spot. Sedimentological specialists of twelve nations participated, led by Dr A. Bennacef of Algeria and Drs P. Rognon and B. Biju-Duval of France. The critical area was in the Central Sahara astride the Tropic of Cancer, near the Niger and Libyan frontiers, where Lower Palaeozoic sediments lap on to the Hoggar pre-Cambrian massif. Two days of discussion in Algiers rounded off the operation, resulting in unanimous agreement that the case had been made.

From the Gautier Mountains to the Tassili Interne, Upper Ordovician glacials (450 million years old) were plainly displayed in a region of tectonic simplicity. The party saw glaciated valleys, sediments grooved by glaciers and icefloes, tillites, iceberg-borne erratics, eskers, and so on. The glacial valleys, opening northwards to the Ordovician sea, may have formed true



North-west Africa, showing the area visited by the members of the expedition.

fjords, but this was uncertain. Their depths reached 300 m, suggesting to some the biggest ever drop in sea level (and therefore the biggest polar ice-caps) of the Earth's history. Enough evidence was at hand to start serious thinking about the isostatic behaviour of the African Ordovician crust.

At times the area north of the Hoggar became a subaerial meltwater plain strewn with glacial debris. It was at least 1,000 km wide because some of the evidence has been observed in the Hassi Messaoud oilfield. Soil polygons, possibly permafrost-motivated sand-volcanoes, endglacial channels terminating in gravelly fans, kettleholes, pingos and so on are exposed in three dimensions. Because of the greater depth of erosion, glaciological knowledge of features based on Quaternary information may be enhanced and entirely new phenomena revealed.

Since the deposits pass laterally and vertically into marine strata, the glaciation must have been at sea level. It was therefore not due to high altitude, but to high latitude. Fossil faunas had previously suggested that the Ordovician seas cooled southwards across Europe into North Africa (Spjeldnaes, 1961, 1967). Palaeomagnetic evidence indeed placed the Ordovician south pole in the general region of NW Africa, from which it has since "wandered" to its present site. (The Ordovician north pole was in the Pacific.) Now we know with certainty that the Hoggar was part of Ordovician "Antarctica". But today the glacial sediments swelter in summer temperatures that must sometimes exceed the record 137° F at Hassi Messaoud.

Proof that the icecap extended westwards into Mauretania and eastwards into Chad is at hand. Some of the Algerian evidence has already been published locally (*Rév. Inst. fr. Pétrol.*, **21**, 363; 1966; *Pub. Serv. Algérie*, Bull. **38**, 39, 53; 1968). Earth scientists eagerly await a full account in a major international journal.

GEOLOGY

Core and Mantle

from our Geomagnetism Correspondent

THE theme of geophysical discussion at the Royal Astronomical Society on February 20 was the nature and behaviour of the Earth's core–mantle interface, and it became very clear that this boundary deserves more attention than it has received so far. Many important processes occur in both the core (for example, the production of the geomagnetic field) and the mantle (global tectonic processes, convection currents and so on)—and any interactions which may occur across the boundary between them are likely to have a significant effect on each part.

The meeting was opened by Dr R. Hide (Meteorological Office), who reviewed some of the problems which a more detailed study of the core–mantle interface might solve. Chief among these is undoubtedly the secular variations in the length of the day, or the Earth's period of rotation on its axis. The basic cause of these variations is thought to be horizontal stresses at the core–mantle boundary which prevent the core and mantle moving completely independently of one another. But how are these stresses produced? Because viscous stresses couple the core and mantle only weakly, it has been suggested that the electric currents responsible for the geomagnetic field leak into the lower mantle thereby causing so-called electromagnetic coupling. But the torques produced on the mantle by fluctuations in the electric currents would only give changes of about 1 ms per decade in the length of the day, whereas changes of several milliseconds in only a few years have been observed. In short, the stresses set up by electromagnetic coupling are ten times too small to explain the known variations in the length of the day.

Another stronger form of coupling is thus required—and Hide suggests that it arises from topographic irregularities, or "bumps", on the core's surface. Calculations show that bumps a few kilometres high would be sufficient to produce stresses a hundred times greater than those from electromagnetic coupling, and thus ten times greater than required to explain length of day variations. Thus there is ample margin for error.

If core surface undulations exist, they are likely to have some effect on the geomagnetic field originating in the core, almost certainly making it more complex than a field produced in a perfectly spherical core. As Hide pointed out, some of the complexities are already familiar, but have been "swept under the rug" pending adequate explanation. What, for example, is the true cause of the non-dipole field low in the Pacific? And why does the reversal frequency of the Earth's field vary so markedly over geological time? Unfortunately the answers to these questions are not yet forthcoming; but they almost certainly depend on perturbations at the core–mantle boundary.

Meanwhile, evidence for the complexity of the dipole field itself is accumulating. According to Dr J. C. Briden (University of Leeds), palaeomagnetists have so far tended to take the pessimistic view that deviations from a geocentric axial dipole in the past are undetectable because their effects are smaller than the uncertainties inherent in palaeomagnetic data. Even so, some progress is being made in pinning down the precise characteristics of the ancient dipole field. Briden reviewed the dipole models suggested so far—spherical harmonics (Gauss), non-central radial dipoles (Alldredge), northward displaced axial dipole (Wilson) and off-centre dipole (Creer)—and added one of his own. An analysis of the geomagnetic field before Wegenerian drift suggests that the dipole was displaced southward.

Hide and Malin (*Nature*, **225**, 605; 1970) have already demonstrated a correlation between the Earth's gravity and non-dipole magnetic fields with a displacement of about 160° between the two. They interpreted this as evidence for the existence of core surface bumps. But could such bumps be observed more directly—seismologically, for example? There is little doubt that undulations a few kilometres in amplitude are undetectable by seismological methods in current use. But Mr D. R. Jones (Atomic Weapons Research Establishment, Aldermaston, and University of Liverpool) has developed a method based on a comparison of P and PcP waves from nuclear explosions, which he claims is theoretically capable of detecting core surface bumps down to an amplitude of about 4 km. So far progress has been limited to the development of the technique; but Jones is now applying it to the thousand or so nuclear explosions known to have occurred.

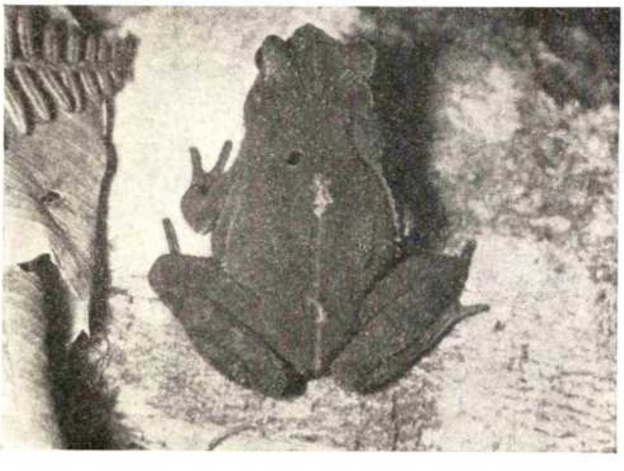
Finally, Dr P. G. Harris (University of Leeds) courageously confronted the audience of geophysicists with a discussion of the geochemistry of the core and mantle. The greatest difficulty, of course, is to determine how chemical properties are likely to be modified by the high pressures in the Earth. The composition of the core is thought to be simpler than that of the mantle; but there are still many unknowns, particularly the nature of the light element(s) which must be combined with the core iron to reduce the density of the core to its known value.

But details of composition apart, Harris's chief point was that there is likely to be chemical disequilibrium between the core and mantle. If the core is highly reduced and the mantle more oxidized, the system could act as a galvanic cell. In this case the potential across the core-mantle interface would be small, though what e.m.f. might be produced at such high pressures is not known. A second possibility is that the electric currents in the core might cause electrolysis and reduction in the mantle, and the growth of the core. Chemical interactions across the core-mantle boundary may or may not occur; but geophysicists cannot afford to dismiss the possibility lightly.

AMPHIBIA

Possible New Toad

SINCE October 1968 a South American toad has been baffling herpetologists in the British Museum (Natural History) and at the Rijksmuseum at Leiden. In August 1968 Brian Ridout, at that time a London zoo keeper, and Adrian Warren, a student of zoology, were collecting small animals along a tributary of the Echerak River, which flows into the Potaro River, a day's walk west of the Kaieteur Falls in Guyana. Mr Warren writes: At an altitude of 1,500 feet a toad jumped from the floor of the dense tropical rain forest; had it not moved it would never have been seen, so effective was its cryptic coloration among the leaves. It was an adult female 64 mm long, with upper surfaces a rich red brown with a lighter stripe down the back, on one side of which a dark light-edged spot stood out (see illustration). Its limbs were faintly barred with dark brown and the lower lip was white. Underneath it was mottled brown and white. This was nothing very remarkable at first glance, but closer inspection revealed some curious features which distinguished it from other South American toads with similar head crests. In August 1969 a similar toad was found in the same area, this time a youngster only 12 mm long.



Female toad found by Ridout and Warren in August 1968.

To the curators of herpetology at the British Museum and at Leiden these toads are unique. They are reluctant to dub them a new species and describe them in the scientific literature because the Echerak specimens have certain similarities to a rare eastern Andean species, *Bufo ceratophrys*, that is known only from males and from no farther east than Marahuaca in Venezuela. Significant differences between this species and the Guyanan toads are the spine on the eyelid, which is present in *B. ceratophrys* but not in the Guyanan form, and skin texture. These could be sexual differences, and until further collecting in Guyana yields the sexually mature male of this strange toad, and a comparison can be made with the males of *B. ceratophrys*, the problem of the identity of the Echerak toads must remain unresolved. Perhaps the answer will come from the area stretching from the interior of Surinam to the Gran Sabana in Venezuela where little herpetological collecting has been done.

TUMOUR VIROLOGY

Transformation and Transcription

from our Cell Biology Correspondent

IN permissive hosts polyoma and SV40 virus cause a lytic infection characterized by the release of many progeny viruses and ultimately death of the host cell. Non-permissive hosts, on the other hand, are transformed; the transformants have new antigenic and growth properties and are tumorigenic. Moreover, they contain one or more copies of the viral genome stably integrated into one or more chromosomes. What distinguishes permissive from non-permissive hosts? One fashionable explanation suggests that the non-permissive host lacks the biochemical environment necessary for the complete and ordered expression of the viral genome. For example, it might be imagined that the non-permissive host lacks a sigma factor necessary for the transcription of part of the virus genome.

At first sight, the observation that the expression of polyoma genes in transformed cells is only 40 per cent of that in cells supporting lytic growth corroborates this model. But polyoma virus has not been rescued from these transformed cells, and so maybe they only contain 40 per cent of the polyoma genome. SV40 virus, however, can be rescued from transformants by fusing them with permissive cells. The transformants must therefore contain a complete set of SV40 genes, and Martin and Axelrod (*Proc. US Nat. Acad. Sci.*, **64**, 1203; 1969) have shown recently that in some SV40 transformed cell lines all regions of the genome are transcribed. In other words, transformation of these cells does not seem to be a consequence of failure to transcribe part of the SV40 genome.

Using a specially devised saturation hybridization technique, they compared the extent of transcription of the SV40 genome in a permissive cell and five lines of mouse cells transformed with SV40. During lytic infection the entire genome is apparently transcribed whereas in the various transformed cell lines between 30 per cent and 100 per cent of the genome is transcribed. There is apparently no correlation between transformation and the gross extent of transcription of the SV40 genome. There is a correlation, however, between the extent of transcription and the extent of SV40 virus rescue on fusion with a permissive cell. SV40 virus is more readily rescued from transformants in which all the SV40 genome is transcribed, and the two transformed lines from which virus has never been rescued are the two lines in which SV40 transcription is lowest (30 to 40 per cent of the entire genome).

Of course, the saturation hybridization technique does not have the resolution necessary to prove that a few crucial RNA molecules are or are not made in the transformants. Nor does it reveal differences in the relative amounts of the various SV40 RNAs or the timing of their sequential appearance. Nevertheless, Martin and Axelrod's experiments seem to rule out the idea that the extent of SV40 transcription in permissive and non-permissive cells is necessarily very different. Perhaps therefore the difference between the permissive and non-permissive environment is at the translational rather than the transcriptional level.

Defective SV40 virions containing less than a complete genome appear spontaneously during passage of the virus. Uchida and his colleagues have shown that some of these defective viruses produce T antigen but not virus coat protein, and moreover the tumorigenicity of the defective virus in hamsters parallels closely the ability to induce T antigen in permissive cells. Uchida and Watanabe (*Virology*, **39**, 721; 1969) have reported that these defective SV40 which can induce T antigen can transform mouse cells *in vitro* with the same efficiency as plaque forming virus. The defective virus cannot, however, be rescued by fusion of transformed cells with permissive cells. The two transformants used by Martin and Axelrod from which virus cannot be rescued and in which only 30–40 per cent of the SV40 genome is transcribed may have been produced by SV40 defectives.

GENETICS

How Genes are Controlled

by our Botany Correspondent

THE well known adage of molecular biology that what is true for *Escherichia coli* is also true for elephants may be far from proven, but encouraging facts are beginning to collect. Some of them were discussed at a meeting on gene expression and its control at the Royal Society, London, on February 19 and 20. Dr D. J. Cove (University of Cambridge) produced evidence that the fungus *Aspergillus nidulans* has similar gene regulatory mechanisms to those of the familiar bacteriophages. Using mutants with deficiencies in the nitrate reductase and other enzyme systems, Cove and his colleagues have found evidence of induction and repression, and even some suggestion of linked genes that may form an operon. He sees no reason to suppose that eukaryotes have regulatory mechanisms much different from those of the prokaryotes—viruses and bacteria.

Moving nearer to the elephants, Dr J. Paul (Beatson Institute for Cancer Research, Glasgow) is investigating the masking of chromosomes in mammals. He has found patterns of hybridization between RNA and DNA which indicate that there must be specific restrictions on the transcription of messenger RNA from the chromatin of different organs in the mouse. This leads to the conclusion that some regions of DNA are masked in the chromatin. More nucleic acid hybridization experiments have indicated to Paul that the masking is done by the histones of the chromatin, which, when the masking is removed, are displaced by non-histone proteins which allow access to RNA polymerase to make messenger RNA. The all important non-histone component, with its regulatory function, apparently has a very complex chemistry, but Paul knows that it is acidic. Replying to a question, Paul said that there was no evidence of any relationship between his non-histone protein and the sigma factor which forms an essential part of the RNA polymerase of λ phage.

Dr J. B. Gurdon (University of Oxford) has used another technique — nuclear transplantation — to demonstrate repression and derepression in the early development of the amphibian *Xenopus laevis*. He described how, in spite of the stability of a normal differentiated cell of *Xenopus*, its nucleus can be transferred to the cytoplasm of an egg to give rise to a normal tadpole. Nuclear transplantation thus results in a change in the expression of the genes.

Recent work in Gurdon's laboratory has revealed some of the metabolic changes that follow nuclear transplantation. When a nucleus is taken from a late blastula and put in egg cytoplasm, its pattern of RNA synthesis changes within a few hours from that of a blastula to that of an egg. These rapid changes in gene expression are apparently brought about by the cytoplasm. When Gurdon injected pure *Xenopus* DNA into egg cytoplasm he obtained a result very similar to the effect of injecting a whole nucleus. Clearly other nuclear material was not needed to change the expression of the genes.

Labelling experiments have shown that cytoplasmic proteins enter a nucleus of *Xenopus laevis* after it has been transplanted. Although he cannot give an overall scheme for the gene regulation involved here, Gurdon assumes that the entry of cytoplasmic proteins influences the activity of the nucleus in some way so that it is quite derepressed. He intends to go on to find out what it is that restricts the activity of the nucleus of a fully differentiated cell.

ANTIBIOTICS

Jamming the Message

from our Molecular Biology Correspondent

MUCH progress has in recent years been made in sorting out at just what point different antibiotics jam the cycle of protein synthesis. A new example of the precise localization of a site of attack on the ribosome comes from Bollen and Herzog (*FEBS Lett.*, **6**, 69; 1970), who have identified a protein in the 30S subunit of *E. coli* that is implicated in sensitivity to the antibiotic, spectinomycin. Resistant mutants from two different strains were obtained and the 30S ribosomal proteins were fractionated on an ion-exchange column. Both mutants had, by comparison with the products from sensitive cells, a single aberrant protein component. This was made still clearer when proteins from resistant and sensitive cells, labelled respectively with tritium and carbon-14, were eluted together. The function of the protein in question is not known, neither is it yet established which activity of the ribosome is hindered by spectinomycin.

At the same time the cataloguing of antibiotics in terms of their mode of action continues. Pestka (*Arch. Biochem.*, **136**, 80; 1970) has examined the effect of a range of antibiotics on the puromycin reaction, which involves the formation of a peptidyl puromycin bond, and so serves as a convenient prototypic protein synthesis model. It seems that chloramphenicol and sparsomycin function as competitive inhibitors of puromycin, whereas six other antibiotics are noncompetitive inhibitors. Sparsomycin has been previously reported to promote the association of aminoacyl-tRNA with the ribosome and may therefore make it unavailable for reaction with puromycin. Whether chloramphenicol operates in a similar manner, or by specific inhibition of the peptidyl-transferase step, is not yet clear.

In a second report (*ibid.*, 89) Pestka examines whether the antibiotics disturb the translocation reaction by analysing the product of a poly-U-stimulated system for phenylalanylphenylalanine, and oligophenylalanines with chain length three or more. The relative amounts of these in the mixture then give a measure of the extent to which translocation—the ability of the ribosome to shift from one coding triplet on the messenger to the next—is inhibited. The presence of GTP and the protein factor *G* is another variable which can be controlled. The steroid antibiotic, fusidic acid, turns out to be the only one that unequivocally blocks the capacity of the *G* factor to induce translocation, though two others have a lesser, and probably secondary, effect. The remaining antibiotics tested inhibit oligopeptide synthesis, but their action is manifested in the absence of the *G* factor and GTP. It is most likely—and indeed in some case has been established previously—that these antibiotics operate by preventing the attachment of incoming aminoacyl-tRNA to the ribosome.

How such effects on the ribosome are physically accomplished by a single molecule of antibiotic is another issue, but it has been widely supposed to involve some steric disturbance, local or gross, of the ribosome structure. The first direct evidence for such an effect has been described by Sherman and Simpson (*Proc. US Nat. Acad. Sci.*, **64**, 1388; 1969). It is known from tritium exchange experiments that a considerable proportion of the exchangeable hydrogen atoms in intact ribosomes is inaccessible, but when the ribosomes are dissociated into their subunits by withdrawal of magnesium all exchange essentially instantaneously. The addition of streptomycin to *E. coli* ribosomes induces a considerable alteration in the tritium exchange kinetics: with low ratios of antibiotic to ribosomes there is a marked increase in exchange, suggesting a fairly extensive conformational loosening (or diminution in the strength of association between the subunits). In these conditions there is little inhibition of poly-U-directed polypeptide synthesis, or misreading (a familiar and striking effect of this antibiotic). When the molar ratio of antibiotic to ribosomes exceeds 0·1, however, both effects are progressively and strongly manifested, and at the same time the rate of tritium exchange begins to diminish, and falls to something considerably lower than the value for the pristine ribosome. Sherman and Simpson suggest that there are two completely different modes of interaction. With ribosomes from streptomycin-resistant cells neither type of effect can be observed.

HEALTH

Unusual Cause of Impotence

from our Social Medicine Correspondent

CIRCUMSTANTIAL evidence has been recently found of a possible link between impotence and the use of certain herbicides and pesticides (Espir *et al.*, *British Medical Journal*, **1**, 379; 1970). Between April and November 1967, four out of five members of a team of farm workers who had used these chemicals complained of difficulty in achieving and maintaining an erection, having previously been perfectly normal in this respect. None had any other symptom and none experienced an appreciable fall-off in libido.

The possibility of a toxic cause was not suspected immediately in the first patient, but fortunately he stopped using the chemicals of his own accord and recovered completely within 2 months of the onset of impotence. When a second patient of similar occupation visited the same general practitioner 7 months later, suspicion fell on the chemicals. He and two subsequent patients were advised to avoid further contact with the pesticides, and except for the third patient, who took a year to recover completely, all recovered their potency within 2 to 3 months. Each patient was treated with methyltestosterone, but Espir *et al.* say there is no clear evidence that this enhanced recovery, which may have occurred spontaneously after the men stopped using the chemicals.

Nine groups of compounds were used on the estate in question during 1967: organophosphorus, substituted phenols, dipyridyls, organochlorides, substituted phenoxy compounds, triazines, carbamates, dithiocarbamates and ureas and uracils. All were used either during early spring and/or late summer and autumn—coincidental with the reported impotence. The men had occasionally disregarded regulations concerning the safe use of chemicals because of the discomfort from the lack of ventilation with protective clothing.

According to Espir *et al.*, remission of impotence, as in the cases described, is not consistent with many of the possible organic diseases. Though multiple sclerosis could not be excluded on this basis, there were no other signs of any neurological disorder and neither was there any evidence of psychological, endocrinological or local structural disease. In view of the circumstantial evidence, and having been unable to find any alternative cause, Espir *et al.* conclude that the reversible impotence was probably due to the toxic effects of the chemicals to which the men were exposed, though there was no evidence of poisoning with these compounds. Organophosphorus compounds were originally thought to be responsible, but Espir *et al.* could not find previous reports of impotence as an isolated manifestation. They therefore cannot say whether the reversible impotence was caused by a single substance or by two in combination.

Additional support for the proposed link is provided in an article in the December issue of the *Medical News Tribune* (**19**, 21; 1969). Referring to this in their addendum, Espir *et al.* say the case in question was an agricultural labourer who, for 3 years, complained of impotence, nausea and some indigestion which started each year in March and lasted for 6 weeks—the time of year when he used insecticides and herbicides. The sprays which the labourer thought upset him were 'Metasystox 55', 'Gesatop' and 'Tecane'.

WORTHINGTON

RNase-free DNase, DNase-free RNase...

the cleanest, sharpest tools for molecular biology come to you direct from Worthington

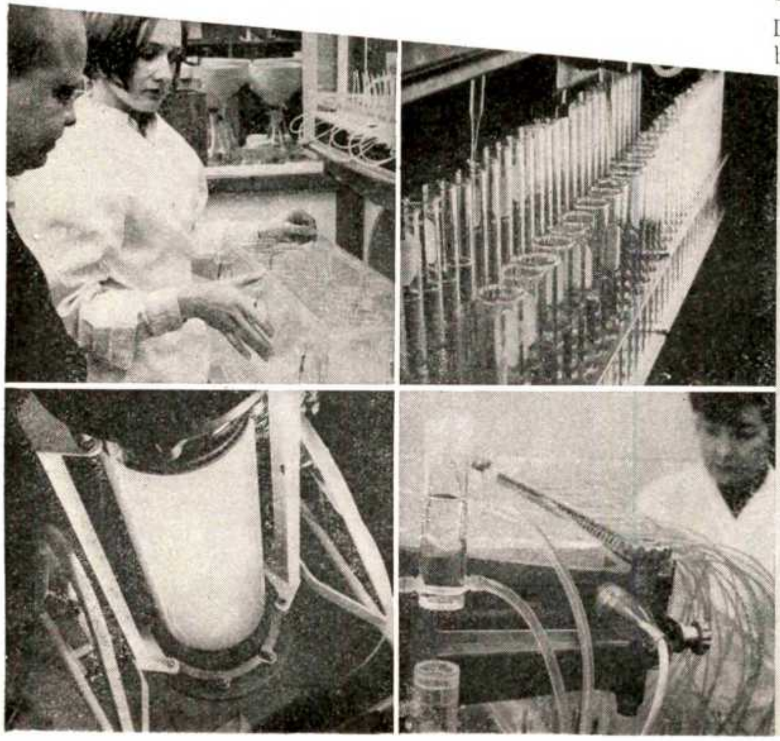
Unlike other biochemical supply houses, mere re-sellers, we make all our own enzymes. We have adapted such specialized analytical techniques as column chromatography, free-flow electrophoresis, and disc gel electrophoresis to routine enzyme production. From our efforts come enzymes with the purity and high activity required for demanding research work.

- For example, our chromatographically-prepared pancreatic ribonucleases, RAF and RASE, are free of that last 1% impurity found in other preparations. Even though we sacrifice half our yield in final purification, our RNA materials are priced competitively.
- Another ribonuclease for nucleic acid chemists, our RT_1 has twice the activity of any other commercially-available product.
- Our standard once-crystallized DNase and our chromatographically-prepared alkaline phosphatase both are upgraded electrophoretically to eliminate trace RNase.

The preparation of each of our 200-plus enzymes is carried through by the one person who best understands the process. His records provide the complete history of each batch of every enzyme. If necessary, we can trace any material back to its source.

Re-sellers can't do this. They merely slap a new label on the bottle and stick it on the shelf.

Distributed in the United Kingdom by Cambrian Chemicals, Limited.



Worthington Biochemical Corporation
Dept. K
Freehold, New Jersey 07728

☐ Send WORTHINGTON ENZYMES FOR RESEARCH booklet.

☐ Send the complete Worthington Enzyme Manual. I am a new customer.

Name

Title

Institution

Address

City

State Zip

DIEHL sigmatron

Electronic Calculating System with Pre-programmed Statistical functions

DIEHL sigmatron is the first calculating system specifically designed for Statistical calculations. By pre-programmed functions, Statistical calculations are reduced to one key operations. For instance, to obtain the Arithmetic Mean and Standard Deviation enter data on the keyboard and touch the pre-programmed key. After entry of the data is completed you need only touch the same pre-programmed key to obtain the results immediately. Linear Regression, Chi Square, Coefficient of Correlation are all reduced to the simplicity of one key operation. DIEHL sigmatron also gives you a powerful and effective calculating performance with ten separate memories, automatic square root, automatic decimal selection up to 8 places and a complete check of each figure, each step and each result.

If you prefer to make your own programs, DIEHL combitron S has 10 Program/Memory registers providing a capacity of 120 steps with branching and looping.

For external input of data, DIEHL dilector – punched tape reader – is available.

Please cut out!

DIEHL Calculating Systems 8500 Nürnberg 2 W · Germany

Please send me details of DIEHL Calculating Systems

Name ______________________ Title ______________________

Address ____

City ______________________ Country ______________________

Diehl

Brain Mechanisms and Motivation

by
KEITH OATLEY
Laboratory of Experimental Psychology,
University of Sussex,
Brighton

Mechanisms in the brain generate specific patterns of motivated behaviour concerned with such matters as hunger, thirst and sex. These mechanisms contain models of relevant aspects of the internal and external environment which enable an animal to direct its behaviour appropriately and purposefully.

DARWIN[1] was one of the first biologists to deal with the problem of purposeful behaviour in animals. He proposed that specific instincts, which gave direction to behaviour, were shaped by natural selection just as surely as were anatomical characteristics. Although he explicitly disclaimed any understanding of the mechanisms which gave rise to instinctive behaviour, this did not prevent many who came after him from using the idea of instincts as if the mere existence of a name for each instinct could explain how the behaviour was produced. Aggressive behaviour, for example, would be attributed to an aggressive instinct.

Brain Areas and Behaviour

Clearly, names such as aggression which act as labels or shorthand descriptions of categories of motivated behaviour have no real explanatory value. Nevertheless the existence of brain mechanisms which generate specific behaviour patterns is beyond doubt. Hess[2] was the first to show that instinctive patterns could be elicited by electrical stimulation of the brain through chronically implanted electrodes. Stimulation of parts of the hypothalamus of cats produced signs of aggressive behaviour such as a defensive posture, hissing, and usually a well directed attack on any convenient target. Subsequently other behaviour patterns, including eating, drinking and sexual behaviour, have been elicited by stimulating hypothalamic and limbic regions in many species. In the same way that chronic electrodes can be implanted intracranially, cannulae made of fine gauge hypodermic tubing can also be directed into almost any area of the brain. Application of transmitter substances through a cannula, like electrical stimulation, can elicit well defined patterns of motivated behaviour from unanaesthetized animals. Grossman[3] found that acetylcholine and related substances, applied directly to the hypothalamus of rats, selectively induced drinking. Noradrenaline applied through the same cannula evoked eating. The most plausible interpretation was that neurones subserving these different types of behaviour were very close together, but used separate transmitter substances. Testosterone and other sex hormones have also been injected directly into the brain[4]. Maternal patterns such as nest building and repeated grooming of the young have been elicited in this way, as have exaggerated sexual responses. The regions from which the various motivational behaviours can easily and reliably be obtained are chiefly the hypothalamus and related structures of the limbic system (Fig. 1). It has further been demonstrated[5] that animals will learn to press a small lever which turns on electrical stimulation to an electrode implanted in parts of these same regions. This learned behaviour has indicated that the stimulation has rewarding properties. In spite of early claims, however, the effect seems not to be one of unalloyed pleasure. The stimulation has aversive aspects as well.

Lesions of the hypothamalus and limbic system also have striking effects. Hetherington and Ranson[6] showed that bilateral destruction of the ventromedial nucleus of the hypothalamus in rats was followed by gross overeating and obesity. Conversely, lesions of the nearby lateral

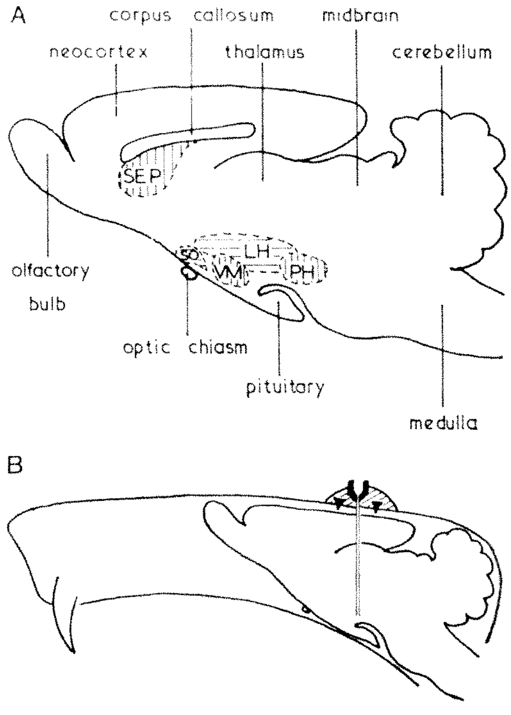


Fig. 1. (*A*) Semi-schematic view of the rat's brain in the sagittal plane. Areas shown with horizontal hatching are further lateral than those with vertical hatching. SEP is the septal area, one of the major parts of the limbic system. SO is the supra-optic nucleus of the hypothalamus. Here or further forward is a probable site for osmoreceptors important in the control of thirst. LH and VM are the lateral hypothalamus and the ventromedial nucleus of the hypothalamus which have been proposed respectively as "appetite" and "satiety" centres for hunger. PH is the posterior area of the hypothalamus, one of the regions from which aggressive behaviour has been elicited. The loci from which specific behaviour patterns can be affected by lesions or stimulation do not always correspond closely to anatomically defined nuclei. (*B*) Diagram illustrating permanent implantation of a cannula (or an electrode) in the rat's hypothalamus. The cannula is aimed using a stereotaxic apparatus, and anchored to the skull by means of jeweller's screws and acrylic cement.

hypothalamic area resulted in a complete cessation of eating and drinking[7].

These findings provoked an explosion of research on the behavioural effects of brain lesions and stimulation. Here it seemed were data that directly related structure to function. Stellar's[8] theoretical treatment unified a growing number of observations in this field, and a schematic diagram illustrating his hypothesis is shown in Fig. 2. Stellar proposed that each type of motivation was controlled by two bilateral brain centres. An excitatory centre turned on behaviour appropriate to some specific motivation, and an inhibitory centre turned the excitatory centre off. Each centre was supplied with internal and external stimuli, and learned and unlearned influences. In the case of hunger, because lesions of the ventro-medial hypothalamus increased eating and electrical stimulation stopped it, this region was identified as the inhibitory or "satiety" centre for eating.

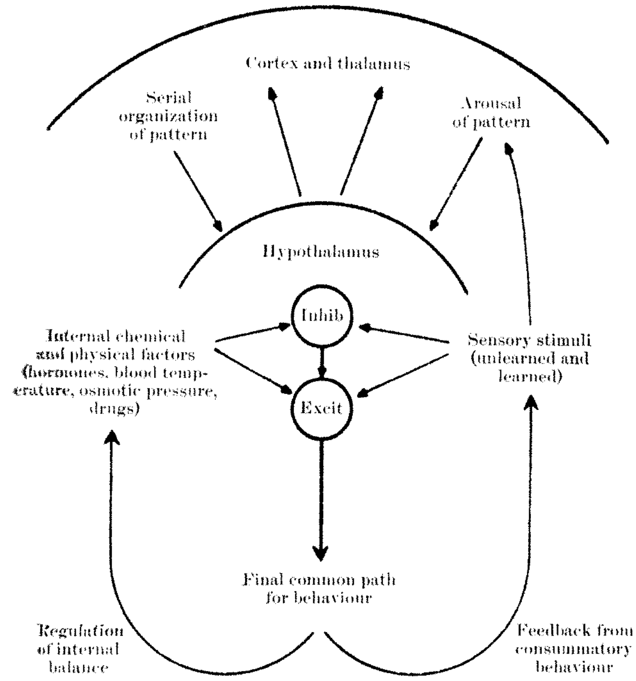

Cortex and thalamus
Serial organization of pattern
Arousal of pattern
Hypothalamus
Inhib
Excit
Internal chemical and physical factors (hormones, blood temperature, osmotic pressure, drugs)
Sensory stimuli (unlearned and learned)
Final common path for behaviour
Regulation of internal balance
Feedback from consummatory behaviour

Fig. 2. Stellar's[8] diagram illustrating his hypothesis about the type of mechanism underlying motivated behaviour.

Difficulties have arisen in connexion with this analysis. Proliferation of experiments has led to a slightly unwelcome proliferation not only of the anatomical extent of these putative hypothalamic centres, but also of their properties. It now seems, for example, that lesions of the lateral hypothalamus (thought to be the excitatory eating centre) do not lead to permanent aphagia. If the lesioned rats are fed by stomach tube, they eventually start to eat again[9]. They also exhibit characteristic and abnormal dietary preferences as recovery proceeds. It has been found[10] that although electrical stimulation of the lateral hypothalamus will induce a rat to eat if food is offered, stimulation by means of the same electrode can also elicit other types of behaviour. Rats, which in some initial tests were found specifically to eat when stimulated, subsequently had food removed during periods of stimulation but were still allowed water, and pieces of wood which they could gnaw. After this experience they were all found either to drink or to gnaw when stimulated without food present, and when it was present in addition to water and wood they distributed their responses between food and at least one of the other substances offered. These results on the lateral hypothalamus, together with several other recent experiments involving the behavioural effects of direct manipulation of the brain, have revealed discrepancies which are not adequately resolved by the notion of centres that simply turn on and turn off different types of behaviour.

Logical Basis of Behaviour

Experiments involving lesions and stimulation have demonstrated dramatic effects on behaviour. They have certainly revealed the presence and location of mechanisms which generate the specific and largely innate behaviour patterns which characterize several types of motivation. But the behaviour that has been produced has not revealed how these mechanisms work. This needs a different line of attack, in which the nature of the system capable of generating the observed behaviour is inferred from experiments where physiological and environmental variables which usually affect the system are manipulated and behaviour is measured carefully.

Claude Bernard[11] deduced the important principle that physiological mechanisms act to maintain constancy of the internal environment of the body in spite of changes in the outer world. Cannon called this principle homeostasis[12], and Richter[13] emphasized how motivated behaviour, such as eating and drinking, contributes to homeostasis just as much as does internal regulation.

Earlier workers had only the vaguest ideas as to how homeostatic mechanisms might work. The advent of control systems in engineering made possible a much greater understanding of the matter. To the extent that behaviour contributes to homeostasis, the mechanisms generating behaviour must be control systems of the kind known as regulators. From this it is possible to postulate a logical basis for behaviour. The essential components of a regulator show how such a system will display the purposiveness so characteristic of motivation. The minimal regulator must have (*a*) a specification of the desired state of the system, (*b*) a means for detecting deviation of the system from this desired state, and (*c*) a means whereby the measurement of deviation shall drive the system in such a direction as to minimize the deviation. The system works by feeding back some aspects of its output to control the system by means of the measurement (*b*).

If behaviour results in the regulation of some variable, then a feedback control device with these characteristics represents the underlying mechanism, and it then remains to be decided which part of the brain corresponds to each of the essential components. If the system is more complicated than first suspected. this will become clear in the course of enquiry. The essential nature of the mechanism as a control system will remain.

The concept of regulation made it possible to organize theories of motivation on a framework which gave apparently satisfactory explanations of behaviour. Thus Hull[14] proposed that needs (departures from optimal bodily states) induced drive, the energizer of behaviour. Responses, produced under the influence of the drive which led to drive-reduction, were reinforced, and their probability of recurrence was increased.

One implication of the theory was that some behaviour is built directly on needs such as hunger. All other types of behaviour, not directly related to deviations of the body from its optimal physiological state, were thought to be learned secondary drives arising by association with primary needs. In an experiment illustrating this, chimpanzees, instead of being rewarded by food for performing a task, learned to work just as hard for tokens which they could subsequently exchange for food in a slot machine[15].

Not all attempts to relate motivation ultimately to physiological needs have been convincing. An alternative version, also using a homeostatic framework, involved some optimal neural state. Hebb[16] and several others have proposed that the level of cortical arousal produced by the reticular activating system rises above its desired level during intense fear and falls below it during boredom. In the normal course of events it is regulated near some optimal level by behaviour such as curiosity and exploration (Fig. 3).

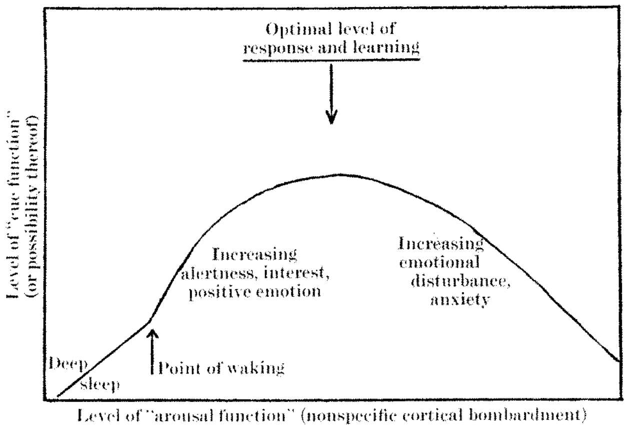


Fig. 3. Hebb's[16] graph of the effectiveness of cue function, the attribute of stimuli used in guiding behaviour, related to the level of arousal, or general energizing influence on behaviour.

Hull's and Hebb's explanations of motivation were successful partly because they were formulated on the plausible basis of homeostatic regulation. Both theories, however, held that drive was the engine impelling behaviour, but not the steering gear. They stated that behaviour was undirected at first and would be learned if it led to drive reduction. Then the learning mechanism determined the direction of behaviour. Several predictions were based on this hypothesis. For example, separate means of inducing drive should be cumulative in their effect on the vigour of response. These predictions have not been confirmed unequivocally, and consequently the general drive idea has not played a large part in more recent theoretical treatments of motivation[17]. Instead emphasis has turned away from drives that push behaviour from behind, towards external incentive events that draw behaviour forward. Such theories seem especially appropriate for motivations like exploration and sex.

Models of the Environment

Further justification for rejecting general drive theories comes from the lesion and stimulation work I have discussed. Behaviour elicited by electrical or chemical stimulation is clearly generated by mechanisms which specify not only the quantitative vigour of response, but also its direction. The conclusion echoes Lashley's[18] suggestion that problems of motivation would not involve libido or general drive but the manifestation of very specific sensori-motor systems.

An experiment by Petrinovitch and Bolles[19] illustrates this. They trained rats that were either hungry or thirsty in a T maze. Thirsty rats learned to find water always in one place in the maze more easily than hungry rats learned to find food there. On the other hand, hungry rats were superior in a task in which reward was placed on alternate sides of the maze on successive trials. Presumably brain mechanisms of rats have evolved in such a way as to match the tendencies of the environment to maintain a source of water in a constant place while varying the location of food.

This experiment also demonstrates that, as well as specificity, motivational mechanisms have features appropriate to the nature of the environment. A similar argument was put in a rather different context by Craik[20]. He proposed a hypothesis on the nature of human thought. In order to generate behaviour appropriate to the environment, Craik argued that the brain must itself contain a model of that environment. The model would represent in a neural symbolism the objects, events and relationships that occur in the world. Evidently animals, including man, conduct their affairs with apparent knowledge of the kinds of things that occur in the world. They predict the outcome of events, and the results of their own actions. A man will say "If I leave now I can just catch the train". A rat behaves as if he knows that the left turn in a maze leads to food. It is difficult to see how behaviour could be appropriately directed towards the environment if the brain did not contain, in some sense, a model of the world.

Though important, Craik's hypothesis has not been exploited much. A suitable application for this type of idea is the problem of motivation. Motivational or, as Darwin called them, instinctive mechanisms must have been shaped during evolution to take account of and represent those aspects of the environment relevant for eating, sleeping, breeding and so forth. Aspects of the environment have come to determine the logical structure of the model contained in the brain. If this is true, then a fundamental method of reaching an understanding of brain mechanisms is to discover what objects, events and relationships are relevant to some category of behaviour. If it is possible to represent formally how these objects, events and relationships are articulated in the environment, evidence can be sought that the brain has a symbolic representation of these same environmental processes.

On this formulation it would not be possible to explain all motivation with a single type of mechanism. The use of hunger as the prototype for motivation has led to theoretical difficulties, as well as the spread of mistaken ideas such as that of an aggressive drive which grows stronger if the opportunity for aggression is denied[21]. The homeostatic principle should be applied when behaviour is directed towards the regulation of some internal variable. The paradigm is useful because it specifies the nature of the mechanism generating behaviour. This advantage should not allow behaviour to be explained by homeostasis when internal regulation is not involved. Instead the role of each type of behaviour should be sought in the life of the animal, as well as the environmental events that need to be represented and symbolically manipulated by the brain.

The characteristics of motivational systems are usually as follows. (1) The mechanisms include a means for generating specific consummatory behaviour closely matched to the properties of the goal object, and released by and guided towards this object. (2) There is some means of controlling the quantity of consummatory behaviour. In some cases this may be by homeostatic regulation. (3) There are mechanisms for generating suitable appetitive behaviour. This behaviour is not entirely random, but involves strategies appropriate to the properties of the goal object. A cat, for example, hunts in a very systematic way. All three of these are innate. In addition learning enters as follows. (4) What is learned about the environment during appetitive behaviour may be partly determined by component (3). (5) Motivation can be induced and reduced by learned stimuli, particularly (for example) in the case of fear, somewhat less so in the case of sex, and probably much less in the case of thirst.

Sexual behaviour illustrates the interaction of several component processes[22,23]. Anatomical and behavioural patterns displayed by an animal will identify it to a partner of the same species and opposite sex. The partner also displays patterns, and each evokes in the other sequences of courtship and copulation, but only if hormonal conditions in both are right. Hormones in this context act as a timing device, encouraging only mature individuals to mate, and stimulating fertilization at a time of the year which suitably anticipates the best season for rearing young. The motivational mechanism represents in its model both a suitable partner and a suitable season, relating these in such a way as to produce behaviour which will continue the species.

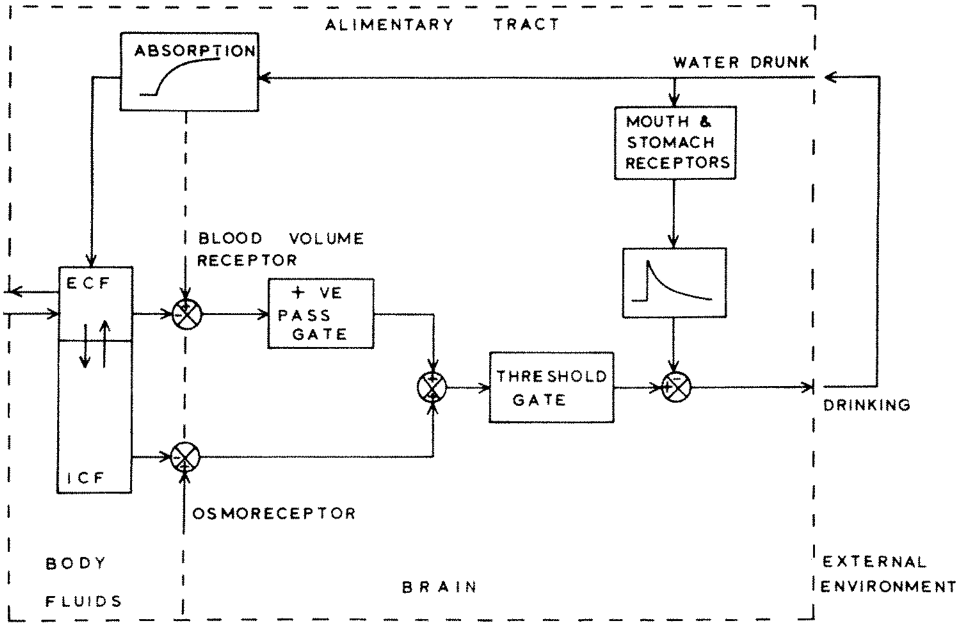


Fig. 4. A model of the control of thirst (simplified from ref. 30). Arrows represent signals, boxes are mechanisms or transfer functions operating on the signals, and the circles containing diagonal crosses are summing junctions which add signals according to the signs given. Deficits of body fluid produce a positive thirst signal. Though only deficits of ECF are taken into account, both deficits and excesses of the cellular space reach the summing unit.

The ability of animals to choose a healthy diet[13] also shows how the mechanism of motivation is matched to the nature of the environment. It was originally thought that animals might be able to discriminate the presence of vitamins in food, and that they might have specific (homeostatic) hungers for each of these. It now seems instead that animals detect in themselves a state of illness or good health, and can associate these states with foods that they have eaten. Thus rats avoid a food with a discriminable taste or smell if illness follows eating that food. This can occur after only a single exposure to a toxic food and even when the eating and illness are separated by several hours. This type of learning contrasts sharply with learning of contingencies in the external world, where associations between events are only made if the events occur within a few seconds of each other. Furthermore, in learning about the external world stimuli affecting the somatic receptors (visual, auditory, tactile) are effective. For learning about the onset of illness these stimuli are ineffective and only tastes and smells can be learned[24]. Eating a balanced diet is the reverse of avoiding illness. Here vitamin-deficient animals avoid foods which do not relieve their illness. A rat suffering from thiamine deficiency offered a choice of diets will try them systematically, one at a time, with intervals of several hours. If the diets have distinctive tastes and any of them contain the vitamin, he will eventually find that one and keep eating it, again as a result of learning[25].

Here again the brain mechanisms of a specific motivation have features which model the workings of the environment. For the learning of most external processes the constant temporal conjunction of events reflects a high probability of causality. In becoming ill or recovering because of the diet, causality has a longer time course, and this must be reflected in learning mechanisms that will make associations over a longer period.

Thirst and Water Balance

Thirst has both homeostatic and non-homeostatic features[26], which indicate the way in which the brain represents the environment. Homeostatic regulation of thirst occurs when body water falls below some desired level. Body fluids are distributed in two principal compartments, cellular and extracellular. Water shifts between the two along osmotic gradients, while solutes are confined to their own compartment. Diminutions of either the cellular[27] or the extracellular space[28] will provoke thirst and receptors exist whereby the brain monitors these changes. Osmoreceptors act as a sample of the cellular space and blood volume receptors measure a sample of the extracellular compartment. When deficits of both fluid

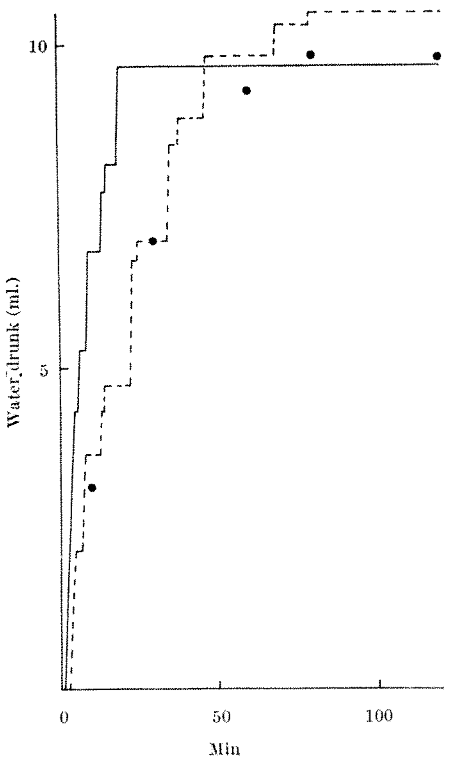


Fig. 5. Unpublished result (myself and Toates) of the output from a computer simulation (solid line) compared with drinking of an individual rat (dotted line) and the mean of a group of rats (solid spots) in response to an intravenous injection of sodium chloride. The simulation parameters and time constants were all fitted to physiological data for individual components of the system, and this overall response gives a good fit. The computer response is slightly faster than that of the rat because the rat responds partly in a non-homeostatic way by spending some of its time grooming instead of drinking.

spaces occur together they are additive in their effect on thirst[29]. This occurs, for example, when an animal is deprived of drinking water. I proposed the additive arrangement[30] in a model of thirst (Fig. 4). This model, which explains the quantity of consummatory behaviour, was simulated on a computer in collaboration with F. M. Toates. Fig. 5 shows an example of a comparison between the output of the simulation and drinking by rats.

The variables and calculations used by the computer follow a physiological understanding of each component process in the system. Distribution of water between cellular and extracellular compartments, for example, is thought to be determined by osmotic gradients. Accordingly, concentrations on either side of the cellular membrane were calculated. A rate of flow of water between the compartments was made proportional to the difference, or gradient between them, and the integral of the rate added to and subtracted from the appropriate fluid spaces. The time constant for this and other component processes was fitted to published data and not further adjusted. The simulation contains three principal parts. The first is the computer representation of the body fluids, including the absorption of water and solutes from the gut. The other two parts are the systems for control of the kidneys and of thirst (which is an elaborated version of the scheme of Fig. 4). Renal and thirst systems embody Claude Bernard's concept of homeostatic regulation within the formal context of feedback control.

The thirst system simulates the brain's model of the body fluids. It exhibits the kind of structure that the brain's model (in Craik's sense) must have in order to be able to act appropriately towards the environment; in this case the internal environment, the body fluids. The brain measures two relevant events, the two compartment volumes, and represents the exchange of water between compartments as a neural addition process. Also represented in the brain's model is the delay of alimentary absorption after water is drunk. This exponential lag in the feedback loop would lead to instability were it not for additional short-term feedback signals. To compensate precisely for absorption the brain represents the delay by a memory of the amount drunk which decays with the same time constant as that of absorption. This decaying memory is added to the absorptive feedback to calculate the total amount drunk.

This homeostatic mechanism represents the brain's working model of the internal environment, and the means of controlling it. Other aspects of thirst are not homeostatic and relate instead to the external environment. The diurnal rhythm of drinking, for example, seems to be independent of changes in body fluids[31]. It is exhibited when rats allowed to drink freely are found to drink about 80 per cent of their total water at night. The rhythm is probably driven by an endogenous oscillator, which is synchronized to, but not entirely dependent on, the external lighting cycle. Mechanisms that determine diurnal rhythms simulate and predict the natural alternation of light and darkness and generate behaviour at the time of day that is suitable for the ecological niche of the animal.

Motivation in animals is beginning to be understood in terms of kinds of mechanisms necessary to generate the appropriate behaviour. The next step is to put this essential component of understanding together with knowledge of the anatomical location of the mechanisms in the brain, and determine, perhaps by micro-electrode recording, the neural embodiment of these systems which represent and control their environment.

I thank Professor N. S. Sutherland for helpful criticism.

[1] Darwin, C., *The Origin of Species* (1859).
[2] Hess, W. R., *Diencephalon: Autonomic and Extrapyramidal Functions* (Grune and Stratton, New York, 1954).
[3] Grossman, S. P., *Amer. J. Physiol.*, **202**, 872 (1962).
[4] Fisher, A. E., *Science*, **124**, 228 (1956).
[5] Olds, J., and Milner, P., *J. Comp. Physiol. Psychol.*, **47**, 419 (1954).
[6] Hetherington, A. W., and Ranson, S. W., *Anat. Rec.*, **78**, 149 (1942).
[7] Anand, B. K., and Brobeck, J. R., *Yale. J. Biol. Med.*, **24**, 123 (1951).
[8] Stellar, E., *Psychol. Rev.*, **61**, 5 (1954).
[9] Teitelbaum, P., and Epstein, A. N., *Psychol. Rev.*, **69**, 74 (1962).
[10] Valenstein, E. S., Cox, V. C., and Kakolweski, J. W. K., *Science*, **159**, 1119 (1968).
[11] Bernard, C., *Introduction à l'Etude de la Médicine Expérimentale* (1865).
[12] Cannon, W. B., *The Wisdom of the Body* (Norton, New York, 1932).
[13] Richter, C. P., *Hervey Lectures*, **38**, 63 (1942–43).
[14] Hull, C. L., *Essentials of Behaviour* (Yale University Press, New Haven, 1951).
[15] Cowles, J. T., *Comp. Psychol. Monog.*, **14**, No. 71 (1937).
[16] Hebb, D. O., *Psychol. Rev.*, **62**, 243 (1955).
[17] Bolles, R. C., *Theory of Motivation* (Harper and Row, New York, 1967).
[18] Lashley, K. S., *Psychol. Rev.*, **45**, 445 (1938).
[19] Petrinovitch, L., and Bolles, R. C., *J. Comp. Physiol. Psychol.*, **47**, 450 (1954).
[20] Craik, K. J. W., *The Nature of Explanation* (Cambridge University Press, 1943).
[21] Lorenz, K., *On Aggression* (Methuen, London, 1966).
[22] Beach, F. A., in *Biological and Biochemical Bases of Behaviour* (edit. by Harlow, H. F., and Woolsey, C. N.), 263 (University of Wisconsin Press, Madison, 1958).
[23] Young, W. C., in *Hormones and Mating Behaviour* (edit. by Young, W. C.), 1173 (Williams and Wilkins, Baltimore, 1961).
[24] Garcia, J., and Irvin, F. R., *Commun. Behav. Biol.*, Part A, 1, 389 (1968).
[25] Rozin, P., *J. Comp. Physiol. Psychol.*, **69**, 126 (1969).
[26] Falk, J. L., *Nebraska Symp. on Motivation*, **9**, 1 (1961).
[27] Gilman, A., *Amer. J. Physiol.*, **120**, 323 (1937).
[28] Fitzsimons, J. T., *J. Physiol.*, **159**, 297 (1961).
[29] Fitzsimons, J. T., and Oatley, K., *J. Comp. Physiol. Psychol.*, **66**, 450 (1968).
[30] Oatley, K., *Med. and Biol. Eng.*, **5**, 225 (1967).
[31] Fitzsimons, J. T., *J. Physiol.*, **138**, 39P (1957).

Amino-acid Sequence of Porcine Pancreatic Elastase and its Homologies with other Serine Proteinases

by
D. M. SHOTTON[*]
B. S. HARTLEY
MRC Laboratory of Molecular Biology,
University Postgraduate Medical School,
Hills Road, Cambridge

The primary structure of porcine elastase has been determined by classical procedures of enzymatic digestion and chemical sequence analysis and shown to be closely related to those of chymotrypsins A and B and trypsin. Differences in side-chains offer an explanation of the differences in substrate specificity.

THE proteolytic enzyme elastase (EC 3.4.4.7) can digest a wide variety of protein substrates[1], but is distinguished from the other pancreatic endopeptidases by its ability to digest elastin, the elastic fibrous protein of connective tissue[2], and by its specificity for substrates with uncharged non-aromatic side-chains[3]. The specificity of elastase thus complements those of the trypsins and the chymotrypsins for substrates with basic and aromatic side-chains, respectively. Elastase is formed in the duodenum by tryptic activation of the zymogen, proelastase, which is secreted by the acinar tissue of the pancreas[4,5]. It consists of a single polypeptide chain of 240 residues, with a molecular weight of 25,900, which is homologous with the single chain of trypsin and the B and C chains of the chymotrypsins. The amino-acid composition is shown in Table 1. In common with all other serine proteinases[6], elastase possesses a serine residue at the active centre which is readily inhibited by diisopropyl phosphorofluoridate[7]. The amino-acid sequences around the active centre serine and histidine residues, the disulphide bridges and at the amino-terminal region have been shown to be closely homologous with the corresponding regions of bovine trypsinogen and chymotrypsinogen A (refs. 8, 9). We wish now to report the determination of the complete amino-acid sequence.

Table 1. AMINO-ACID COMPOSITION OF ELASTASE

Amino-acid	No. of residues	Amino-acid	No. of residues
Lysine	3	Glycine	25
Histidine	6	Alanine	17
Arginine	12	Cysteine	8
Aspartic acid	6	Valine	27
Asparagine	18	Methionine	2
Threonine	19	Isoleucine	10
Serine	22	Leucine	18
Glutamic acid	4	Tyrosine	11
Glutamine	15	Phenylalanine	3
Proline	7	Tryptophan	7

Total: 240 residues.

Determination of Amino-acid Sequence

Highly purified elastase was prepared from 'Trypsin 1–300', an acetone powder of porcine pancreas (Nutritional Biochemicals, Cleveland, Ohio)[1,8], using crystallization rather than chromatography as the final stage in the purification[10,50].

The complete amino-acid sequence was determined by studies of the peptide fragments produced by digestion of the elastase molecule by various proteolytic enzymes. The peptide mixtures thus obtained were fractionated by column chromatography and then by high voltage paper electrophoresis and paper chromatography. The sequences of the purified peptides were determined by the most appropriate current techniques.

In the first instance peptide fragments were obtained from reduced and aminoethylated diisopropyl phosphoryl elastase (DIP-elastase) by digestion with trypsin. This procedure had the advantage that the conversion of cystine to aminoethyl-cysteine residues provided extra sites of tryptic cleavage in regions of the molecule where the sequence was already known[9], resulting in a greater number of smaller, more soluble and more easily sequenced peptides than would otherwise have been obtained. This digest was fractionated on a 'Zeo-Karb 225' (×2) ion-exchange column with a pyridine–acetate gradient, and from it peptides accounting for almost all of the elastase molecule were isolated and sequenced.

The missing fragments were obtained by subjecting reduced and ^{14}C-carboxymethylated DIP-elastase to cyanogen bromide cleavage at the two methionine residues. The three fragments produced were separated by gel filtration on 'Sephadex *G*-50', as were the soluble tryptic peptides obtained from them. Fourteen of the fifteen tryptic peptides of elastase were isolated in this way, further purified by high voltage paper electrophoresis and, where necessary, subjected to sequence analysis.

The missing tryptic peptide was that from the carboxyl terminus of the molecule. It contained fifteen amino-acid residues. It was insoluble in most normal solvents, including 8 M urea, and was isolated by prolonged tryptic digestion of the purified carboxyl-terminal cyanogen bromide fragment of sixty-eight residues, followed by removal of the soluble peptides by washing in 8 M urea. Its sequence was determined from studies of the overlapping soluble peptides obtained by peptic digestion of this insoluble core. Nine of its amino-acid residues occupy an α-helical conformation in the three dimensional structure of tosyl-elastase[11], which may explain its peculiar solubility properties.

In order to overlap the tryptic peptides to determine the complete sequence, peptides containing internal arginine residues were isolated from a peptic digest of native elastase, fractionated on 'Sephadex *G*-50' and purified by high voltage paper electrophoresis, as were several other peptides used to determine or confirm the sequence at various positions. Peptides containing internal lysine residues were obtained from a combined tryptic plus chymotryptic digest of reduced and aminoethylated DIP-elastase which had been fully acetylated with ^{14}C-acetic anhydride, this digest being fractionated on a 'Zeo-Karb 225' column with a pyridine–acetate gradient. These lysine peptides were prepared and purified by Dr K. J. Stevenson during other studies (H. Kaplan, K. J. S. and B. S. H., manuscript in preparation).

In determining the sequences of the purified peptides, much use was made of the dansyl–Edman procedure for stepwise sequential analysis[12], and larger peptides were frequently subjected to secondary digestion either by the enzymes trypsin, α-chymotrypsin, papain, pepsin or thermolysin, or by partial acid hydrolysis. The amide groups of asparagine and glutamine residues were unambiguously located by measurements of the electrophoretic mobilities of peptides containing them[13]. It is noteworthy that extensive deamidation occurred at each of the four -Asn-Gly- sequences in elastase, the deamidated varieties of peptides containing these sequences being isolated in much greater yields than the amidated varieties, as also noted by Ambler[47]. This suggests that the amide groups of asparagine are made much more labile by an adjacent glycine residue, and that evidence for -Asp-Gly- sequences should not be uncritically accepted at face value.

---

[*] Present address: Laboratory of Molecular Enzymology, Department of Biochemistry, University of Bristol, Bristol B58 1TD.

Table 2. THE AMINO-ACID SEQUENCES OF PORCINE ELASTASE AND BOVINE TRYPSIN, CHYMOTRYPSIN A, AND CHYMOTRYPSIN B

	⑯	⑰	18	19	20	21	㉒	23	24	25	26	㉗	㉘	㉙	㉚	㉛	㉜	㉝	34	35	36	36A	36B	36C	37	28	39
PE :	VAL	VAL	GLY	GLY	Thr	GLU	ALA	Gln	Arg	ASN	SER	TRP	PRO	Ser	GLN	ILE	SER	LEU	GLN	Tyr	ARG	Ser	Gly	Ser	SER	Trp	Ala
BT :	ILE	VAL	GLY	GLY	Tyr	Thr	Cys	Gly	Ala	ASN	THR	Val	PRO	TYR	GLN	VAL	SER	LEU	ASN	—	—	—	—	—	SER	GLY	TYR
BCA:	ILE	VAL	Asn	GLY	Glu	GLU	ALA	Val	Pro	Gly	SER	TRP	PRO	TRP	GLN	VAL	SER	LEU	GLN	Asp	LYS	—	—	—	THR	GLY	PHE
BCB:	"	"	"	"	"	ASP	"	"	"	"	"	"	"	"	"	"	"	"	"	"	Ser	—	—	—	"	"	"

	㊵	41	㊷	㊸	㊹	㊺	㊻	㊼	48	49	50	(51)	(52)	(53)	(54)	(55)	56	57	(58)	59	60	61	62	63	64	65	65A
PE :	HIS	Thr	CYS	GLY	GLY	THR	LEU	ILE	Arg	GLN	ASN	TRP	VAL	Met	THR	ALA	ALA	HIS	CYS	VAL	ASP	Arg	Glu	LEU	THR	Phe	ARG
BT :	HIS	PHE	CYS	GLY	GLY	SER	LEU	ILE	ASN	Ser	GLN	TRP	VAL	VAL	SER	ALA	ALA	HIS	CYS	Tyr	Lys	SER	GLY	ILE	Gln	VAL	ARG
BCA:	HIS	PHE	CYS	GLY	GLY	SER	LEU	ILE	ASN	Glu	ASN	TRP	VAL	VAL	THR	ALA	ALA	HIS	CYS	Gly	Val	THR	Thr	Ser	Asp	VAL	—
BCB:	"	"	"	"	"	"	"	"	Ser	"	ASP	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	—
PCC:																	ALA	HIS	CYS	ILE	ASN	SER	GLY	THR	SER	Arg	Thr

	(66)	(67)	(68)	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92
PE :	VAL	VAL	Val	GLY	GLU	His	ASN	LEU	ASN	Gln	Asn	Asn	GLY	Thr	GLU	GLN	TYR	VAL	Gly	VAL	Gln	LYS	ILE	VAL	VAL	HIS	PRO
BT :	Leu	—	—	GLY	GLN	Asp	ASN	ILE	ASN	Val	Val	Glu	GLY	ASN	GLN	GLN	PHE	ILE	Ser	Ala	Ser	LYS	Ser	ILE	VAL	HIS	PRO
BCA:	VAL	VAL	Ala	GLY	GLU	Phe	ASP	Gln	Gly	Ser	Ser	SER	Glu	Lys	Ile	GLN	Lys	LEU	Lys	ILE	Ala	LYS	VAL	Phe	Lys	Asn	Ser
BCB:	"	"	"	"	"	"	"	"	"	Leu	Glu	THR	"	ASP	Thr	"	Val	"	"	"	Gly	"	"	"	"	"	PRO

	93	94	95	96	97	98	99	99A	99B	100	101	(102)	(103)	(104)	(105)	(106)	107	(108)	109	110	111	(112)	113	114	115	116	117
PE :	Tyr	TRP	ASN	THR	ASP	Asp	VAL	Ala	Ala	Gly	Tyr	ASP	ILE	Ala	LEU	LEU	ARG	LEU	ALA	Gln	Ser	Val	THR	LEU	ASN	SER	Tyr
BT :	Ser	TYR	ASN	SER	ASN	THR	LEU	—	—	ASN	ASN	ASP	ILE	Met	LEU	ILE	LYS	LEU	Lys	SER	ALA	ALA	SER	LEU	ASN	SER	Arg
BCA:	Lys	TYR	ASN	SER	Leu	THR	ILE	—	—	ASN	ASN	ASP	ILE	Thr	LEU	LEU	LYS	LEU	Ser	THR	ALA	ALA	SER	Phe	Ser	GLN	Thr
BCB:	"	PHE	Ser	Ile	"	"	VAL	—	—	Arg	"	"	"	"	"	"	"	"	ALA	"	Pro	"	Gln	"	"	GLU	"

	(118)	119	120	(121)	122	123	(124)	125	126	127	128	129	130	131	132	133	134	135	(136)	137	(138)	(139)	(140)	(141)	(142)	143	144
PE :	VAL	Gln	Leu	Gly	Val	LEU	PRO	Arg	ALA	Gly	Thr	Ile	Leu	ALA	Asn	Asn	SER	Pro	CYS	Tyr	ILE	THR	GLY	TRP	GLY	LEU	THR
BT :	VAL	Ala	Ser	ILE	Ser	LEU	PRO	THR	—	SER	Cys	Ala	Ser	—	ALA	GLY	THR	Gln	CYS	Leu	ILE	SER	GLY	TRP	GLY	Asn	THR
BCA:	VAL	Ser	Ala	VAL	Cys	LEU	PRO	SER	ALA	SER	ASP	Asp	Phe	ALA	ALA	GLY	THR	Thr	CYS	Val	Thr	THR	GLY	TRP	GLY	LEU	THR
BCB:	"	"	"	"	"	"	"	"	"	Asp	GLU	"	"	Pro	"	"	Met	Leu	"	Ala	"	"	"	"	"	Lys	"

	145	146	147	148	149	150	151	152	153	154	(155)	156	157	(158)	159	(160)	161	(162)	163	164	165	166	167	(168)	169	170	170A
PE :	ARG	—	THR	ASN	Gly	GLN	Leu	Ala	Gln	Thr	LEU	GLN	GLN	ALA	Tyr	LEU	PRO	Thr	VAL	Asp	Tyr	Ala	Ile	CYS	Ser	SER	Ser
BT :	LYS	Ser	SER	Gly	Thr	Ser	Tyr	PRO	ASP	Val	LEU	Lys	Cys	Leu	Lys	Ala	PRO	ILE	LEU	SER	ASN	SER	Ser	CYS	LYS	SER	—
BCA:	ARG	Tyr	THR	ASN	Ala	ASN	Thr	PRO	ASP	ARG	LEU	GLN	GLN	ALA	SER	LEU	PRO	LEU	LEU	SER	ASN	THR	ASN	CYS	LYS	Lys	—
BCB:	LYS	"	Asn	Ala	Leu	Lys	"	"	"	LYS	"	"	"	"	THR	"	"	ILE	VAL	"	"	"	ASP	"	ARG	"	—

	170B	171	172	173	174	175	(176)	177	178	179	(180)	(181)	(182)	(183)	(184)	184A	185	186	187	188	188A	189	190	191	192	(193)	(194)
PE :	Ser	TYR	TRP	GLY	SER	Thr	VAL	LYS	ASN	Ser	MET	VAL	CYS	ALA	GLY	—	Gly	Asn	GLY	VAL	ARG	SER	Gly	CYS	GLN	GLY	ASP
BT :	—	Ala	TYR	Pro	Gly	Gln	ILE	THR	Ser	Asn	MET	Phe	CYS	ALA	GLY	Tyr	Leu	Glu	GLY	Gly	LYS	ASP	SER	CYS	GLN	GLY	ASP
BCA:	—	TYR	TRP	GLY	THR	LYS	ILE	LYS	ASP	Ala	MET	ILE	CYS	ALA	GLY	—	Ala	Ser	GLY	VAL	—	SER	SER	CYS	Met	GLY	ASP
BCB:	—	"	"	"	SER	ARG	VAL	THR	"	Val	"	"	"	"	"	—	"	"	"	"	—	"	"	"	"	"	"
PCC:																						GLU	THR	CYS	GLU	GLY	ASP

	195	(196)	(197)	(198)	(199)	(200)	(201)	202	203	204	205	206	207	208	(209)	(210)	(211)	(212)	(213)	(214)	(215)	216	217	217A	218	219	220
PE :	SER	GLY	GLY	PRO	LEU	His	CYS	Leu	Val	ASN	GLY	Gln	TYR	Ala	Val	His	GLY	VAL	Thr	SER	PHE	Val	SER	Arg	Leu	GLY	CYS
BT :	SER	GLY	GLY	PRO	Val	VAL	CYS	Ser	Gly	Lys	—	—	—	—	LEU	Gln	GLY	ILE	VAL	SER	TRP	GLY	SER	—	—	GLY	CYS
BCA:	SER	GLY	GLY	PRO	LEU	VAL	CYS	Lys	Lys	ASN	GLY	Ala	TRP	Thr	LEU	Val	GLY	ILE	VAL	SER	TRP	GLY	SER	—	Ser	Thr	CYS
BCB:	"	"	"	"	"	"	"	Gln	"	"	"	"	"	"	"	Ala	"	"	"	"	"	"	"	—	"	"	"
PCC:	SER	GLY	(GLY,	PRO)	LEU	VAL																					

	221	221A	222	223	224	225	226	(227)	(228)	(229)	230	(231)	232	233	(234)	235	236	(237)	(238)	239	240	241	242	243	244	245
PE :	Asn	Val	THR	Arg	LYS	PRO	Thr	VAL	PHE	THR	ARG	VAL	SER	ALA	TYR	ILE	SER	TRP	ILE	ASN	ASN	Val	ILE	ALA	SER	ASN
BT :	Ala	Gln	Lys	Asn	LYS	PRO	GLY	VAL	TYR	THR	LYS	VAL	Cys	Asn	TYR	VAL	SER	TRP	ILE	Lys	GLN	THR	ILE	ALA	SER	ASN
BCA:	Ser	—	THR	Ser	Thr	PRO	GLY	VAL	TYR	Ala	ARG	VAL	THR	ALA	Leu	VAL	Asn	TRP	VAL	GLN	GLN	THR	LEU	ALA	Ala	ASN
BCB:	"	—	"	"	"	"	Ala	"	"	"	"	"	"	"	"	Met	Pro	"	"	"	GLU	"	"	"	"	"

The numbering is that of chymotrypsinogen A. "Insertions" in the sequences of other enzymes are numbered 36A, 36B, etc. "Deletions" are indicated thus —. Only those residues of chymotrypsinogen B which differ from chymotrypsinogen A are indicated. At each sequence position residues which are chemically similar (defined as Arg = Lys, Asp = Glu, Asn = Gln, Asp = Asn, Glu = Gln, Ser = Thr, Val = Ile, Ile = Leu and Tyr = Phe = Trp) between any pair of enzymes are shown in capitals. Residues which are identical are underlined, except for identities between chymotrypsinogen A and chymotrypsinogen B which are not shown. Residues whose sidechains are internal, judged to be inaccessible to water molecules in the molecular models of both elastase[11] and α-chymotrypsin[36], are indicated by circles round the residue numbers. PE = porcine elastase; BT = bovine trypsin[17]; BCA = bovine chymotrypsin A[19]; BCB = bovine chymotrypsin B[21]; PCC = porcine chymotrypsin C[34]. Disulphide bridges[48] are as follows: E, T, CA and CB: 42–58, 136–201, 168–182 and 191–220; T only: 22–157 and 128–232; CA and CB only: 1–122.

Table 3. THE N-TERMINAL SEQUENCES OF TRYPSINOGENS AND CHYMOTRYPSINOGENS

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
PT:								Phe-	Pro-	Thr-	Asp-	Asp-	Asp-	Asp-	Lys-		
OTA:								Phe-	Pro-	Val-	Asp-	Asp-	Asp-	Asp-	Lys-		
OTB:										Val-	Asp-	Asp-	Asp-	Asp-	Lys-		
BT:										Val-	Asp-	Asp-	Asp-	Asp-	Lys-	Ile-	Val-
PCA:	Cys-	Gly-	Val-	Pro-	Ala-	Ile-	Pro-	Pro-	Val-	Leu-	Ser-	Gly-	Leu-	Ser-	Arg-	Ile-	Val-
PCB:	Cys-	Gly-	Val-	Pro-	Ala-	Ile-	Pro-	Pro-	Val-	Leu-	Ser-	Gly-	Leu-	Ser-	Arg-	Ile-	Val-
BCA:	Cys-	Gly-	Val-	Pro-	Ala-	Ile-	Gln-	Pro-	Val-	Leu-	Ser-	Gly-	Leu-	Ser-	Arg-	Ile-	Val-
BCB:	Cys-	Gly-	Val-	Pro-	Ala-	Ile-	Gln-	Pro-	Val-	Leu-	Ser-	Gly-	Leu-	Ala-	Arg-	Ile-	Val-
PCC:	Cys-	Gly-	Val-	Pro-	Ser-	Phe-	Gln-	Pro-	Asn-	Leu-	Ser-	—	—	Ala-	Arg-	Val-	
BCC:	Cys-	Gly-	Ala-	Pro-	Ile-	Phe-	Gln-	Pro-	Asn-	Leu-	Ser-	—	—	Ala-	Arg-	Val-	

Only residues which differ from the sequence in porcine trypsinogen or porcine chymotrypsinogen A are indicated. PT = porcine trypsinogen[29]; TAO and OTB are two ovine trypsinogens[30] and BT = bovine trypsinogen[16]. PCA = porcine chymotrypsinogen A[31]; PCB = porcine chymotrypsinogen B[27]; BCA = bovine chymotrypsinogen A[18]; BCB = bovine chymotrypsinogen B[21]; PCC = porcine chymotrypsinogen C and BCC = "bovine chymotrypsinogen C" (procarboxypeptidase fraction II)[32].

These and the previous studies[8,9] have led to the complete determination and unambiguous overlapping of the amino-acid sequence of elastase (Table 2). Confirmation of the accuracy of this determination is given by three independent lines of evidence. First, tryptic peptides produced from each of the purified cyanogen bromide fragments of elastase have all been identified and agree with those predicted from the local overlaps. Second, there are extensive sequence homologies between elastase, trypsin and the chymotrypsins. These are not fortuitous, but are closely related to the functions and three-dimensional structures of the enzymes, and would be lessened by any re-ordering of the elastase peptides. Third, the three-dimensional electron density map of tosyl-elastase[14] contains detailed information about the order of large, small, polar, nonpolar and aromatic amino-acid residues along the polypeptide chain, many of which can be unambiguously identified from their electron density alone. A model of the elastase molecule built from the sequence data (Table 2) satisfactorily accounts for all the electron density in the map, there being no point at which the crystallographic and sequence evidence are in conflict[11,15].

The sequence of elastase shown in Table 2 aligned with those of bovine trypsinogen[16,17], bovine chymotrypsinogen A (refs. 18-20) and bovine chymotrypsinogen B (ref. 21) in a way which maximizes the homologies between the four enzymes and introduces only two extra sequence positions into the table. Three corrections have been made to the previously published elastase sequence data[9]. Between Cys-168 and Tyr-171 there are four, not five, serine residues; residue 186 is asparagine, not aspartic acid; and between Asn-204 and Glu-206 there is a glycine residue, Gly-205. (To aid in the comparison between the various serine proteinases we have, throughout this paper, used the numbering of the amino-acid residues in the chymotrypsinogen A sequence to describe the position of corresponding amino-acid residues in these homologous enzymes: see Table 2.)

The ambiguity in the previously published sequence of residues 197 and 198 has been resolved, the sequence being -Gly-Pro-, and the overlap between Asn-186 and Gly-187 has been established. The table also shows recent corrections to the sequences of bovine trypsinogen (residue 189 is aspartic acid, not asparagine[22]) and bovine chymotrypsinogen A (residue 102 is aspartic acid, not asparagine[23]) which are of great importance for a correct understanding of the nature of the specificity and catalytic mechanism of those enzymes[11,22-24].

Homologies between Pancreatic Endopeptidases

Bovine pancreatic juice contains, in roughly equal amounts, the zymogens of trypsin, chymotrypsin A, chymotrypsin B (ref. 25) and a fourth serine endopeptidase, less well characterized, which is part of the procarboxypeptidase A complex[26]. In porcine juice, trypsinogen, chymotrypsinogen A and chymotrypsinogen B appear to resemble their bovine counterparts, while chymotrypsinogen C may be very similar to the "procarboxypeptidase endopeptidase" of the cow[27]. In neither of these studies was proelastase or elastase discovered. The enzyme undoubtedly exists in good yield in acetone powders of pig pancreas, and the apparent discrepancy may arise from the fact that the enzyme precipitates as a complex with another acidic component when extracts of pancreas are dialysed free of salt[1]. It might therefore be accidentally removed before ion-exchange chromatography, and for this reason one cannot be certain that elastase is absent in bovine pancreatic juice, though its biological value for a herbivore, such as the cow, is less obvious than for an omnivore like the pig. Early reports of elastase activity in bovine pancrease[1,28] deserve greater credence than they have sometimes received, and it seems likely that both bovine and porcine pancreatic juice contain five serine proteinase zymogens: trypsinogen, proelastase and three chymotrypsinogens, A, B and C.

Table 3 shows the homology which exists between the N-terminal sequences of the chymotrypsinogens and trypsinogens of various species. The N-terminal sequences of porcine chymotrypsinogens A and B are identical to that of bovine chymotrypsinogen A except that Pro-7 replaces Gln-7 of the latter. Bovine and porcine chymotrypsinogen C resemble each other quite closely but are less homologous with chymotrypsinogens A and B both in sequence (Tables 2 and 3) and in activity[33]. They are, nevertheless, best classified as chymotrypsinogens. Some further sequences[34] of porcine chymotrypsinogen C around the active centre His-57 and Ser-195 are included in Table 2. The sequence of the small activation peptide of proelastase is as yet unknown[5]. Splitting of the bonds between residues 15 and 16 activates each zymogen, releasing activation peptides in the case of proelastase and the trypsinogens.

In Table 4 a general analysis is made of the degree of homology* between those enzymes the sequence of which is completely established. It shows that bovine chymotrypsin A and chymotrypsin B, which show almost identical substrate specificities[35], are closely related isoenzymes showing a degree of homology (78 per cent identity) which is much higher than that between the α and β chains of human haemoglobin (44 per cent identity). On the other hand, the chymotrypsins, trypsin and elastase are clearly enzymes of different specificity, but show between 35 per cent and 43 per cent identity in amino-acid sequence (contrast, for example, sperm whale myoglobin and human haemoglobin α chain, which show 18 per cent identity), suggesting that they too have been produced by divergent evolution from a common ancestor following gene duplication events.

Table 2 shows that these amino-acid side-chain homologies are not uniformly distributed throughout the polypeptide chain, but tend to be concentrated around those residues that are important for catalysis and in those parts of the molecule that are internal, as judged by the apparent inaccessibility of the amino-acid side-chains to water molecules in the molecular models of both elastase[11] and α-chymotrypsin[36]. Table 5 gives an analysis of this distribution of homologous residues between internal and external positions. 71 per cent of the internal positions, but only 19 per cent of the external ones, are homologous

---

* Homologous residues are those which are identical or chemically similar to their counterparts in the other enzymes. Chemical similarity is defined in this paper as Arg = Lys, Asp = Glu, Asn = Gln, Asp = Asn, Glu = Gln, Ser = Thr, Val = Ile, Ile = Leu, and Tyr = Phe = Trp.

Table 4. OVERALL HOMOLOGIES BETWEEN SERINE PROTEINASES

	Total No. of residues	Per cent identity (or per cent chemical similarity*) to†, and mutation distance‡ from: Trypsin	Chymotrypsinogen A§	Chymotrypsinogen B§	Any other of these four
Elastase	240	35% (48%) 244	39% (51%) 215	38% (47%) 220	51% (68%)
Trypsin	223		43% (53%) 211	38% (49%) 220	56% (67%)
Chymotrypsinogen A§	230			78% (85%) 62	59% (69%)¶
Chymotrypsinogen B§	230				53% (64%)‖

* Defined as for Table 2.

† Calculated as per cent of the minimum length required to accommodate the sequences being compared when aligned as in Table 2, discounting common deletions.

‡ Minimum number of nucleotide base changes required to convert the gene for one sequence into that for the other, based on the genetic code as reported by Crick[49], computed with a program kindly written by Miss Joyce Cox. Deletions are scored as being two bases different from any amino-acid residue.

§ Residues 16–245 only.

¶ Excluding chymotrypsinogen B.

‖ Excluding chymotrypsinogen A.

Table 5. HOMOLOGIES IN INTERNAL AND EXTERNAL POSITIONS BETWEEN SERINE PROTEINASES*

	No. of residues	Per cent residues identical (or chemically similar†) in all four enzymes	Per cent residues showing no homology between any pair of enzymes‡
In internal positions†	75	56% (71%)	4%
In external positions†	167	10% (19%)	30%
Overall	242	24% (35%)	22%

* Porcine elastase, bovine trypsin and bovine chymotrypsinogens A and B (residues 16–245 only).

† Defined as for Table 2.

‡ Except between chymotrypsinogen A and chymotrypsinogen B.

in all four enzymes, while only 4 per cent of the internal positions show no homology between any two enzymes of different specificities, compared with 30 per cent of the external positions. Of the one hundred residues suspected of being internal by Smillie *et al.*[21] on the basis of their being hydrophobic in both trypsin and the chymotrypsins, sixty are in fact in internal positions, along with another fifteen residues which were thought to be external.

The sequence homologies (Table 2) allow one to build models of trypsin and elastase based on the principle that the conformation of the polypeptide chains are identical to that of α-chymotrypsin. Such models were built[22], and showed no obvious steric or chemical defects. For example, the internal side-chains could be close packed with no distortion of the main chain and no charged groups replaced uncharged groups which are internal in the α-chymotrypsin structure. These models suggested that the differences in specificity between the enzymes could be explained by relatively minor side-chain substitutions on the enzyme surface. In the trypsin model, the replacement of Asp-189 for Ser-189 in the substrate binding site of chymotrypsin[24] provides a negative charge perfectly placed to form an ion-pair with the positive charge of lysyl or arginyl substrates[22]. In the elastase model the bulky side chains of Val-216 and Thr-226, replacing Gly-216 and Gly-226 of chymotrypsin, block the entrance to this substrate binding cavity. This enzyme should not, therefore, demonstrate marked specificity for large basic or aromatic side-chains on the amino-acid residue N-terminal to the bond split, as do trypsin and chymotrypsin. The available data on the specificity of elastase[3,52,53] support this conclusion. The X-ray data[11,14] justify these conclusions from the hypothetical model of elastase and thereby add weight to the conclusions from the trypsin model.

The distribution of the amino-acid side-chains of elastase is shown in Fig. 1. With the exception of the two buried aspartic acid residues (102 and 194) the functions of which are discussed in the third article of this series[11], all the charged amino-acid residues occupy surface positions in the molecule. The other hydrophilic residues show a similar but less marked tendency. By contrast the hydrophobic amino-acids are fairly evenly distributed between the inside and the outside of the molecule, but while those on the surface are mostly found in non-homologous positions, most of the internal ones are similar to, if not identical with, their counterparts in the other three enzymes. Such analysis of the side-chain distributions in trypsin and the chymotrypsins yield essentially similar results and will not be detailed here. A more detailed discussion of the organization of the tertiary structure of elastase and the conformation of those residues important for its activity is given later in the series[11].

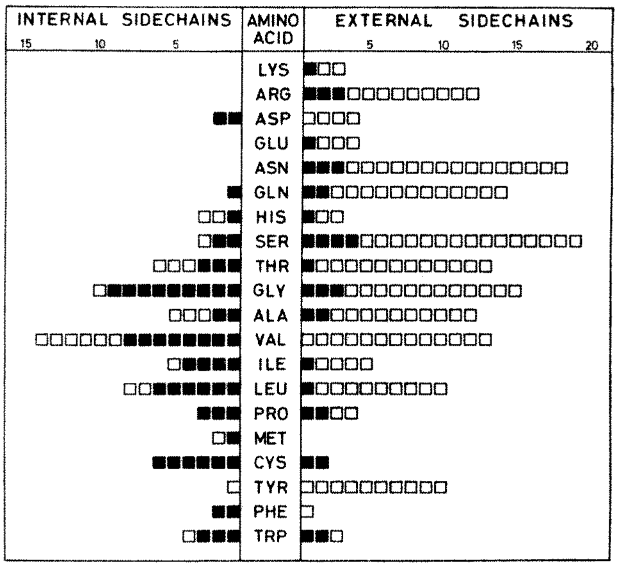


Fig. 1. The distribution between internal and external positions of the side-chains of the amino-acid residues in the porcine elastase molecule. ■, Residues homologous with (identical or chemically similar to) corresponding residues in bovine trypsin, chymotrypsinogen A and chymotrypsinogen B. □, Residues not homologous with all three other enzymes. "Internal", "external" and "chemically similar" are defined as for Table 2.

Evolution of the Serine Proteinases

The five pancreatic endopeptidases thus seem to be a single family of enzymes with almost identical tertiary structures, this identity being attributable to the homology of their amino-acid sequences, especially of the internal hydrophobic residues. The differences in specificity between the chymotrypsins, trypsin and elastase seem to result from relatively few amino-acid replacements at the substrate binding sites, which are accompanied by quite extensive but relatively unimportant changes in the rest of the enzyme surface. The same pattern of organization probably exists in all the mammalian serine proteinases, because thrombin, secreted as prothrombin by the liver into the blood stream, exhibits a high degree of sequence homology with the pancreatic enzymes in positions which would be expected from similar tertiary structures[22,27].

Serine proteinases from other organisms, the spiny Pacific dogfish[38], the turtle[39], a sea anemone *Metridium senile*[40], a mould *Streptomyces griseus*[41] and a soil bacterium *Sorangium* sp.[42] have been shown to possess some homologies with the mammalian serine proteinases in substrate kinetics and specificity and in amino-acid sequence, notably the possession of an -Asp-Ser-Gly- sequence at the serine residue of the active centre. This suggests that these enzymes of the "chymotrypsin" type may be of common distant evolutionary origin, the products of gene duplication events followed by divergent evolution. Another class of serine proteinases exists which is characterized by a -Thr-Ser-Met- sequence at the active centre serine residue and is represented by the bacterial extracellular proteinases subtilisin BPN′ from *Bacillus amyloliquofaciens* and subtilisin Carlsberg from *B. subtilis*, and probably by a proteinase from a mould *Aspergillus oryzae*[43]. While homologous with one another, these enzymes show no homology in sequence[44] or in tertiary structure[45] with the mammalian serine proteinases, and yet have a similar grouping of an aspartic acid, a histidine and a serine residue at the catalytic site and seem to function by a similar enzymic mechanism produced by convergent evolution. Yet another class of serine proteinases may exist in yeasts and plants[46]. In addition the mammalian serine esterases are a separate class of enzymes which display active centre sequence homologies with the mammalian serine proteinases. Further sequence studies on these various types and classes of enzymes from widely divergent species are needed to reveal the degree of similarity which exists between them and to clarify and substantiate our present rudimentary knowledge about their classification and evolutionary origins.

We thank Mr R Jakes for technical assistance. One of us (D. M. S.) acknowledges the financial support of a Medical Research Council scholarship and a Beit Memorial medical research fellowship received during the course of this work.

Received October 23, 1969; revised January 8, 1970.

[1] Lewis, U. T., Williams, D. E., and Brink, N. G., *J. Biol. Chem.*, **222**, 705 (1956).

[2] Partridge, S. M., and Davis, H. F., *Biochem. J.*, **61**, 21 (1955).

[3] Naughton, M. A., and Sanger, F., *Biochem. J.*, **78**, 156 (1961).

[4] Grant, N. H., and Robbins, K. C., *Arch. Biophys. Biochem.*, **66**, 396 (1957).

[5] Moon, H. D., and McIvor, B. C., *J. Immunol.*, **85**, 78 (1960).

[6] Hartley, B. S., *Ann. Rev. Biochem.*, **29**, 45 (1960).

[7] Naughton, M. A., Sanger, F., Hartley, B. S., and Shaw, D. C., *Biochem. J.*, **77**, 149 (1960).

[8] Smillie, L. B., and Hartley, B. S., *Biochem. J.*, **101**, 232 (1966).

[9] Brown, J. R., Kauffman, D. L., and Hartley, B. S., *Biochem. J.*, **103**, 497 (1967).

[10] Shotton, D. M., Hartley, B. S., Camerman, N., Hofmann, T., Nyburg, S. C., and Rao, L., *J. Mol. Biol.*, **32**, 155 (1968).

[11] Shotton, D. M., and Watson, H. C., *Nature*, **225**, 811 (1970). (Third article of this series.)

[12] Gray, W. R., in *Methods in Enzymology* (edit. by Hirs, C. H. W.), **11**, 469 (Academic Press, New York, 1967).

[13] Offord, R. E., *Nature*, **211**, 591 (1966).

[14] Watson, H. C., Shotton, D. M., Cox, J. M., and Muirhead, H., *Nature*, **225**, 806 (1970). (Second article of this series.)

[15] Shotton, D. M., and Watson, H. C., *Phil. Trans. Roy. Soc.*, B, **257**, 111 (1970).

[16] Walsh, K. A., Kauffman, D. L., Kumar, K. S. V. S., and Neurath, H., *Proc. US Nat. Acad. Sci.*, **51**, 301 (1964).

[17] Mikeš, O., Tomašek, V., Holeyšovský, V., and Šorm, F., *Biochim. Biophys. Acta*, **117**, 281 (1966).

[18] Hartley, B. S., *Nature*, **201**, 1284 (1964).

[19] Hartley, B. S., and Kauffman, D. L., *Biochem. J.*, **101**, 229 (1966).

[20] Meloun, B., Kluh, I., Kostka, V., Morávek, L., Prusík, Z., Vančček, J., Keil, B., and Šorm, F., *Biochim. Biophys. Acta*, **130**, 543 (1966).

[21] Smillie, L. B., Furka, A., Nagabhushan, N., Stevenson, K. J., and Parkes, C. O., *Nature*, **218**, 343 (1968).

[22] Hartley, B. S., *Phil. Trans. Roy. Soc.*, B, **257**, 77 (1970).

[23] Blow, D. M., Birktoft, J. J., and Hartley, B. S., *Nature*, **221**, 337 (1969).

[24] Birktoft, J. J., Blow, D. M., Henderson, R., and Steitz, T. A., *Phil. Trans. Roy. Soc.*, B, **257**, **57** (1970).

[25] Keller, P. J., Cohen, E., and Neurath, H., *J. Biol. Chem.*, **233**, 344 (1958).

[26] Brown, J. R., Greenshields, R. N., Yamasaki, M., and Neurath, H., *Biochemistry*, **2**, 867 (1963).

[27] Gratecos, D., Guy, O., Rovery, M., and Desnuelle, P., *Biochim. Biophys. Acta*, **175**, 82 (1969).

[28] Banga, I., *Acta Physiol. Acad. Sci., Hung.*, **3**, 317 (1952).

[29] Charles, M., Rovery, M., Guidoni, A., and Desnuelle, P., *Biochim. Biophys. Acta*, **69**, 115 (1963).

[30] Bricteux-Gregoire, S., Schyns, R., and Florkin, M., *Biochim. Biophys. Acta*, **127**, 277 (1966).

[31] Charles, M., Gratecos, D., Rovery, M., and Desnuelle, P., *Biochim. Biophys. Acta*, **140**, 395 (1967).

[32] Peanasky, R. J., Gratecos, D., Baratti, J., and Rovery, M., *Biochim. Biophys. Acta*, **181**, 82 (1969).

[33] Folk, J. E., and Schirmer, W. E., *J. Biol. Chem.*, **240**, 181 (1965).

[34] Tobita, C. T., and Folk, J. E., *Abstr. Seventh Intern. Congr. Biochem.*, **4**, 599 (1967).

[35] Enenkel, A. G., and Smillie, L. B., *Biochemistry*, **2**, 1449 (1963).

[36] Matthews, B. W., Sigler, P. B., Henderson, R., and Blow, D. M., *Nature*, **214**, 652 (1967).

[37] Magnusson, S., *Biochem. J.*, **110**, 25*P* (1968).

[38] Neurath, H., Bradshaw, R. A., and Arnon, R., *Abstr. Intern. Union Biochem. Symp. Copenhagen* (in the press).

[39] Barnard, E. A., and Hope, W. C., *Biochim. Biophys. Acta*, **178**, 364 (1969).

[40] Gibson, D., and Dixon, G. H., *Nature*, **222**, 753 (1969).

[41] Smillie, L. B., *Abstr. Intern. Union Biochem. Symp.*, *Copenhagen* (in the press).

[42] Smillie, L. B., and Whitaker, D. R., *J. Amer. Chem. Soc.*, **89**, 3350 (1967).

[43] Sanger, F., *Proc. Chem. Soc.*, 76 (1963).

[44] Smith, E. L., Markland, F. S., Kasper, C. B., DeLange, R. J., Landon, M., and Evans, W. H., *J. Biol. Chem.*, **241**, 5974 (1966).

[45] Wright, C. S., Alden, R. A., and Kraut, J., *Nature*, **221**, 235 (1969).

[46] Shaw, D. C., and Wells, J. R. E., *Biochem. J.*, **104**, 5C (1967).

[47] Ambler, R. P., *Biochem. J.*, **89**, 349 (1963).

[48] Hartley, B. S., Brown, J. R., Kauffman, D. L., and Smillie, L. B., *Nature*, **207**, 1157 (1965).

[49] Crick, F. H. C., *J. Mol. Biol.*, **38**, 367 (1968).

[50] Shotton, D. M., in *Methods in Enzymology* (edit. by Perlmann, G. E., and Lorand, L.) (Academic Press, New York, in the press).

[51] Gertler, A., and Birk, Y., *Europ. J. Biochem.* (in the press).

[52] Geneste, P., and Bender, M. L., *Biochem. Biophys. Res. Commun.* (in the press).

[53] Sampath Narayanan, A., and Anwar, R. A., *Biochem. J.*, **114**, 11 (1969).

Three-dimensional Fourier Synthesis of Tosyl-elastase at 3·5 Å Resolution

by

H. C. WATSON*
D. M. SHOTTON*
JOYCE M. COX
HILARY MUIRHEAD*

MRC Laboratory of Molecular Biology,
Hills Road, Cambridge

X-ray diffraction data have been collected from four isomorphous crystalline derivatives of the proteolytic enzyme elastase, and a map of the three-dimensional electron density of the tosyl derivative has been calculated by Fourier synthesis of 2,750 independent terms. It shows clearly the conformation of the elastase polypeptide chain, the size and orientation of the amino-acid side-chains, and the position of the tosyl group which labels the active centre of the enzyme.

PORCINE pancreatic elastase (EC 3.4.4.7) is a serine proteinase[1] homologous with trypsin and the chymotrypsins[2]. Preliminary studies[3] showed that elastase could be easily crystallized in the orthorhombic spacegroup $P2_12_12_1$, with unit cell dimensions $a = 51{\cdot}5$ Å, $b = 58{\cdot}0$ Å and $c = 75{\cdot}5$ Å, corresponding to a unit cell of four molecules, with one molecule per asymmetric unit. The large and well ordered crystals diffracted X-rays strongly out to a resolution greater than 2 Å and were very stable to X-irradiation, suggesting that elastase was ideally suited for high resolution X-ray crystallographic studies.

The determination of the structure of elastase, as well as providing a significant contribution to our knowledge of protein conformation in general and of the relationship between amino-acid sequence and tertiary structure in particular, would, by comparison with the known structure of α-chymotrypsin[4,5], answer the longstanding question as to whether related enzymes with different but homologous sequences possess very similar tertiary structures, and would explain the common catalytic mechanism exhibited by these two enzymes. Besides providing information about the detailed conformation of elastase itself, which would make possible the correlation and interpretation of the various chemical, kinetic and physicochemical data obtained on this enzyme, it would also reveal in atomic terms the structural differences responsible for the different substrate specificities and activities exhibited by elastase and α-chymotrypsin.

---

* Present address: Molecular Enzymology Laboratory, Department of Biochemistry, University of Bristol, Bristol B58 1TD.

We describe here the preparation and evaluation of isomorphous crystalline derivatives of elastase, and the determination of the three-dimensional electron density distribution of one of these derivatives, tosyl-elastase, at a resolution of 3·5 Å. Details of the three-dimensional conformation of the elastase molecule, determined by interpreting the crystallographic data in terms of the amino-acid sequence of the enzyme[3], are given in the third article in this series[6], which also contains a comparison of the structures of elastase and α-chymotrypsin.

Preparation and Characterization of Elastase Derivatives

The scheme used for the preparation of derivatives of elastase for three-dimensional crystallographic study is shown in Fig. 1. The inhibited derivatives *p*-toluenesulphonyl elastase (tosyl-elastase) and *p*-chloromercuribenzenesulphonyl-elastase (PCMBS-elastase), in each of which the sulphonyl group is covalently attached to the active centre serine residue of the enzyme, were prepared following the techniques developed by Sigler *et al.* for the synthesis of similar derivatives, which were used in the structural determination of α-chymotrypsin[7,8]. Although these derivatives could be prepared by soaking crystals of elastase in high concentrations of inhibitors, they were routinely prepared by inhibition in solution using the procedure described in Fig. 1. There was more than 98 per cent inhibition with this method, giving virtually full occupancy of these single site derivatives. Crystals were prepared from 0·5–1·0 per cent solutions of the dialysed and freeze dried derivatives that had been redissolved in 0·01 M sodium acetate, *p*H 5·0, by the addition of sodium sulphate to a final concentration of 0·02–0·1 M. Good crystals, isomorphous with crystals of the native enzyme, grew overnight to a size of 1–2 mm and were then transferred to a standard working solution of 1·2 M sodium sulphate–0·01 M sodium acetate (*p*H 5·0), in which they were stable. Sodium, rather than ammonium, sulphate was used in order to avoid the preferential complexing with ammonia of metal ions used to prepare further heavy atom derivatives by conventional soaking techniques[9,10]. The tosyl and PCMBS derivatives form a perfect isomorphous pair which differ only in the replacement of a methyl group in tosyl-elastase by a chloromercury group in PCMBS-elastase. Throughout this work tosyl-elastase, rather than the native enzyme, was used as the parent structure in order fully to exploit this high isomorphism, and to provide an active site marker to aid in the interpretation of the three-dimensional electron density map. Further heavy atom derivatives suitable for use with the multiple isomorphous replacement technique[11] were prepared by soaking crystals of tosyl and PCMBS-elastase in uranyl nitrate solutions (Fig. 1). This heavy atom salt, which proved very useful in solving the structure of hen egg white lysozyme, was chosen because of the good X-ray scattering properties and high anomalous scattering component of the uranyl ion, probably $UO_2(OH)_n^{(n-2)-}$, bound in such derivatives[12]. Four other derivatives of tosyl-elastase were investigated but not used. Uranyl pentafluoride[13] at 30 mM concentration produced weak binding at the same sites as 5 mM uranyl nitrate, 5 mM phenylmercury acetate gave a usable multisite derivative, and both potassium chloroplantinite and mercury acetate at 5 mM concentration caused extensive crystal deterioration as a result of excessive binding, while at 0·5 mM negligible binding was observed.

X-ray diffraction photographs of the principal reciprocal lattice planes of these crystalline derivatives were taken on a Buerger precession camera using CuKα radiation from a rotating anode X-ray tube, and were measured by a computer-controlled flying spot microdensitometer[14]. Easily interpretable difference Patterson projections at 2·8 Å or 3·5 Å resolution were calculated from these data, as shown, for example, in Fig. 2. The accuracy of the interpretations and the potential usefulness of these derivatives in providing three-dimensional phase information was evaluated by calculating difference Fourier projections, using the phase information from one derivative to phase the amplitude data of another. From the results of these studies, details of which are given elsewhere[15], it was evident that the PCMBS group occupied the same single site as the tosyl group with full occupancy, unlike the situation encountered with this derivative of α-chymotrypsin[8], and that in both uranyl derivatives the uranyl ions bound to another single site with almost full occupancy. All three heavy atom derivatives were therefore excellent for use in determining the structure of tosyl-elastase. The difference Fourier projections also suggested the presence of a secondary uranyl binding site of relatively low occupancy, but because the corresponding peaks failed to appear in subsequent three-dimensional difference Fourier syntheses this site was omitted from the three-dimensional phase calculations. The reliability factors of the centric 0*kl* reflexions, calculated after parameter refinement by the Hart process[16], were 42 per cent and 43 per cent for the single site mercury and uranyl derivatives respectively.

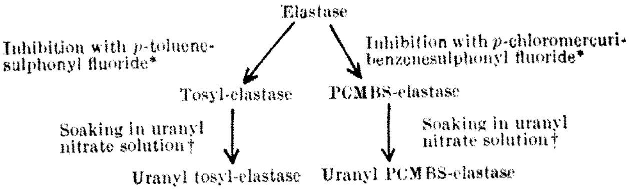

Elastase
Inhibition with *p*-toluenesulphonyl fluoride*
Inhibition with *p*-chloromercuribenzenesulphonyl fluoride*
Tosyl-elastase
PCMBS-elastase
Soaking in uranyl nitrate solution†
Soaking in uranyl nitrate solution†
Uranyl tosyl-elastase
Uranyl PCMBS-elastase

Fig. 1. Preparation of elastase derivatives.

* Covalent inhibition was achieved by slowly adding a two-fold molar excess of sulphonyl fluoride in dioxane, to give a final dioxane concentration of 5 per cent, to a 0·5 per cent solution of elastase in 0·05 M sodium phosphate buffer (*p*H 7·2) containing 2·5 per cent dioxane, and stirring for 18 h at 0° C.

† Uranyl derivatives were prepared by soaking selected crystals of the sulphonyl derivatives in 5 mM uranyl nitrate–1·2 M sodium sulphate–0·01 M sodium acetate (*p*H 5·0) for 2 weeks at 20° C.

Three-dimensional Data Collection

Atoms which are separated by more than the Van der Waals radii are resolved by an accurate structure determination at 3·5 Å resolution. Although confident interpretations of the positions and orientations of many of the amino-acid side-chains could not be made from the 3·5 Å electron density maps of ribonuclease S[17] and lysozyme (personal communication from D. C. Phillips), the overall configuration of the polypeptide chains of these enzymes could be easily discerned. In spite of the greater molecular size of elastase, it was considered that the quality of the heavy atom derivatives was such that the structure of tosyl-elastase at 3·5 Å resolution could be determined with sufficient accuracy to permit a meaningful comparison to be made with the known structure of α-chymotrypsin[4,5].

The stability of elastase crystals to X-irradiation was assessed by collecting two complete sets of 3·5 Å resolution three-dimensional diffractometer data in sequence from a single crystal of tosyl-elastase by the method described below. This involved approximately 11,000 measurements over an irradiation time of about 200 h. The agreement residual of the structure amplitudes of Bijvoet pairs of reflexions

$$R_{(+,-)} = 2 \cdot \frac{\Sigma(|\mathrm{F}|_{hkl} - |\mathrm{F}|_{hk\bar{l}}}{\Sigma(|\mathrm{F}|_{hkl} + |\mathrm{F}|_{h\bar{k}l})}$$

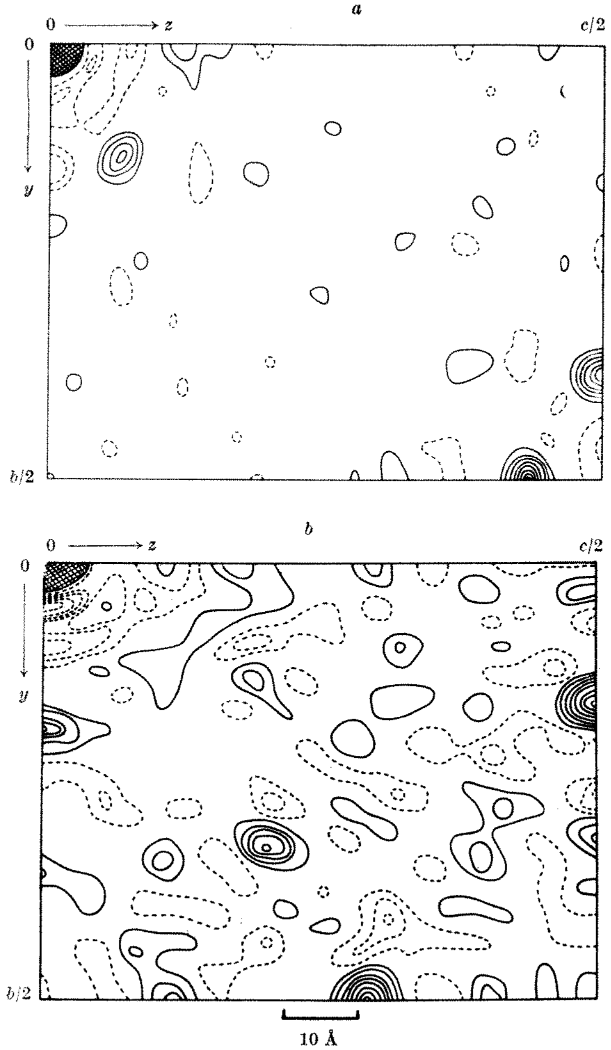


Fig. 2. $0kl$ difference Patterson projections, calculated using tosyl-elastase as the parent structure, (a) of PCMBS-elastase at 2·8 Å resolution, and (b) of uranyl tosyl-elastase at 3·5 Å resolution.

was 1·8 per cent for each data set, while the standard deviation of the averaged values of Bijvoet pairs between the first and the second data set was 3·3 per cent. It was therefore clear that complete 3·5 Å data could be collected from a single crystal without introducing significant errors due to radiation damage. The use of a single crystal of each derivative eliminated several sources of error inherent in the normal practice of collecting three-dimensional data from several crystals of each derivative, including variability in isomorphism, salt concentration and heavy atom site occupancies between crystals, and scaling and weighting errors introduced when data from several crystals are combined.

A complete set of three-dimensional data to 3·5 Å resolution was measured from a single crystal of each of the four elastase derivatives, each crystal used being approximately 0·8 mm in length along each axis. The 5,300 X-ray diffraction intensities within one complete quadrant of the reciprocal sphere of radius 3·5 Å^{-1}, comprising 2,800 independent reflexions, were measured on a Hilger and Watts four-circle diffractometer, which was fitted with helium filled input and output collimators and connected on line to a time-shared Ferranti Argus 312 computer, using CuKα radiation from a Phillips sealed fine focus X-ray tube run at 40 kV and 25 mA. Each reflexion was measured by an ordinate analysis procedure using a diffractometer control program especially written for this study by one of us (H. C. W.) with the help of Mr J. F. W. Mallett. (Programs supplied with the Hilger and Watts PDP8-controlled diffractometer system (Y290) (ref. 18) have now been modified for this ordinate analysis procedure and are available from the authors.) By this procedure the crystal was moved, in steps of 0·02° on the ω circle of the diffractometer, through the setting calculated to satisfy the Bragg angle requirements for the reflexion, the recorded intensity ordinates for the steps being temporarily stored, and then analysed as illustrated in Fig. 3 to produce values for the peak and background intensities. The total scan width (typically 0·8°) was initially set to be twice the previously determined maximum peak width of the strongest reflexions.

The reflexion counting times for each of the four crystals were adjusted to reduce the mean errors from counting statistics to approximately 2 per cent. The counts recorded for corresponding reflexions from the four crystals were thus approximately equal, and in subsequent refinement processes the contributions from the various derivatives were, with proper justification, given equal weight. After each measurement had been made, the reflexion indices, the peak and background intensities, and the position of the peak within the scan were recorded on paper tape.

In comparison with conventional computer controlled background–peak–background counting methods this ordinate analysis procedure, which requires very little extra computer memory, enables a substantial reduction of the reflexion counting time, and hence radiation damage, while retaining the same counting statistics. (A further improvement can be effected by using the more sophisticated ordinate treatment proposed by Diamond[19], although this process requires approximately 4 K more computer memory. Our experience suggests, however, that the Diamond method produces a significant improvement over the method described here only when the data set contains a large number of very weak reflexions.) Using this procedure the complete 3·5 Å data sets from the four elastase derivatives were collected on one diffractometer during July 1968.

Data Processing and Phase Refinement

The measured X-ray intensities were corrected for Lorentz, polarization and absorption[20] factors using a

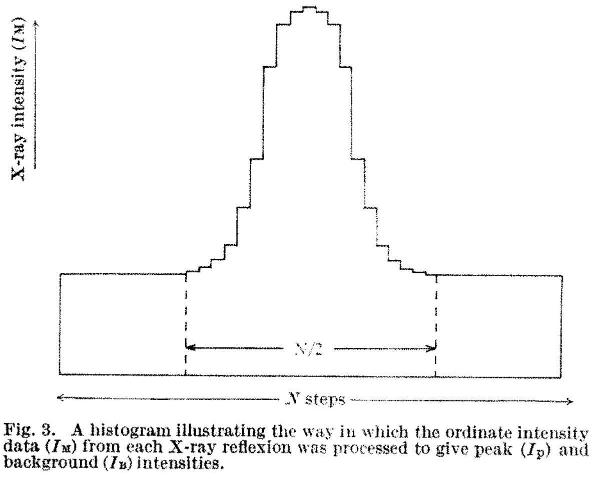


Fig. 3. A histogram illustrating the way in which the ordinate intensity data (I_M) from each X-ray reflexion was processed to give peak (I_P) and background (I_B) intensities.

$$I_P = \left(\sum_{M=n+1}^{M=n+N/2} I_M \right)_{\text{Max}}, \text{ where } n = 0,1,2,---N/2$$

$$I_B = \sum_{M=1}^{M=N} (I_M) - I_P$$

slightly modified version of a general diffractometer output processing program written by Mr T. Gossling. Measurements were rejected if either peak edge was within four steps of a scan extremity. No attempt was made to recover the hundred and fifty or so very weak reflexions rejected from each data set by this procedure, nor the much smaller number of good reflexions which were accidentally rejected because of paper tape punching errors.

The data were checked for internal consistency by comparing the measurements of individual Bijvoet pairs, a very small number of pairs being rejected when the differences were greater than would be expected from anomalous scattering effects. Unpaired reflexions were not rejected by this procedure.

One of the reasons for collecting the X-ray diffraction data from the "double" derivative, uranyl PCMBS-elastase, was to provide an alternative source of the phase information lost by the rejection of reflexions from the data sets of the single heavy atom derivatives, PCMBS-elastase and uranyl tosyl-elastase, thus avoiding the necessity of recollecting this data.

The agreement residuals between Bijvoet pairs for each derivative, $R_{(+,-)}$, which show clearly the effects of anomalous dispersion on the data sets of the heavy atom derivatives, are given in Table 1. This table also includes the values of the scale and temperature factors required to equate the derivative data to that of the parent, and the mean observed differences in structure amplitudes between parent and derivative data, which were calculated by a least squares procedure on 1,400 selected medium intensity reflexions chosen in equal numbers from equal ranges of $\sin^2\theta/\lambda^2$.

Table 1. EXPERIMENTAL DATA STATISTICS

Derivative	$R_{(+,-)}$	c	b	$\overline{\lvert\Delta F_H\rvert}$
Tosyl-elastase (parent)	0·018	1·00	0·00	
PCMBS-elastase	0·031	1·09	−4·36	3·5
Uranyl tosyl-elastase	0·050	0·86	−0·84	5·1
Uranyl PCMBS-elastase	0·054	0·91	−3·13	6·3

$R_{(+,-)}$ is the agreement residual between the structure amplitudes of Bijvoet pairs of reflexions, defined as in the text, calculated from the complete 3·5 Å data sets. c and b are the scale and temperature factors required to equate the derivative data to that of the parent, defined by the expression $F_{H\,\mathrm{corr}} = c \times F_{H\,\mathrm{obs}} \cdot e^{-b\cdot\sin^2\theta/\lambda^2}$. $\overline{\lvert\Delta F_H\rvert}$ is the mean difference in corrected structure amplitudes between parent and derivative data, the mean observed structure amplitude of tosyl-elastase, $\lvert\overline{F_P}\rvert$, being 31·0. The values of c, b, $\overline{\lvert\Delta F_H\rvert}$ and $\lvert\overline{F_P}\rvert$ were calculated from 1,400 medium intensity reflexions selected in equal numbers from equal ranges of $\sin^2\theta/\lambda^2$.

Three-dimensional difference Patterson syntheses, including that of the "double" derivative, were easily interpretable and were used to check the mercury and uranyl single site coordinates derived from the two-dimensional studies described here. These coordinates, plus heavy atom occupancies and isotropic temperature factors, were then refined using alternate cycles of phase determination and least squares refinement[21–23] on the 1,400 selected reflexions, anomalous scattering being ignored. Difference Fourier syntheses were calculated for all three heavy atom derivatives to identify possible subsidiary binding sites, but none were found. The parameters derived from the last cycle of the least squares refinement are given in Tables 2 and 3.

To determine the correct structural enantiomorph, the anomalous dispersion data were included in the phase determination of the 1,400 selected reflexions in the two possible senses[22,27]. The results of these calculations are summarized in Table 3. Because the heavy atom derivatives used contained only two independent heavy atom sites, the mean figure of merit[21] increased for both structural enantiomorphs, although the correct enantiomorph produced the better value. The root mean square lack of closure for the phase triangles of each derivative[21,22,24] became worse for the wrong enantiomorph, however, but improved significantly when the anomalous scattering was included correctly, suggesting that this factor distinguishes more effectively between the two possibilities than does mean figure of merit.

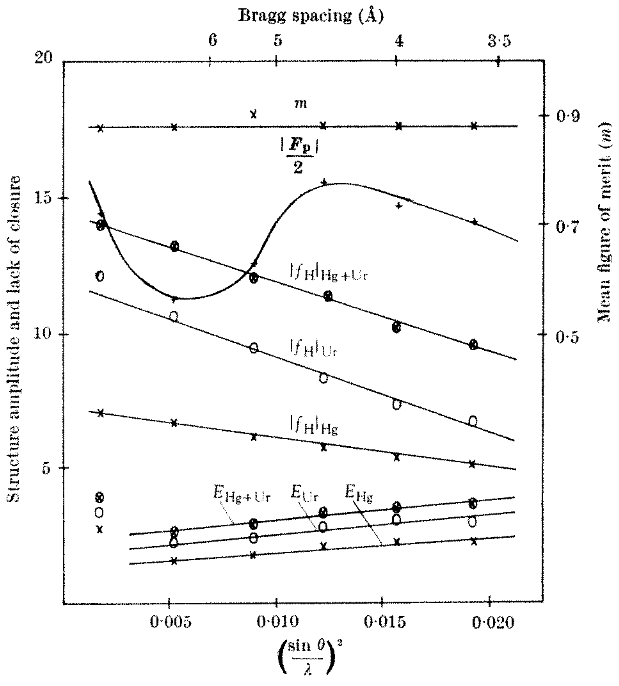


Fig. 4. The variation in the mean structure amplitudes of the parent structure and of the heavy atoms, and in the accuracy of the phase determination as a function of $\sin^2\theta/\lambda^2$. m, $\lvert\overline{F_P}\rvert$, $\lvert\overline{f_H}\rvert$ and E are defined as in Tables 2 and 3. The values of m are given by the right ordinate, and those of $\lvert\overline{F_P}\rvert$, $\lvert\overline{f_H}\rvert$ and E, which are on the same non-absolute scale, by the left ordinate.

The centroid phases[24] of all 2,750 independent tosyl-elastase structure amplitudes were then calculated from the final heavy atom parameters, incorporating the anomalous scattering data[27]. Fig. 4 shows the variations of the mean parent and heavy atom structure amplitudes for the complete data sets, and of the accuracy of the phase determination as a function of resolution. The mean figure of merit averaged over all reflexions, which remained constant over the range of $\sin^2\theta/\lambda^2$ studied, was 0·88, corresponding to a mean error in phase angle of 28°. The figure of merit would have been greater had the centrosymmetric reflexions not been treated in the same

Table 2. PARAMETERS FROM THE LAST CYCLE OF REFINEMENT

Derivative	Heavy atom	x	y	z	k	Z	B	$\lvert\overline{f_H}\rvert$
PCMBS-elastase	Hg	0·910	0·313	0·487	1·007	3·68	22	5·8
Uranyl tosyl-elastase	Ur	0·120	0·585	0·398	1·008	6·48	37	8·8
Uranyl PCMBS-elastase	Hg	0·915	0·315	0·468	0·999	4·65	28	11·5
	Ur	0·121	0·585	0·398		5·85	25	

x, y and z are the fractional co-ordinates of the heavy atom sites in one elastase molecule. k is a scale factor by which the derivative structure amplitudes are multiplied to put them on the same non-absolute scale as the parent structure amplitudes. Z and B are the values of the heavy atom occupancies and isotropic temperature factors obtained from the refinement, the occupancies being on the same arbitrary scale. $\lvert\overline{f_H}\rvert$ is the average value of the root mean squares of the heavy atom structure amplitudes, the mean observed structure amplitude of tosyl-elastase $\lvert\overline{F_p}\rvert$, on the same scale, being 31·0. The data in this table were obtained by refinement of the 1,400 selected reflexions.

Table 3. EFFECT OF INCLUSION OF ANOMALOUS DISPERSION DATA ON THE PHASE DETERMINATION

Anomalous dispersion data	$\bar{m}$	E_{Hg}	E_{Ur}	E_{Hg+Ur}	R_{Hg}	R_{Ur}	R_{Hg+Ur}
Not included (from last cycle of refinement)	0·778	2·10	3·31	4·03	0·40	0·41	0·38
Included incorrectly	0·837	3·38	3·54	4·27	0·61	0·45	0·42
Included correctly	0·913	1·98	2·84	3·19	0·37	0·34	0·30

$\bar{m}$ is the mean figure of merit[21]. E is the average root mean square lack of closure of the phase triangles for each derivative[21,22,24]. $R = \Sigma\frac{\lvert(F_D - F_P) - f_H\rvert}{\Sigma\lvert F_D - F_P\rvert}$

The values of E and R for PCMBS-elastase, uranyl tosyl-elastase and uranyl PCMBS-elastase are indicated by the subscripts Hg, Ur and Hg + Ur respectively. The data in this table were obtained by refinement of the 1,400 selected reflexions. The values of the ratios of the imaginary part to the real part of the heavy atom scattering factors were taken from the *International Tables for X-ray Crystallography*, **3**, 215 (1962).

way as the non-centrosymmetric reflexions. The achievement of such accurate phases was greatly facilitated by the $P2_12_12_1$ spacegroup of elastase, which has a uniquely defined origin and gives a large number of centric reflexions. The mean heavy atom structure amplitude, $|f_H|$ decreased, and the root mean square lack of closure for each derivative, E, increased as $\sin^2 \theta/\lambda^2$ increased, but even at the highest limit of this study the values of $|f_H|$ were significantly greater than the respective values of E, indicating that all three derivatives were providing accurate phase information at 3·5 Å resolution. It is significant that while uranyl tosyl-elastase contributed more than PCMBS-elastase to the phase determination at low resolution, the convergence of the $|f_H|$ and E curves in Fig. 4 indicates that PCMBS-elastase will continue to provide accurate phase information to a higher resolution than will uranyl tosyl-elastase, because of its extremely high isomorphism with the parent compound, tosyl-elastase.

Electron Density Map

The "best" Fourier synthesis[24] of tosyl-elastase was calculated, in sections perpendicular to the a axis, at intervals of $a/60$, $b/60$ and $c/60$, using a Fortran program written especially for protein structure calculations by Dr R. A. Crowther. These sections were displayed in contour form on a computer controlled oscilloscope, and recorded on 35 mm film[25]. They were enlarged by back-projection and copied onto perspex sheets to the scale 2 cm = 1 Å for subsequent interpretation and model building.

The molecular boundaries of the tosyl-elastase molecules are clearly visible in the electron density map, each molecule being an oblate ellipsoid having approximate dimensions 53 Å along the x axis, 40 Å along the y axis and 38 Å along the z axis. Neighbouring molecules have relatively few points of contact and are separated by wide interstices filled with salt solution in which no strong features appear in the electron density map. By contrast, the density associated with the atoms of tosyl-elastase stands out clearly. The course of the single polypeptide chain can be traced unambiguously from one end to the other as a column of continuous high density, which is on average three to four contours above the general background level. At regular intervals along the chain side-chains can be seen branching out in various directions. The peaks of the main chain density are located at these branch points, which clearly correspond to the α-carbon atom positions, and not at the positions of the peptide carbonyl groups[12,26]. At no point is the course of the polypeptide chain less well defined than at any other, except for one small loop near the amino terminus which projects outwards from the surface and has no contacts with neighbouring molecules. Although the electron density of this loop is only two contours above the general background level it is nevertheless easily interpretable. Several of the amino-acid side-chains are sufficiently well defined for them to be identified unambiguously. The four cystine disulphide bridges are each clearly visible as a column of high density, on average six contours above the general background level, linking two parts of the main chain, while both methionine residues can be recognized by their high sulphur peaks. The positions, orientations and characteristic planarity of the aromatic rings of the tryptophan, tyrosine, phenylalanine and histidine residues are in most cases clearly defined. Glycines are usually distinguishable from residues bearing side-chains. The side-chains of the other residues appear as stalks or bulges of various sizes, and in general cannot be identified from the electron density alone. Fig. 5 is a stereophotograph of the central part of seven sections from the complete three-dimensional electron density map of tosyl-elastase, which illustrates many of these features. It shows clearly the low background density of the intermolecular interstices and the clearly defined electron density of the several short stretches of extended chain which traverse these sections. The positions of the prominent sulphonyl group of the tosyl group, which is attached to the active centre serine residue, and of other residues of catalytic importance are indicated. An illustrated discussion of the detailed interpretation of the whole map, and of the relationship between conformation and

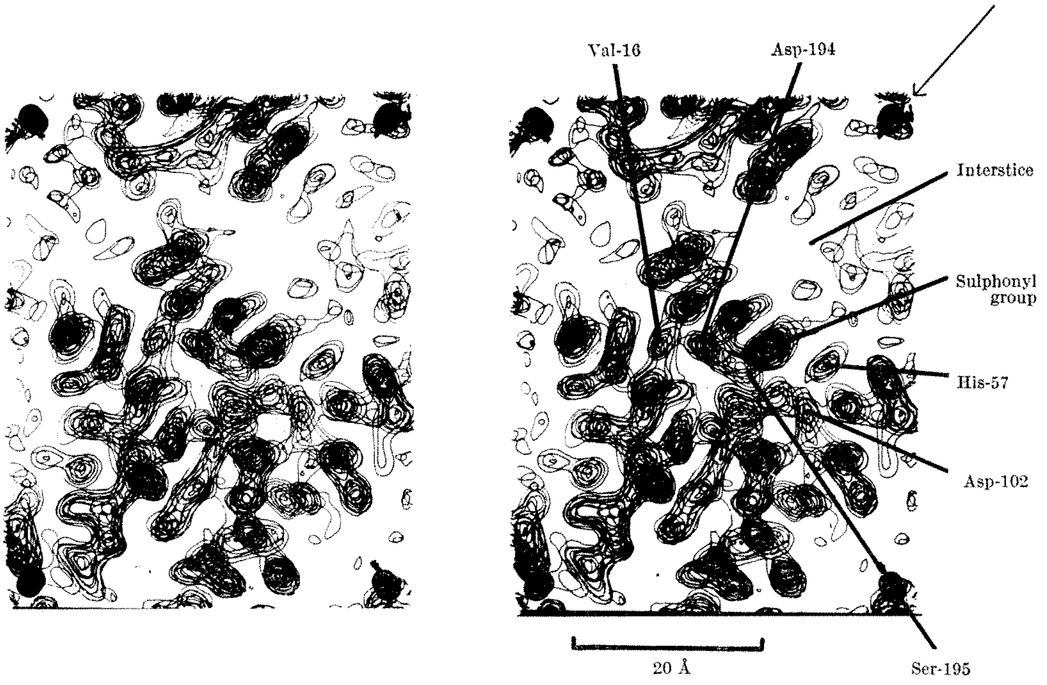


Fig. 5. A stereophotograph of the central region ($y = +10/60$ to $+50/60$, $z = +10/60$ to $+50/60$) of a stack of seven sections ($x = -13/60$ to $-7/60$) from the complete three-dimensional electron density map of tosyl-elastase. The positions of the large intermolecular interstice adjacent to the active centre, the dense sulphonyl group of the tosyl inhibitor attached to the γ-oxygen atom of the active centre serine residue, Ser-195, and the side chains of some of the catalytically important residues, Val-16, His-57, Asp-102, Asp-194 and Ser-195, are marked. The arrow indicates the direction, perpendicular to the x axis, from which the configuration of these catalytically important residues is portrayed in Fig. 4 of the third article of this series[6]. The chymotrypsinogen A numbering scheme has been used[2].

function of these catalytically important residues is given in the third article of this series[6].

By contrast with the situation found in α-chymotrypsin[5], the active centre of the elastase molecule is not blocked by intermolecular contacts, but faces one of the largest interstices in the crystal structure, this cavity being approximately 18 Å in diameter. It is hoped to exploit the accessibility of the active centre of elastase in future studies, at a higher resolution, of enzyme–substrate complexes and of "small" molecules the structures of which are difficult to determine by conventional methods.

We thank our numerous colleagues at the MRC Laboratory of Molecular Biology for their help and especially Dr B. S. Hartley for suggesting this project and for his continued encouragement, Dr P. B. Sigler for sulphonyl fluoride inhibitors, Dr C. C. F. Blake for advice on the synthesis of uranyl derivatives, and the directors and staff of the Computer Units of the Imperial College of Science and Technology, London, and the Institute of Theoretical Astronomy, Cambridge, for provision of computing facilities.

One of us (D. M. S.) acknowledges the financial support of an MRC scholarship and a Beit Memorial Medical research fellowship.

Received December 4, 1969.

[1] Hartley, B. S., *Ann. Rev. Biochem.*, **29**, 45 (1960).

[2] Shotton, D. M., and Hartley, B. S., *Nature*, **225**, 802 (1970). (First article of this series.)

[3] Shotton, D. M., Hartley, B. S., Camerman, N., Hofmann, T., Nyburg, S. C., and Rao, L., *J. Mol. Biol.*, **32**, 155 (1968).

[4] Matthews, B. W., Sigler, P. B., Henderson, R., and Blow, D. M., *Nature*, **214**, 652 (1967).

[5] Sigler, P. B., Blow, D. M., Matthews, B. W., and Henderson, R., *J. Mol. Biol.*, **35**, 143 (1968).

[6] Shotton, D. M., and Watson, H. C., *Nature*, **225**, 811 (1970). (Third article of this series.)

[7] Sigler, P. B., Skinner, H. C. W., Coulter, C. L., Kallos, J., Braxton, H., and Davies, D. R., *Proc. US Nat. Acad. Sci.*, **51**, 1146 (1964).

[8] Sigler, P. B., Jeffery, B. A., Matthews, B. W., and Blow, D. M., *J. Mol. Biol.*, **15**, 175 (1966).

[9] Banaszak, L. J., Watson, H. C., and Kendrew, J. C., *J. Mol. Biol.*, **12**, 130 (1965).

[10] Sigler, P. B., and Blow, D. M., *J. Mol. Biol.*, **14**, 640 (1965).

[11] Green, D. W., Ingram, V. M., and Perutz, M. F., *Proc. Roy. Soc.*, A, **225**, 287 (1954).

[12] Blake, C. C. F., Koenig, D. F., Mair, G. A., North, A. C. T., Phillips, D. C., and Sarma, V. R., *Nature*, **206**, 757 (1965).

[13] Holmes, K. C., and Leberman, R., *J. Mol. Biol.*, **6**, 439 (1963).

[14] Arndt, U. W., Crowther, R. A., and Mallett, J. F. W., *J. Sci. Instrum.* (Series 2), **1**, 510 (1968).

[15] Shotton, D. M., and Watson, H. C., *Phil. Trans. Roy. Soc.*, B., **257**, 111 (1970).

[16] Hart, R. G., *Acta Cryst.*, **14**, 1188 (1961).

[17] Wyckoff, H. W., Hardman, K. D., Allewell, N. M., Inagami, T., Johnson, L. N., and Richards, F. M., *J. Biol. Chem.*, **242**, 3984 (1967).

[18] Edwards, D. B. G., Bowden, K. F., Standeven, J., and Mills, O. S., *Computer Bulletin*, 54 (1966).

[19] Diamond, R., *Acta Cryst.*, A, **25**, 43 (1969).

[20] North, A. C. T., Phillips, D. C., and Mathews, F. S., *Acta Cryst.*, **24**, 351 (1968).

[21] Dickerson, R. E., Kendrew, J. C., and Strandberg, B. E., *Acta Cryst.*, **14**, 1188 (1961).

[22] Lipscomb, W. N., Coppola, J. C., Hartsuck, J. A., Ludwig, M. L., Muirhead, H., Searl, J., and Steitz, T. A., *J. Mol. Biol.*, **19**, 423 (1966).

[23] Muirhead, H., Cox, J. M., Mazzarella, L., and Perutz, M. F., *J. Mol. Biol.*, **28**, 117 (1967).

[24] Blow, D. M., and Crick, F. H. C., *Acta Cryst.*, **12**, 794 (1959).

[25] Gossling, T. H., *Acta Cryst.*, **22**, 465 (1967).

[26] Birktoft, J. J., Blow, D. M., Henderson, R., and Steitz, T. A., *Phil. Trans. Roy. Soc.*, B, **257**, 67 (1970).

[27] North, A. C. T., *Acta Cryst.*, **18**, 212 (1965).

Three-dimensional Structure of Tosyl-elastase

by
D. M. SHOTTON
H. C. WATSON
Molecular Enzymology Laboratory, Department of Biochemistry, University of Bristol, Bristol, BS8 ITD

Amino-acid sequence studies and crystallographic evidence are used to construct an atomic model of elastase, which is compared with the structure of α-chymotrypsin. The single polypeptide chain of elastase is folded into two distinct and structurally similar halves displaying opposite polarity.

It has long been predicted that enzymes with homologies of amino-acid sequence and common catalytic mechanisms should have similar tertiary structures. For the pancreatic serine proteinases, there is experimental support for these predictions. Sigler *et al.* have found that the two disulphide bridges present in trypsin but absent in chymotrypsin and elastase can be built into the atomic model of α-chymotrypsin with little or no distortion of the polypeptide chain[1], Schroeder and Shaw[2] have observed an autolytic cleavage in trypsin between Lys-145* and Ser-146, a point immediately adjacent to the position of autolytic cleavages in δ-chymotrypsin, and Hartley[3] has shown that the trypsin and elastase side chains can be substituted for those present in a skeletal model of α-chymotrypsin with no gross distortion of the polypeptide chain.

This article describes a combination of the results of the chemical and crystallographic studies of porcine pancreatic elastase (EC 3.4.4.7), reported in the two preceding articles[4,5], which has led to the construction of an atomic model of this enzyme. The information thus obtained has allowed a comparison to be made between the structures of elastase and α-chymotrypsin, a homologous pancreatic serine proteinase[4], and has permitted a detailed analysis of the conformation adopted by the single polypeptide chain of elastase.

Interpretation of the Electron Density Map

Tosyl-elastase is an inhibited derivative of elastase which has a *p*-toluenesulphonyl (tosyl) group attached to the γ-oxygen of the active centre serine residue, Ser-195. A map of the electron density distribution of crystalline tosyl-elastase at 3·5 Å resolution was prepared as described in the second article in this series[5]. The entire course of the single polypeptide chain could be followed easily and unambiguously through the electron density map, and it was quickly apparent that the elastase molecule has the same general conformation as α-chymotrypsin. The N and C-termini of the chain, the four disulphide bridges and the sulphonyl group of the tosyl inhibitor residue were obvious features of the electron density distribution[5],

---

* To aid in the comparison between the various mammalian serine proteinases, we use the numbering of the amino-acid residues in the chymotrypsinogen A sequence to describe the position of amino-acid residues in these homologous proteins[4].

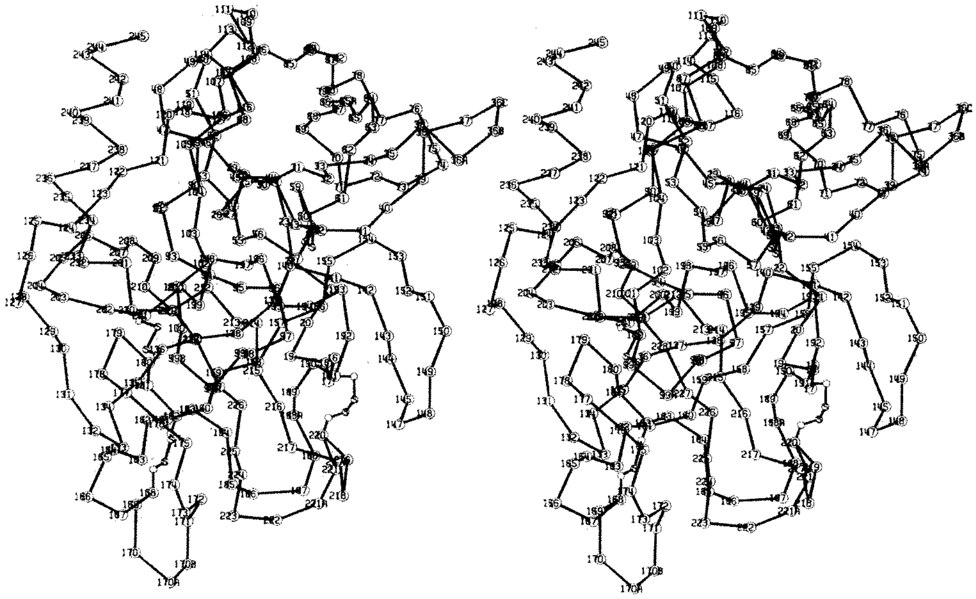


Fig. 1

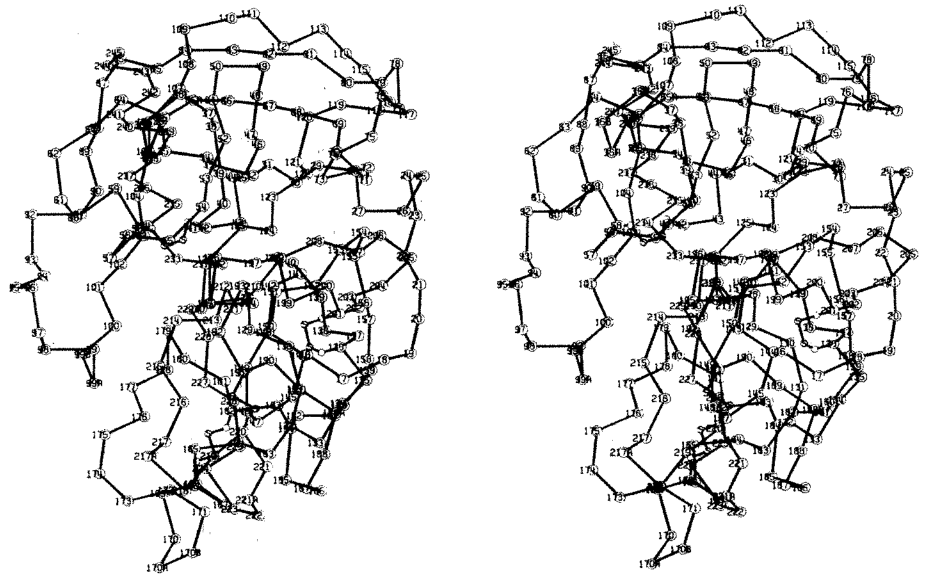


Fig. 2

Figs. 1 and 2. Computer-prepared stereo views of the conformation of the elastase polypeptide chain, prepared from the approximate α-carbon atomic coordinates of tosyl-elastase, measured from the preliminary model. Residues are numbered on the chymotrypsinogen A scheme, "insertions" relative to the chymotrypsinogen A sequence being numbered 36A, 36B and so on[2]. Residue 146 is not present in elastase. For clarity all side chains are omitted except those forming the four disulphide bridges. In both views the crystallographic x axis is vertical. Fig. 1 is a view down the y axis and Fig. 2 is a view from 90° to the right, down the z axis.

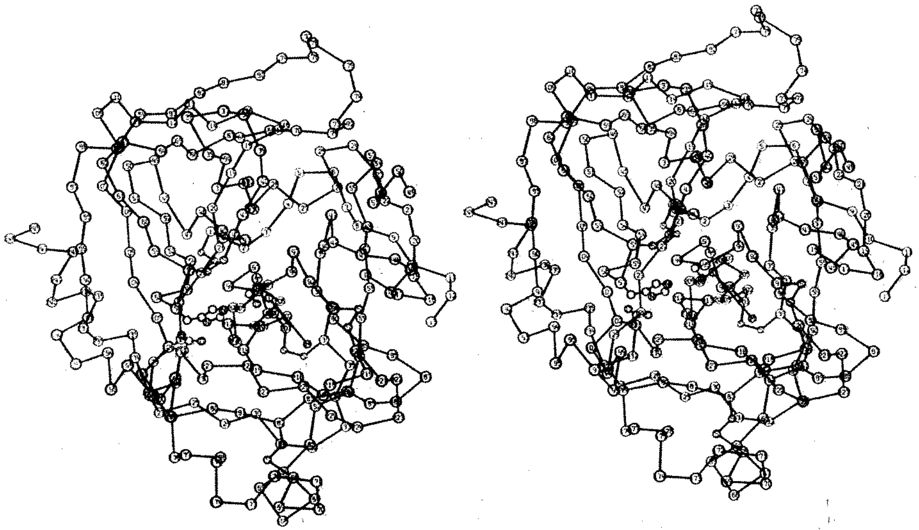


Fig. 3

Fig. 3. A computer-prepared stereo view of the conformation of the α-chymotrypsin polypeptide chain, prepared from the atomic coordinates of the tosyl α-chymotrypsin model of Blow and his collaborators[7-9]. The numbering scheme is that of chymotrypsinogen A[2]. The side chains of some of the important residues are shown, and the position of the tosyl inhibitory group in the substrate binding cleft is indicated by the line joining the positions of the methyl carbon (M) and the sulphonyl sulphur (I). The molecular orientation is similar to that of tosyl elastase shown in Fig. 2, and the similarity of three-dimensional conformation can be readily seen.

providing reference points from which to start a detailed interpretation. Although some of the amino-acid side chains could be identified with reasonable certainty from their electron density alone[5,6], we relied heavily on our knowledge of the amino-acid sequence of elastase[4] for the detailed interpretation of the electron density distribution of tosyl-elastase. This interpretation was made without knowledge of, or reference to, the detailed structure of α-chymotrypsin determined elsewhere by Blow and his collaborators[1,7-9].

With the aid of a half-silvered mirror device similar to that described by Richards[10], a skeletal model of tosyl-elastase was constructed on the scale 2 cm = 1 Å, using standard components designed by one of us (H. C. W.) in conjunction with Dr J. C. Kendrew. Throughout the building process the accuracy of the interpretation was checked and confirmed by the excellent correlation obtained between the expected amino-acid side chain[4] and the observed electron density[5,6]. The aromatic side chains formed very obvious markers which greatly facilitated the interpretation. This model satisfactorily accounts for all the electron density in the map, there being no point where the chemical and crystallographic evidence are in conflict. The distal parts of some long side chains, especially those of arginines and lysines, are not represented in the map, due to a degree of free-rotational disorder in these groups rather than to lack of resolution. As expected at 3·5 Å resolution, the dimpling of aromatic rings and forking of the side chains of valine, threonine, leucine and isoleucine residues are not apparent, and the carbonyl oxygens of the peptide bonds are not resolved from the rest of the main chain density. We are therefore unable from the 3·5 Å resolution map to determine the exact orientation of peptide groups, and thus can only discuss hydrogen bonding patterns within the molecule in general terms.

General Conformation and Comparison with α-Chymotrypsin

The elastase polypeptide chain is folded on itself in a series of large loops to form a compact globular molecule in which there are only two small regions of α-helical conformation, between residues 164 and 170 and at the C-terminus between residues 237 and 245, the rest of the chain tending to be fully extended. This is shown in Figs. 1 and 2, which are computer-prepared stereo views of the conformation of the elastase polypeptide chain, in which the single polypeptide chain can be traced from the buried N-terminal residue, Val-16, which forms an internal ion pair with the carboxyl group of Asp-194, to the α-helical "tail", terminating in residue Asn-245.

Elastase shows extensive amino-acid sequence homologies with the B and C chains of α-chymotrypsin[4]. We have been able to directly compare the three-dimensional structures of elastase and α-chymotrypsin. (To do this, our skeletal model of elastase was taken to the MRC Laboratory of Molecular Biology, Cambridge, where direct comparisons were made with the model of α-chymotrypsin built by Blow and his collaborators.) These comparisons show that the conformations adopted by the single polypeptide chain of elastase and the B and C chains of α-chymotrypsin are extremely similar, as are also the orientations of the amino-acid side chains of corresponding residues within these chains, thus confirming the predictions referred to in the introduction. This similarity can be seen by comparing the stereo view of the elastase structure given in Fig. 2 with a stereo view of the α-chymotrypsin molecule in a similar orientation, given in Fig. 3.

Where differences between corresponding internal residues in the two enzymes do occur, these are generally of a most conservative nature and do not involve charged residues. Where the size difference between dissimilar residues is great, compensating substitutions are found in adjacent regions so that the identity of tertiary structures is preserved. For example, the difference at position 29 between a serine residue in elastase and a tryptophan in α-chymotrypsin is offset by the occurrence of a histidine residue in elastase and a valine in α-chymotrypsin at position 200.

Because the elastase polypeptide chain contains twelve more residues than the B and C chains of α-chymotrypsin[4], some differences in local conformation in the vicinity of these "insertions" must occur. Fig. 5 (described in detail in the last section) shows that the insertions in elastase relative to α-chymotrypsin all occur on the molecular surface, usually at the ends of loops which "expand" to accommodate these changes without disturbing the identity of conformation of the rest of the chain.

The analyses of the sequence homologies between the pancreatic serine proteinase given in the first article in this series[4] help to explain the remarkable similarity of tertiary structure exhibited by these two enzymes. While the overall identity of corresponding residues in elastase and α-chymotrypsin is only 39 per cent, the degree of homology in the molecular interior is 81 per cent. Most of the differences which exist between them are therefore located on the molecular surface, which is fairly hydrophilic in nature, and seems merely to provide an insulating layer one polypeptide chain thick serving to shield the hydrophobic interior from the disruptive hydrating effects of the solvent.

These results suggest that the mammalian serine proteinases are a family of very similar enzymes built to a basic plan which has been conserved during divergent evolution. It therefore seems almost certain that the three-dimensional structure of trypsin will closely resemble those of elastase and α-chymotrypsin, for it shows roughly the same degree of amino-acid sequence homology to these two enzymes as they do to each other[4]. As in the α-chymotrypsin model[1], the two "extra" disulphide bridges of trypsin can be built into the elastase model with scarcely any distortion of the polypeptide chain. The distance in the elastase model between the α-carbon atoms of residues 22 and 157 is 6·6 Å, and between those of residues 127 and 232 is 6·2 Å, while the mean distance between α-carbons of the four cystines is 5·8 Å.

Elastase is produced from its zymogen, proelastase, by a tryptic cleavage which removes a small activation peptide from the N-terminus of the polypeptide chain, in an activation process resembling that of trypsinogen[11]. Proelastase binds specifically to elastin, a natural substrate for elastase[11], showing that the substrate binding site is already formed and accessible in the zymogen. It is therefore likely that the conformation of the proelastase chain is very similar to that of the active enzyme.

Substrate Binding Site

Steitz *et al.*[12] have shown that N-formyl-L-tryptophan binds to α-chymotrypsin so that the aromatic side chain lies in a deep hydrophobic cleft adjacent to the catalytic site, while the carboxyl group lies next to the active centre serine residue, Ser-195. They have concluded, therefore, that this cavity, which is also the site occupied by the toluene group of tosyl-α-chymotrypsin, is the binding site for the aromatic side chains of chymotrypsin substrates. This cavity is also present in the elastase molecule, but its mouth is partially occluded by the side chain of a valine residue at position 216, which is absent in trypsin and the chymotrypsins, where the corresponding residues are glycines. Further, the bottom of the cavity in elastase is partly filled by the side chain of Thr-226, again absent in the chymotrypsins and trypsin, where the corresponding residues are also glycines. These extra side chains in elastase make it sterically impossible for bulky aromatic side chains to be accommodated in this cavity, explaining the complete lack of activity of elastase towards such substrates[13]. Elastase has a substrate specificity directed towards uncharged non-aromatic amino-acids[14,22], especially alanine[15,16]. It is clear from the tosyl elastase model that N-formyl-L-alanine is able to bind to elastase in an identical fashion to that in which N-formyl-L-tryptophan binds to α-chymotrypsin[9,12], with the amido-nitrogen able to form a hydrogen bond with the carbonyl group of Ser-214, and the side chain methyl group lying above and in Van der Waals contact with the side chain of Val-216. These substrate interactions would position the carbonyl group of the peptide bond to be split in a favourable position for a nucleophilic attack on its carbonyl carbon by the γ-oxygen of the active centre serine residue of elastase. It seems likely that the binding of peptides to elastase also involves several other sites of weak interaction.

These findings support the explanation of the substrate specificity of trypsin given by Hartley[3], who has shown that residue 189, a serine residue in elastase and the chymotrypsins, which lies at the bottom of this cavity, is an aspartic acid residue in trypsin. It is suggested that the large basic side chain of a trypsin substrate enters the substrate binding cavity of trypsin to form an ion pair with the carboxyl group of this buried aspartic acid, explaining the strong specific binding of substrates observed for this enzyme.

One obvious prediction which can be made from these studies is that a trypsin-like serine proteinase, which contained a basic residue at position 189 instead of an aspartic acid, and glycine residues at positions 216 and 226, would be an excellent enzyme for cleaving peptide bonds on the C-terminal side of aspartic and glutamic acid residues.

Catalytic Site

Crystals of native elastase show substantial activity towards N-benzoyl-L-alanine methyl ester (about 40 per cent that of the enzyme in solution) (D. M. S., manuscript in preparation) and are also inhibited by soaking with various sulphonyl fluoride derivatives[5]. In the crystal lattice the active centre is not obstructed by intermolecular contacts with neighbouring molecules, in contrast to the situation found in crystals of bovine α-chymotrypsin[1], but faces one of the largest interstices in the crystal lattice. In addition, the differences in conformation between crystalline native elastase and tosyl-elastase, determined by difference Fourier projections, are very small and confined to the local environment of the tosyl group[6]. These findings indicate that the conformation of crystalline tosyl-elastase closely resembles that of the uninhibited enzyme in solution. This provides the justification for making deductions about the catalytic mechanism of elastase from the crystallographically determined structure of tosyl-elastase.

In elastase, as in α-chymotrypsin, the active centre is formed in a slight groove where catalytically important residues from different regions of the sequence are brought into close spatial proximity. Blow *et al.*[17] have proposed that in α-chymotrypsin a "charge relay system" exists between a buried aspartic acid residue, Asp-102, and the active centre serine residue, Ser-195, mediated by the imidazole ring of the active centre histidine residue, His-57, whose ring nitrogen atoms are within hydrogen bonding distance of the carboxyl group of the aspartic acid and the γ-oxygen atom of the serine. This would enable charge to be transferred from the buried aspartic through a hydrophobic environment to the serine γ-oxygen atom, explaining the extraordinary nucleophilicity of the active centre serine residue. In the three-dimensional structure of tosyl-elastase the α-carbon atoms of Asp-102, His-57 and Ser-195 occupy the same relative conformation as those in α-chymotrypsin, suggesting that in native elastase a similar charge relay system exists. The common catalytic mechanism of these two enzymes[18] is thus explained simply to be the result of the catalytically functional groups occupying identical configurations. The presence of the side chain of Val-216 in elastase, however, prevents the tosyl group of tosyl-elastase occupying the same relative conformation as it does in tosyl-α-chymotrypsin[9]. Instead of being tucked into the substrate binding cavity near the catalytic site, it points in the opposite direction, interacting with the imidazole ring of His-57 and displacing it outwards from its presumed native configuration by a rotation of about 180° around its α—β bond (see Figs. 3 and 4). In this position the imidazole ring lies almost parallel to and in Van der Waals

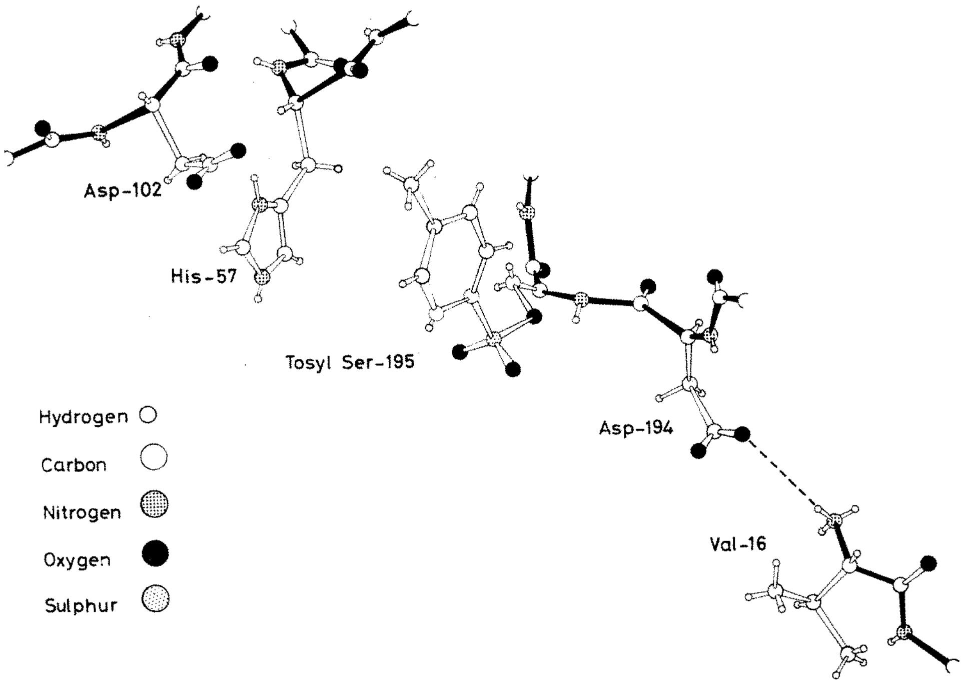


Fig. 4. A diagram showing the positions and orientations of the side-chains of the three catalytically important amino-acid residues of tosyl-elastase and of the two residues which form the internal ion pair. In native elastase, where the bulky tosyl group is absent, the imidazole ring of His-57 is thought to lie between the side chains of Asp-102 and Ser-195, rather than pointing out into solution.

contact with the toluene ring of the tosyl group, and is thus unable to form a charge relay system. The carboxyl group of Asp-102 points towards Ser-195, but forms a hydrogen bond with another nearby side chain, that of Ser-214, instead of with the imidazole ring of His-57. The relative positions of these groups can be seen in the electron density sections of the tosyl-elastase Fourier shown in the second article[5], and are illustrated in Fig. 4 and elsewhere[6]. In the isomorphous PCMBS elastase derivative[5] the chloromercuri group, which replaces the methyl group at the end of the toluene ring of tosyl-elastase, lies about 4 Å from the $N^{\delta 1}$ nitrogen atom of the His-57 side chain.

A similar grouping of a serine, a histidine and an aspartic acid residue has been observed at the active centre of subtilisin BPN′[19], a member of a separate class of bacterial serine proteinases, suggesting that the bacterial and the mammalian serine proteinases have independently acquired a common catalytic mechanism.

Hess and his collaborators[20] have shown that in acetylated δ-chymotrypsin, prepared by tryptic cleavage of the Arg-15—Ile-16 peptide bond in fully acetylated chymotrypsinogen, deprotonation of the α-amino group of the Ile-16, $pK_a = 8{\cdot}3$, results in loss of enzymic activity and in a conformational change, observed by changes in optical rotation, to a state resembling that of the inactive zymogen. Blow and his collaborators[1,7] have shown that in α-chymotrypsin the α-amino group of Ile-16 forms an internal ion-pair with the carboxyl group of Asp-194, adjacent to the active centre serine residue, and have proposed that this ion-pair stabilizes the active conformation of chymotrypsin. In tosyl-elastase at *p*H 5·0, Val-16 and Asp-194 form an internal ion-pair identical to that found in α-chymotrypsin (see Figs. 1–4). It might therefore be thought that the role of this ion pair in creating and maintaining the active conformation in these two enzymes is identical. The pK_a of the α-amino group of Val-16 in elastase has been determined as 9·7 (H. Kaplan, K. J. Stevenson and B. S. Hartley, manuscript in preparation), substantially higher than that of Ile-16 in chymotrypsin, and has been shown to have a very low nucleophilic reactivity, suggesting it is buried in the molecular interior, at all *p*H values up to 10·5, at which *p*H the protein starts to denature. Further, Kaplan and Dugas[15] have shown that, in contrast with the situation in chymotrypsin, the optical rotation does not change on deprotonation of the α-amino group, and that both native and fully acetylated elastase remain fully active towards N-benzoyl-L-alanine methyl ester at all *p*H values between 8 and 10. This indicates that, at least for this substrate, the rate-limiting step in the enzymic activity of elastase is independent of the state of ionization of the α-amino group of Val-16, and hence of the integrity of the Val-16–Asp-194 ion pair.

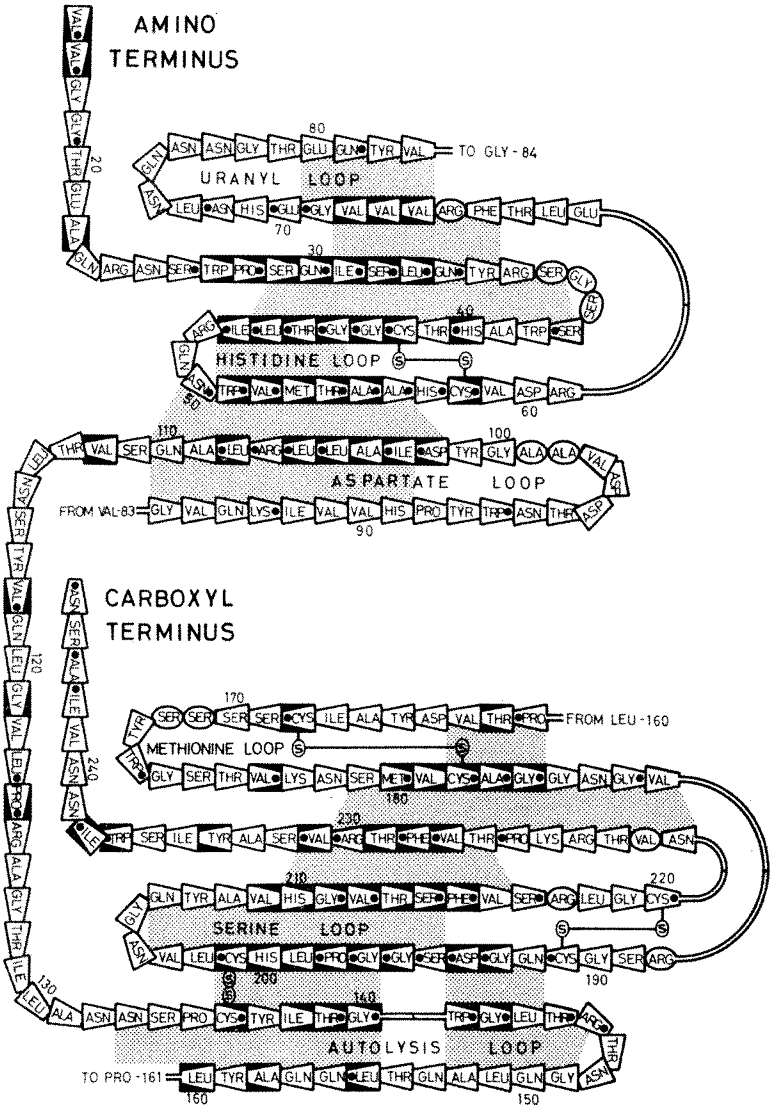


Fig. 5. A diagrammatic representation of the conformation of the elastase polypeptide chain, which is folded into two distinct halves in which looping of the chain brings neighbouring regions into close antiparallel contact. All neighbouring antiparallel regions in the molecule are represented as such in the diagram, those regions where chains run within hydrogen bonding distance of one another being shaded with spots. Internal residues, judged to be inaccessible to water molecules in the atomic models of elastase and α-chymotrypsin[3], are boxed with heavy outlines. Residues which are homologous in elastase, trypsin, chymotrypsin A and chymotrypsin B[2] are indicated by a black dot next to the residue name. Disulphide bridges are drawn. The numbering system is that of chymotrypsinogen A[2]. "Insertions" in elastase are indicated by ovals, and numbered 36A, 36B, etc. In elastase, residue 146 is a deletion, while in chymotrypsin A residues 147 and 148 have been removed by autolytic cleavages. Two breaks in the chain have had to be made in the diagram, between residues 83 and 84 and between residues 160 and 161, in order to represent the three-dimensional conformation of elastase on a plane surface. The diameter of the elastase molecule corresponds to about 10 residues length of extended polypeptide chain.

Organization of the Elastase Molecule in Two Similar Halves

The most striking feature of the general conformation of the elastase molecule is the manner in which the chain is organized into two clearly defined halves, in intimate contact and presumably held together in a stable conformation by hydrogen bonds and hydrophobic interactions. This can be clearly seen in Fig. 2, where the N-terminal half lies above and slightly to the left of the C-terminal half. Within each half of the elastase molecule the chain is folded into a series of large loops, in such a way that the chain runs antiparallel to neighbouring regions in the sequence at a distance of about 5 Å for several residues. These loops have also been noted in α-chymotrypsin[7], where the higher resolution of the structure determination permits the allocation of specific hydrogen bonds[8,9], which are of an irregular antiparallel β-pleated-sheet type. Five of the six most prominent loops in elastase are named in conformity with the usage of other workers[3,9] (see Fig. 5), and we have named the sixth loop, made by residues 66 to 83, the uranyl loop, because in two of the heavy atom derivatives of elastase it forms the principal binding site for a uranyl ion[5,6], which is bound within contact distance of the side chains of Val-67, Glu-70, Glu-80 and Tyr-82, and of the main chain carbonyl oxygen of Leu-73.

In order to clarify the principles of organization involved in the apparently aimless meanderings of the elastase polypeptide chain (Figs. 1 and 2), an attempt has been made in Fig. 5 to represent the conformation within the two halves of the molecule. To do this, all the neighbouring antiparallel regions of the molecule were first represented as such in the diagram, the alignment of the amino-acid residues being kept as close as possible to that observed in the elastase model, and then loops indicating the positions of the remaining stretches of the chain were added, giving reasonably accurate representations of the ends of the various loops in the molecule. The seventy-five internal residues, judged to be inaccessible to water in the atomic models of both elastase and α-chymotrypsin[4], and the eighty-two residues which are homologous in elastase, trypsin, chymotrypsin A and chymotrypsin B[4], are especially indicated in Fig. 5, and can be seen to be concentrated in the middle of the two parts of the diagram. These two regions thus clearly represent the hydrophobic internal homologous "cores" of the two halves of the molecule around which the rest of the molecule is folded.

It can be clearly seen in Fig. 5, which should be studied in conjunction with Figs. 1 and 2, that the two halves of the molecule are similar, each containing six areas of antiparallel interactions. The surprising result to emerge from this analysis is that the organization within the N-terminal half of the molecule, traced from the N-terminus to the middle of the chain, resembles that within the C-terminal half of the molecule, traced from the C-terminus to the middle of the chain. Loops possessing similar properties and positions within the elastase molecule occupy corresponding positions in the two halves of Fig. 5. For instance the histidine and the serine loops are both internal and tend to be both hydrophobic and highly homologous with the other serine proteinases (except at the ends of the loops, which protrude onto the molecular surface, where the hydrophobic homologous character is immediately lost). Both are slightly helical and are stabilized by disulphide bridges, and each bears one of the catalytically essential residue, namely His-57 or Ser-195.

Within each half of the molecule the antiparallel chains form a twisted sheet which curves round to unite with itself to form a "cylinder" around the surface of which the chains run in an oblique fashion. This can be visualized diagrammatically for the N-terminal half of the molecule by bending Fig. 5 so as to connect residues 83 and 84, and similarly for the C-terminal half by connecting residues 160 and 161, but it is much more easily seen in the stereo views of elastase (Figs. 1 and 2). This is especially clear in the N-terminal half of the molecule, where in Fig. 1 the twisted sheet of antiparallel chains can be seen spiralling away from the front left-hand chain of the aspartate loop (residues 84 and 91), and up to the right, to eventually reunite with the adjacent section of the chain (residues 80 to 83) where it forms part of the uranyl loop. In the C-terminal half of the molecule this same pattern is repeated upside down, but is more difficult to distinguish. These "cylinders" lie one above the other, and are oriented approximately at right angles to one another. It is obvious that to describe the elastase structure in these terms is a gross oversimplification, and has value only in as much as it permits an understanding of the basic similarity of organization exhibited by the two halves of the molecule.

Except where the histidine and serine loops run fairly close to one another for a few residues, there is no place in the molecule where adjacent chains run parallel rather than antiparallel. The tendency of antiparallel chains to spiral round one another to form slightly helical loops and twisted sheets has been observed in other enzymes, notably in carboxypeptidase A[21] and to a lesser extent in subtilisin BPN′[19], while the peculiar "cylindrical" arrangement of antiparallel chains described above for elastase has been previously observed in α-chymotrypsin[8,9]. The diagram used by Blow and his collaborators primarily to illustrate the hydrogen bonding pattern between the antiparallel loops, rather than to give an accurate representation of the three-dimensional conformation of α-chymotrypsin, differs significantly from Fig. 5, however. In that diagram the correspondence between similar loops is not clear, and an apparent similarity is shown between the arrangement of chains in the two halves of the molecule when both halves are traced from amino to carboxyl end, in contrast to the situation observed in elastase and illustrated in Fig. 5.

The similarity in conformation between the two halves of the elastase molecule is striking, especially because it cannot be accounted for by gene duplication. It indicates the existence of similar packing forces generated by two quite different stretches of polypeptide chain, in which the polarity of the chain is not of prime importance.

It is clear from the preceding discussion and Figs. 1, 2 and 5 that the proelastase polypeptide chain may be folded with equal ease from the N-terminal or the C-terminal end, but that both these procedures will necessitate threading either the serine or the histidine loop through pre-folded sections of the chain to achieve the native conformation, an intrinsically unlikely occurrence. It seems more likely, as has been suggested for chymotrypsin[7], that after synthesis folding occurs not as a one-step process but in stages, the internal hydrophobic homologous loops (Fig. 5) first forming to give nuclei of highly stabilized local conformation around which the more variable hydrophilic regions of the chain can then condense to give the specific conformation required for enzymic activity.

We thank Dr D. M. Blow and his collaborators for allowing us to make detailed comparisons between our preliminary elastase model and their α-chymotrypsin model and for permission to use the stereo view of α-chymotrypsin shown in Fig. 3, and Professor R. E. Dickerson, who prepared the drawings for Figs. 1, 2 and 3. We also thank Dr P. L. Wendell, Mr N. White and Mrs S. K. Watson for help in the construction of the model. One of us (D. M. S.) acknowledges the financial support of a Beit Memorial medical research fellowship received during the course of this work, which was also supported by a grant from the Science Research Council.

Received December 4, 1969.

[1] Sigler, P. B., Blow, D. M., Matthews, B. W., and Henderson, R., *J. Mol. Biol.*, **35**, 143 (1968).

[2] Schroeder, D. D., and Shaw, E., *J. Biol. Chem.*, **243**, 2943 (1968).

[3] Hartley, B. S., *Phil. Trans. Roy. Soc.*, B, **257**, 77 (1970).

[4] Shotton, D. M., and Hartley, B. S., *Nature*, **225**, 802 (1970). (First article of this series.)

[5] Watson, H. C., Shotton, D. M., Cox, J. M., and Muirhead, H., *Nature*, **225**, 806 (1970). (Second article of this series.)

[6] Shotton, D. M., and Watson, H. C., *Phil. Trans. Roy. Soc.*, B, **257**, 111 (1970).

[7] Matthews, B. W., Sigler, P. B., Henderson, R., and Blow, D. M., *Nature*, **214**, 652 (1967).

[8] Blow, D. M., *Biochem. J.*, **112**, 261 (1969).

[9] Birktoft, J. J., Blow, D. M., Henderson, R., and Steitz, T. A., *Phil. Trans. Roy. Soc.*, B, **257**, 67 (1970).

[10] Richards, F. M., *J. Mol. Biol.*, **37**, 225 (1968).

[11] Gertler, A., and Birk, Y., *Europ. J. Biochem.* (in the press).

[12] Steitz, T. A., Henderson, R., and Blow, D. M., *J. Mol. Biol.*, **46**, 337 (1969).

[13] Shotton, D. M., in *Methods in Enzymology* (edit. by Perlmann, G. E., and Lorand, L.) (Academic Press, New York, in the press).

[14] Naughton, M. A., and Sanger, F., *Biochem. J.*, **78**, 156 (1961).

[15] Kaplan, H., and Dugas, H., *Biochem. Biophys. Res. Commun.*, **34**, 681 (1969).

[16] Geneste, P., and Bender, M. L., *Biochem. Biophys. Res. Commun.* (in the press).

[17] Blow, D. M., Birktoft, J. J., and Hartley, B. S., *Nature*, **221**, 337 (1969).

[18] Bender, M. L., and Marshall, T. H., *J. Amer. Chem. Soc.*, **90**, 201 (1968).

[19] Wright, C. S., Alden, R. A., and Kraut, J., *Nature*, **221**, 235 (1969).

[20] Oppenheimer, H. L., Labouesse, B., and Hess, G. P., *J. Biol. Chem.*, **241**, 2720 (1966).

[21] Reeke, G. N., Hartsuck, J. A., Ludwig, M. L., Quiocho, F. A., Steitz, T. A., and Lipscomb, W. N., *Proc. US Nat. Acad. Sci.*, **58**, 2220 (1967).

[22] Sampath Narayanan, A., and Anwar, R. A., *Biochem. J.*, **114**, 11 (1969).

Multiple Forms of Human Brain Mitochondrial Monoamine Oxidase

by
G. G. S. COLLINS
M. SANDLER
E. D. WILLIAMS
M. B. H. YOUDIM

Bernhard Baron Memorial Research
Laboratories and Institute of Obstetrics
and Gynaecology,
Queen Charlotte's Maternity Hospital,
London W6; and
Royal Postgraduate Medical School,
London W12

Monoamine oxidase exists in at least four molecular forms in different areas of the human brain. Variations in their enzymatic properties may have important clinical implications, perhaps reflecting their relative ability to degrade different monoamine substrates *in vivo*.

CERTAIN biologically active monoamines such as dopamine[1], noradrenaline[2] and 5-hydroxytryptamine[3] are present in the central nervous system of many animal species and may act as transmitter substances[4]. The function of cerebral monoamine oxidase (EC 1.4.3.4) (MAO) has yet to be fully elucidated although it is likely to provide one of the major intraneuronal mechanisms for inactivating these monoamines[4-6]. The part the enzyme and its substrates play in cerebral pathology is even less well understood, although evidence exists which seems to involve them unequivocally in certain diseases of the central nervous system; thus, high oral dosage of L-dopa will bring about clinical improvement in some Parkinsonian patients[7,8], presumably by replacing depleted dopamine stores in the basal ganglia[9]; some MAO inhibitors cause a lightening of affect in depressive illness[10,11].

Using polyacrylamide gel electrophoresis, we have separated rat brain mitochondrial MAO into several bands of activity[12] and suggested that the variable effects of MAO inhibitors in depressive illness might be a result of their ability to inhibit particular MAO isoenzymes of this type. The hypothesis was supported by the differing sensitivities that the bands of activity showed to the drugs investigated. Furthermore, each isoenzyme possessed a characteristic substrate specificity, heat stability and *p*H optimum. Our experiments have now been extended to human brain mitochondrial MAO and its multiple forms in whole brain and various constituent parts.

Preparation of Isoenzymes

Autopsy material was collected within 12 h of death. In five cases, the brain was sliced and samples were taken from the basal ganglia, cerebellar cortex, cerebral cortex, centrum ovale, hypothalamus and pons. The samples were placed in sterile screw-capped containers and frozen at −70° C until required. While dissection was careful, some white matter was included in the samples of cerebellar cortex. From two cases, the whole brain was frozen until assay.

Mitochondria were prepared by homogenization of 5–10 g tissue samples in ice-cold sucrose as described by Hawkins[13]. After washing in sucrose they were suspended in Tris-HCl buffer (0·05 M, *p*H 9·1) containing 1·0 per cent 'Triton *X*-100' and benzylamine[14]. The enzyme was then solubilized and purified as described previously[12]. In each enzyme preparation, four bands of activity were separated by polyacrylamide disc electrophoresis[12,15], respectively designated MAO_{1-4}; two migrated to the anode, one remained at the origin and a fourth migrated towards the cathode (Fig. 1). These results seem to be analogous with those reported for rat brain MAO[12], whereas pig brain MAO is homogeneous[16]. Protein fractions with enzyme activity were extracted from the gels[12] and stored at 4° C until required.

Properties of Human Brain MAO

A comparison was made between the specific activities of solubilized mitochondrial MAO from whole human brain and those of MAO derived from the brain areas chosen for investigation, using ^{14}C-tryptamine, ^{14}C-tyramine, ^{14}C-dopamine and ^{14}C-benzylamine as substrates[17] (Table 1). Whole brain mitochondrial preparations deaminated tryptamine least, and benzylamine most avidly.

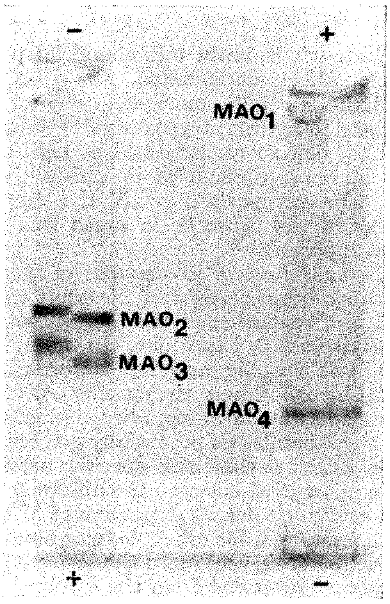


Fig. 1. Multiple forms of solubilized whole human brain mitochondrial monoamine oxidase (MAO) separated on 5 per cent polyacrylamide gel electrophoresis. MAO_{1-3} migrated from cathode to anode and MAO_4 from anode to cathode. 250 μl. of enzyme in 0·05 M Tris-HCl buffer, *p*H 9·1, was mixed with 'Sephadex *G*-200' and introduced on to the gel. Electrophoresis was continued at room temperature for 1–2 h at 6 mA per tube. The gels were developed for MAO activity with a solution containing 4 mg nitroblue tetrazolium, 1 mg sodium sulphate and 4 mg tryptamine-HCl in 5 ml. of 0·05 M phosphate buffer, *p*H 7·4 (ref. 41).

Table 1. SPECIFIC ACTIVITIES OF MITOCHONDRIAL MAO FROM VARIOUS AREAS OF THE HUMAN BRAIN

	Tryptamine	Tyramine	Benzylamine	Dopamine
Whole brain	0·729 ± 0·068	1·735 ± 0·184	5·607 ± 0·610	2·359 ± 0·183
Basal ganglia	0·573 ± 0·060	1·476 ± 0·161	2·848 ± 0·212	4·624 ± 0·371
Centrum ovale	0·899 ± 0·093	2·059 ± 0·300	5·277 ± 0·318	3·709 ± 0·381
Cerebellar cortex	0·613 ± 0·058	0·391 ± 0·052	1·502 ± 0·311	1·893 ± 0·212
Cerebral cortex	1·124 ± 0·111	2·218 ± 0·219	1·427 ± 0·218	1·714 ± 0·210
Hypothalamus	5·164 ± 0·702	7·719 ± 0·782	50·556 ± 8·172	36·148 ± 5·720
Pons	0·318 ± 0·034	0·080 ± 0·050	1·625 ± 0·199	1·972 ± 0·204

The specific activities are expressed as nmole of substrate deaminated per min incubation per mg of protein ± *S.E.*, and are the mean of between three and six experiments using at least two different brains. Protein nitrogen was measured[39] using bovine serum albumin as standard.

Table 2. SPECIFIC ACTIVITIES OF THE MULTIPLE FORMS OF HUMAN BRAIN MITOCHONDRIAL MAO

	^{14}C-Tryptamine	^{14}C-Tyramine	^{14}C-Benzylamine	^{14}C-Dopamine
Whole brain				
MAO$_1$	0·820 ($1·51 \times 10^{-5}$ M)	1·656 ($4·22 \times 10^{-5}$ M)	4·395 ($1·18 \times 10^{-4}$ M)	2·092 ($1·89 \times 10^{-5}$ M)
MAO$_2$	0·229 ($1·18 \times 10^{-5}$ M)	0·279 ($6·13 \times 10^{-5}$ M)	0·875 ($2·17 \times 10^{-4}$ M)	0·969 ($2·58 \times 10^{-5}$ M)
MAO$_3$	0·251 ($4·27 \times 10^{-5}$ M)	0·221 ($9·17 \times 10^{-5}$ M)	0·424 ($2·00 \times 10^{-4}$ M)	6·351 ($1·97 \times 10^{-5}$ M)
MAO$_4$	0·674 ($4·01 \times 10^{-5}$ M)	0·452 ($4·02 \times 10^{-5}$ M)	1·099 ($6·82 \times 10^{-4}$ M)	58·54 ($2·71 \times 10^{-6}$ M)
Basal ganglia				
MAO$_1$	0·774 ($1·56 \times 10^{-5}$ M)	0·550 ($4·00 \times 10^{-5}$ M)	1·471	1·450 ($2·04 \times 10^{-5}$ M)
MAO$_2$	0·237	0·216	1·891	0·127 ($2·77 \times 10^{-5}$ M)
MAO$_3$	0·304 ($4·49 \times 10^{-5}$ M)	0·353	0·781	43·08 ($2·17 \times 10^{-5}$ M)
MAO$_4$	0·713	0·221	7·721 ($7·14 \times 10^{-4}$ M)	338·0 ($3·18 \times 10^{-6}$ M)
Centrum ovale				
MAO$_1$	0·971	2·118	4·915	3·136
MAO$_2$	0·337	0·529 ($7·17 \times 10^{-5}$ M)	0·452	1·476
MAO$_3$	0·416	0·465	0·520	19·68
MAO$_4$	0·318	0·492	1·136	67·22
Cerebellar cortex				
MAO$_1$	0·773	0·433 ($4·69 \times 10^{-5}$ M)	0·765 ($1·19 \times 10^{-4}$ M)	1·330
MAO$_2$	0·246	0·138	0·217 ($2·22 \times 10^{-4}$ M)	0·410
MAO$_3$	0·249	0·115	0·152 ($1·86 \times 10^{-4}$ M)	0·864
MAO$_4$	0·718	0·136 ($4·50 \times 10^{-5}$ M)	0·201 ($7·74 \times 10^{-4}$ M)	4·459 ($2·09 \times 10^{-6}$ M)
Cerebral cortex				
MAO$_1$	2·436 ($2·12 \times 10^{-5}$ M)	5·944 ($4·17 \times 10^{-5}$ M)	1·315	0·949
MAO$_2$	2·101	2·437 ($6·29 \times 10^{-5}$ M)	2·143	1·414
MAO$_3$	0·239	0·074	0·401	0·784
MAO$_4$	0·660	0·191	1·069	4·937
Hypothalamus				
MAO$_1$	3·718 ($1·92 \times 10^{-5}$ M)	11·86 ($4·99 \times 10^{-5}$ M)	27·07 ($1·27 \times 10^{-4}$ M)	3·580
MAO$_2$	2·614	8·469 ($3·47 \times 10^{-5}$ M)	8·940 ($1·84 \times 10^{-4}$ M)	2·331
MAO$_3$	0·893	2·509 ($6·49 \times 10^{-5}$ M)	1·107 ($3·17 \times 10^{-4}$ M)	9·761
MAO$_4$	0·985	2·797 ($4·07 \times 10^{-5}$ M)	6·467 ($6·43 \times 10^{-4}$ M)	150·5 ($2·46 \times 10^{-6}$ M)
Pons				
MAO$_1$	0·271 ($1·72 \times 10^{-5}$ M)	0·265 ($4·78 \times 10^{-5}$ M)	2·659 ($2·04 \times 10^{-4}$ M)	1·410 ($9·94 \times 10^{-6}$ M)
MAO$_2$	0·148	0·047 ($7·43 \times 10^{-5}$ M)	0·125 ($2·14 \times 10^{-4}$ M)	0·858 ($2·19 \times 10^{-5}$ M)
MAO$_3$	0·216 ($5·31 \times 10^{-5}$ M)	0·069 ($7·56 \times 10^{-6}$ M)	0·283 ($2·81 \times 10^{-4}$ M)	1·344 ($3·07 \times 10^{-5}$ M)
MAO$_4$	0·413	0·467 ($4·01 \times 10^{-5}$ M)	0·260 ($5·72 \times 10^{-4}$ M)	13·42 ($2·64 \times 10^{-6}$ M)

Each value is expressed as nmole of substrate deaminated per min incubation per mg protein and is the mean of at least two experiments from four different brains except for the whole brain where two only were used. The variation in activities between the brains did not exceed 20 per cent of the mean value. The figures in parentheses are the corresponding Michaelis constants measured graphically.

Compared with whole brain mitochondrial preparations, hypothalamic MAO deaminated all substrates more rapidly. Although high concentrations of both noradrenaline[2] and 5-hydroxytryptamine[18,19] are present in this brain area, neither amine could be used as substrate because of inherent difficulties in radioactive methods of assay[20]. In comparison with the hypothalamus, MAO activity of the other brain areas was low, as has already been reported[21,22].

The specific activities of isoenzymes of MAO prepared from the various brain areas were also determined (Table 2). The rates of oxidation of the four substrates by the bands of activity seemed to differ. In most areas, MAO$_1$ had the highest specific activity towards tryptamine, tyramine and benzylamine; the specific activity of MAO$_4$ toward dopamine was, however, always greater than that of the other three bands. Of potentially greater significance was the observation that the specific activity of the individual bands varied between the different brain areas. The specific activity towards dopamine of MAO$_4$ deriving from the basal ganglia, for example, was almost six times that of the whole brain and more than seventy times that of the same band prepared from the cerebral cortex. On the other hand, MAO$_4$ prepared from basal ganglia had a lower specific activity toward tyramine than that prepared from the whole brain. Similar differences occurred between the different brain areas with the other substrates.

Michaelis constants of the multiple enzyme forms prepared from whole human brain are also shown in Table 2. There seemed to be differences in the affinities of the bands for the four substrates. In contrast, the marked differences in specific activities of the bands between the brain areas were not accompanied by corresponding changes in the Michaelis constants (Table 2), so that $v_{\max}$ for the substrates would seem to differ between the brain areas investigated. It is unlikely that the lack of correlation between Michaelis constants and specific activities is a reflexion of differing purities of the enzyme samples, for not only were they electrophoretically pure but, before electrophoresis, all were submitted to gel filtration[12] to remove as far as possible proteins of differing molecular weights.

The thermal sensitivity characteristics of whole brain mitochondrial MAO, of MAO isolated from the brain areas under study and of the multiple forms from whole brain are shown in Fig. 2. A wide range of stabilities was displayed by the various brain preparations, hypothalamic MAO being most labile and that from basal ganglia most stable. From the stabilities of the individual enzyme forms, we might expect that hypothalamic MAO has a preponderance of activity in MAO$_1$ and MAO$_3$ whereas in the basal ganglia activity is confined mainly to MAO$_2$ and MAO$_4$.

With kynuramine as substrate[28], we measured the effect on the different brain enzymes of iproniazid and pargyline, two irreversible inhibitors of MAO[23,24]; of harmaline, a reversible inhibitor[25]; and of 'M & B 9302' (clorgyline), a non-hydrazine inhibitor which gives rise to an unusual biphasic inhibition curve with rat and human brain homogenates[26,27] (Table 3). There was little difference between the Ki values of enzyme preparations from the various brain areas. When the inhibition of whole brain

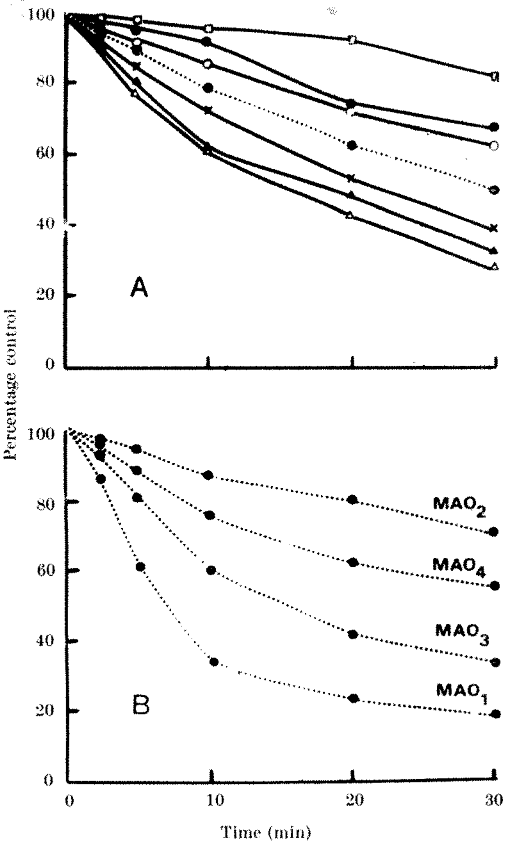


Fig. 2. The effect of temperature on human brain mitochondrial MAO activity. The enzyme was suspended in phosphate buffer (0·05 M, *p*H 7·4), heated to 50° C in a water bath and the residual activity measured using kynuramine as substrate[28]. *A*, Human brain MAO from various brain areas. Basal ganglia, □—□; cerebral cortex, ●—●; cerebellar cortex, ○—○; centrum ovale, ▲—▲; pons, × — ×; hypothalamus, △—△; whole brain, ● - - - ●. *B*, Multiple forms of whole brain MAO. The results are expressed as percentages of the activity of the unheated enzyme.

MAO by 'M & B 9302' was measured using ^{14}C-dopamine as substrate, however, a triphasic curve was obtained (Fig. 3), providing further evidence that human brain MAO exists in at least three forms; each plateau may represent inhibition of one of the isoenzymes. When ^{14}C-tyramine was substrate the plateaux were less distinct, whereas with kynuramine a normal sigmoidal dose–response curve was obtained. This result supports the conclusions of Collins and Youdim[29] that inhibition of the multiple forms of rat and human liver MAO is substrate dependent.

Interpretations

MAO seems to exist in multiple forms in many tissues[12,15,29–36]. We have now been able to show that MAO isoenzymes are similarly present in human brain and that their specific activities differ from one area to another, although the principles underlying these differences are not immediately obvious. Although there may be differences in affinity constants of systems isolated by physical means *in vitro*, the complete system, which could of itself act as an integrated enzyme protein, may differ markedly from the individual isoenzymes in its affinities for substrates or inhibitors.

Table 3. INHIBITION OF HUMAN BRAIN MITOCHONDRIAL MAO

	Iproniazid	'M & B 9302'	Pargyline	Harmaline
Whole brain	$5·68 \times 10^{-6}$	$1·64 \times 10^{-6}$	$2·09 \times 10^{-8}$	$1·25 \times 10^{-5}$
Basal ganglia	$9·89 \times 10^{-6}$	$1·34 \times 10^{-6}$	$3·45 \times 10^{-8}$	$1·59 \times 10^{-5}$
Centrum ovale	$4·90 \times 10^{-6}$	$2·04 \times 10^{-6}$	$2·42 \times 10^{-8}$	$2·81 \times 10^{-5}$
Cerebral cortex	$5·62 \times 10^{-6}$	$1·88 \times 10^{-6}$	$4·08 \times 10^{-8}$	$3·65 \times 10^{-5}$
Cerebellar cortex	$9·50 \times 10^{-7}$	$4·69 \times 10^{-6}$	$4·96 \times 10^{-8}$	$1·59 \times 10^{-5}$
Hypothalamus	$4·37 \times 10^{-6}$	$1·70 \times 10^{-6}$	$3·67 \times 10^{-8}$	$2·82 \times 10^{-5}$
Pons	$4·20 \times 10^{-6}$	$6·40 \times 10^{-7}$	$2·28 \times 10^{-8}$	$1·67 \times 10^{-5}$

The values represent inhibitor constants (K_i values) and are calculated from double reciprocal plots using at least two inhibitor concentrations[40].

If the amino-acid content of an isoenzyme differed in the various brain areas, then electrophoresis of whole brain MAO would separate many bands of activity; only four isoenzymes were, however, detected. Two of us (M. B. H. Y. and G. G. S. C.) have shown[37] for the multiple forms of rat liver MAO that they are conformational isoenzymes, that is, proteins differing only in the way the constituent polypeptide chains are folded[38]. If, as would seem a reasonable extrapolation, the bands of activity of human brain MAO also represent conformational isoenzymes, a unitary explanation of the differences in specific activities presents itself. It is possible that each amine substrate in the human brain interacts with one or more of the isoenzymes thereby causing small but not readily reversible changes in conformation with a corresponding alteration of specific activity but not of affinity for the substrate. The interaction could occur either during the folding of the polypeptide chain or, perhaps, during the catalytic process itself. We may therefore have a simple self-regulatory mechanism by which an increase in the intraneuronal concentration of a particular amine is accompanied by the activation of a relatively specific isoenzyme of MAO.

Our hypothesis receives support from the rates of oxidation of dopamine by MAO$_4$ in the different brain areas. The basal ganglia contain a high concentration of dopamine whereas the amount in the cerebral cortex is low[1]; the specific activity of MAO$_4$ towards dopamine is more than seventy times higher in the basal ganglia than in the cerebral cortex.

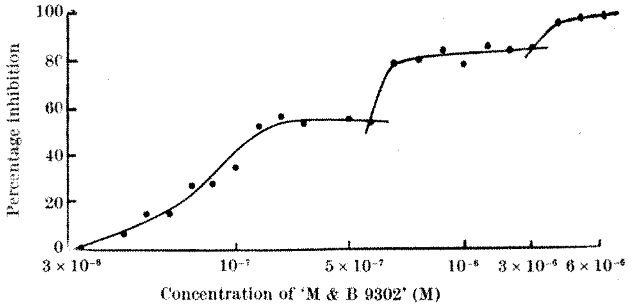


Fig. 3. The inhibition of whole human brain mitochondrial MAO by 'M & B 9302'. The enzyme preparation was incubated with the inhibitor for 20 min at 37° C before addition of the substrate (^{14}C-dopamine) to give a final concentration of $6·79 \times 10^{-4}$ M. The results are expressed as percentage inhibition of the control enzyme activity.

As we recently suggested for depressive illness[12], such an approach might lead to the synthesis of a specific inhibitor tailored to an individual isoenzyme band. Whether the therapeutic effectiveness of MAO inhibitors in depression arises from the localized accumulation of a particular substrate after preferential inhibition of a specific isoenzyme, perhaps at a particular anatomical site, can only be decided by future observation.

In conclusion, we suggest that our observation of high local specific activity of an isoenzyme band, in an area rich in its particular substrate, is unlikely to be an isolated phenomenon. Parallel observations with noradrenaline and 5-hydroxytryptamine are urgently needed.

We thank Dr D. R. Maxwell of May and Baker, Ltd, for a supply of 'M & B 9302'. Our work was supported by grants from the Medical Research Council, the Wellcome Trust and Hoechst Pharmaceuticals, Ltd.

Received October 28; revised December 30, 1969.

[1] Bertler, Å., and Rosengren, E., *Acta Physiol. Scand.*, **47**, 350 (1959).
[2] Vogt, M., *J. Physiol.*, **123**, 451 (1954).
[3] Amin, A. H., Crawford, T. B. B., and Gaddum, J. H., *J. Physiol.*, **126**, 596 (1954).

[4] Bloom, F. E., and Giarman, N. J., *Ann. Rev. Pharmacol.*, **8**, 229 (1968).

[5] Kopin, I. J., *Pharmacol. Rev.*, **16**, 179 (1964).

[6] Andén, N.-E., Carlsson, A., and Häggendal, J., *Ann. Rev. Pharmacol.*, **9**, 119 (1969).

[7] Calne, D. B., Stern, G. M., Laurence, D. R., Sharkey, J., and Armitage, P., *Lancet*, i, 744 (1969).

[8] Cotzias, G. C., Papavasiliou, P. S., and Gellene, R., *New England J. Med.*, **280**, 337 (1969).

[9] Hornykiewicz, O., *Pharmacol. Rev.*, **18**, 925 (1966).

[10] Pare, C. M. B., and Sandler, M., *J. Neurol. Neurosurg. Psychiat.*, **22**, 247 (1959).

[11] Hendley, E. D., and Snyder, S. H., *Nature*, **220**, 1330 (1968).

[12] Youdim, M. B. H., Collins, G. G. S., and Sandler, M., *Nature*, **223**, 626 (1969).

[13] Hawkins, J., *Biochem. J.*, **50**, 577 (1952).

[14] Youdim, M. B. H., and Sourkes, T. L., *Canad. J. Biochem.*, **43**, 1305 (1966).

[15] Youdim, M. B. H., Collins, G. G. S., and Sandler, M., in *FEBS Symp. 18, Enzymes and Isoenzymes* (edit. by Shugar, D.) (Academic Press, New York, in the press).

[16] Tipton, K. F., and Spires, I. P. C., *Biochem. Pharmacol.*, **17**, 2137 (1968).

[17] Robinson, D. S., Lovenberg, W., Keiser, H., and Sjoerdsma, A., *Biochem. Pharmacol.*, **17**, 109 (1968).

[18] Bogdansky, D. F., and Udenfriend, S., *J. Pharmacol. Exp. Ther.*, **116**, 7 (1956).

[19] Gaddum, J. H., and Giarman, N. J., *Brit. J. Pharmacol.*, **11**, 88 (1956).

[20] Southgate, J., and Collins, G. G. S., *Biochem. Pharmacol.*, **18**, 2285 (1969).

[21] Weiner, N., *J. Neurochem.*, **6**, 79 (1960).

[22] Weiner, N., *Arch. Biochem. Biophys.*, **91**, 182 (1960).

[23] Zeller, E. A., Barsky, J., Berman, E. R., and Fouts, J. R., *J. Pharmacol. Exp. Ther.*, **106**, 427 (1952).

[24] Taylor, J. D., Wykes, A. A., Gladish, Y. C., and Martin, W. B., *Nature*, **187**, 941 (1960).

[25] Udenfriend, S., Witkop, B. G., and Weissbach, H., *Biochem. Pharmacol.*, **1**, 160 (1958).

[26] Johnson, J. P., *Biochem. Pharmacol.*, **17**, 1285 (1968).

[27] Hall, D. W. R., Logan, B. W., and Parsons, G. H., *Biochem. Pharmacol.*, **18**, 1447 (1969).

[28] Kraml, M., *Biochem. Pharmacol.*, **14**, 1684 (1965).

[29] Collins, G. G. S., and Youdim, M. B. H., *Biochem. J.*, **114**, 80*P* (1969).

[30] Gorkin, V. Z., *Pharmacol. Rev.*, **18**, 115 (1966).

[31] Youdim, M. B. H., and Sandler, M., *Biochem. J.*, **105**, 43P (1967).

[32] Squires, R. F., *Biochim. Pharmacol.*, **17**, 1401 (1968).

[33] Collins, G. G. S., Youdim, M. B. H., and Sandler, M., *FEBS Letters*, **1**, 215 (1968).

[34] Kim, H. C., and D'Iorio, A., *Canad. J. Biochem.*, **46**, 295 (1968).

[35] Ragland, J. B., *Biochem. Biophys. Res. Commun.*, **31**, 203 (1968).

[36] Gomes, B., Igaue, I., Kloepfer, H. G., and Yasunobu, K. T., *Arch. Biochem. Biophys.*, **132**, 16 (1969).

[37] Youdim, M. B. H., and Collins, G. G. S., *Biochem. J.* (in the press).

[38] Epstein, C. J., and Schechter, A. N., *Ann. NY Acad. Sci.*, **151**, 85 (1968).

[39] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

[40] Dixon, M., and Webb, E. C. *Enzymes.*, second ed. (Longmans, London, 1963).

[41] Glenner, G. C., Burtner, H. J., and Brown, G. W., *J. Histochem. Cytochem.*, **5**, 591 (1957).

Re-evaluation of the Synaptic Activation of an Electrogenic Sodium Pump

by

JacSUE KEHOE*

Department of Anatomy,

P. ASCHER*

Department of Pharmacology,
University of Cambridge

A hyperpolarizing synaptic potential observed in *Aplysia* neurones is shown to be the result of an increase in potassium permeability. A re-evaluation is made of the criteria used to implicate the activation of an electrogenic pump.

ELECTROGENIC explanations of ionic pumps have been proposed[1-5]. Such hypotheses imply that a change in the rate of pumping can lead to a change in membrane potential[6] and it has indeed been shown that some hyperpolarizations—for example, that following repetitive action-potentials[7-10] or intracellular injection of Na^+ (refs. 11 and 12)—could be accounted for by increased activity of the Na^+–K^+ pump. Is it possible that "pump" potential changes could be triggered independently of any change in ionic permeabilities? Such a hypothesis has been proposed to account for a variety of potential changes caused by synaptic transmitters[13-24], hormones[25] and light[26]. For example, the recent experiments of Pinsker and Kandel[21] seemed to imply activation of the Na^+–K^+ pump in a synaptic potential. The experiments we report show, however, that this potential is due to a change in K^+ permeability, and thus emphasize the need of re-evaluating many of the potentials attributed to the activation of an electrogenic pump.

The two-component, postsynaptic inhibition described by Pinsker and Kandel[21] occurs in identifiable cells of the abdominal ganglion of *Aplysia californica*. It consists of a rapid, early component (the "early IPSP") which is the result of a Cl^- permeability change and of a "late IPSP" which appeared to be due to the synaptic activation of an electrogenic Na^+–K^+ pump. We felt that it would be worthwhile to take a second look at this abdominal ganglion inhibition because it markedly resembles a pleural ganglion two-component inhibition—both phases of which were previously shown to be due to ionic permeability changes[27-30]. A list of the most important data published follows.

Pleural ganglion[27-29]	Abdominal ganglion[21]
Rapid component	*Early IPSP*
a. Inverts at −60 mV	*a.* Inverts at −65 mV
b. Cl^- dependent	*b.* Cl^- dependent
c. Selectively blocked by (+)-tubocurarine (dTC)	*c.* Selectively blocked by dTC
Slow component	*Late IPSP*
a. Inverts at −80 mV	*a.* Cannot be inverted
b. K^+ dependent	*b.* Not K^+ dependent
c. Selectively blocked by tetraethylammonium[28] and methylxylocholine[29]	*c.* Selectively blocked by ouabain, cooling and prolonged washing in K^+-free

The major discrepancy between the two sets of data concerns the interpretation of the slow phase of the two inhibitions. In the pleural ganglion it was attributed to an increase in permeability to K^+ ions because one could define an inversion potential which varied according to the Nernst equation when the external K^+ concentration was varied from 5 mM to 20 mM (ref. 27). In the abdominal ganglion, on the other hand, the "late IPSP" was attributed to the activation of an electrogenic pump because it (*a*) was not inverted by hyperpolarization of the postsynaptic membrane, (*b*) was not associated with a change in membrane conductance, and (*c*) was suppressed by prolonged washing in K^+ free seawater, by ouabain 2×10^{-4} M and by cooling to 7°–10° C (ref. 21).

---

* Visiting workers from Laboratorie de Neurophysiologie Cellulaire; Centre d'Etudes de Physiologie Nerveuse, CNRS, Paris.

The interpretation of the effects of ouabain and cold seems, however, subject to question in view of some of our own unpublished observations on the dTC-resistant component of the pleural inhibition.

The effects of ouabain on this component are shown in Fig. 1. In normal seawater, this response inverts at −80 mV. Ten min after the addition of 2×10^{-4} M ouabain, the inversion potential is shifted to −70 mV—indicating a decrease in the internal K^+ concentration after blockage of the Na^+-K^+ pump. It can be noted that if the effect of ouabain on the dTC-resistant inhibition had been analysed only at −70 mV, ouabain would appear to have completely eliminated the inhibition.

Cooling also has marked effects on the K^+ dependent inhibition of the pleural ganglion. The response is progressively reduced and slowed down by cooling and it disappears altogether when the temperature reaches 7°–10° C. The Cl^- dependent phase is considerably less affected. Both components of the synaptic inhibition can be mimicked by an injection of acetylcholine (ACh)[27] and, as seen in Fig. 2, cooling also selectively blocks the K^+ dependent phase of the ACh potential.

Because both ouabain and cooling have rather marked effects on a potential that is known to be due to membrane permeability changes, we thought it possible that their effects on the abdominal ganglion inhibition had been misinterpreted and we consequently felt it necessary to perform additional experiments on the abdominal cells.

In these experiments, both the "postsynaptic" neurones (L1, L2, L3, L4, L5, L6) and the presynaptic neurone (L10)[31] were penetrated with double barrelled microelectrodes filled with 0·5 M K_2SO_4. We could thus evaluate the synaptic potentials triggered by intracellular stimulation of L10 at different levels of polarization of the postsynaptic neurone. We found, as had Pinsker and Kandel[21], that it seemed often impossible to invert the "late IPSP" (see Fig. 3*A*). After dTC had eliminated the Cl^- dependent component, however, an initial hyperpolarization of the remaining multiphased response could be inverted around −90 mV (±5 mV). The terminal component, on the other hand, remained hyperpolarizing until −100 mV (±5 mV), at which level it was no longer detectable (Fig. 3*B*).

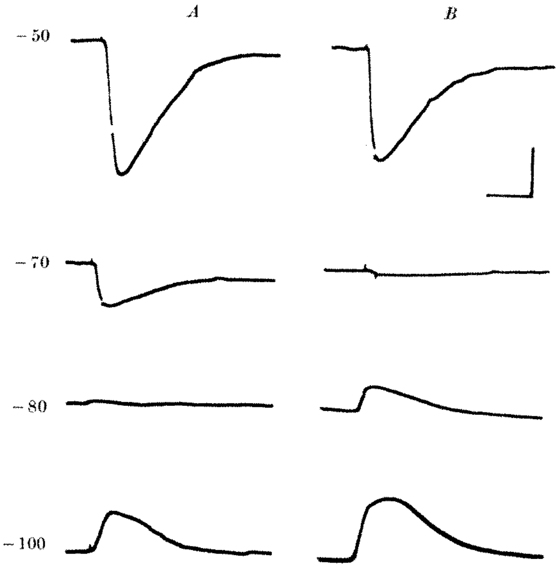


Fig. 1. Effects of ouabain (2×10^{-4} M) on the K^+ dependent dTC-resistant component of the synaptic inhibition of the pleural neurones. *A* shows the postsynaptic responses to intracellular stimulation of the presynaptic neurone[13,14] in a preparation where dTC chloride (10^{-3} g/ml.) has eliminated the chloride dependent component. Polarization of the postsynaptic neurone to the levels indicated on the left shows that normally the inversion potential of the dTC resistant component is −80 mV. In *B*, 10 min after the addition of ouabain, the inversion potential was shifted from −80 to −70 mV. Calibration: 5 mV; 4 s.

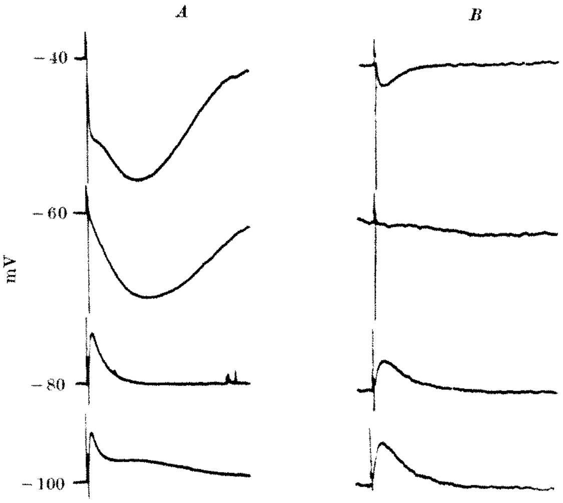


Fig. 2. Effects of cold on the ACh potential in pleural neurones. *A*, Responses to an electrophoretic injection of ACh (5×10^{-2} M) on the pleural neurones. The early component of the ACh potential inverts at −60 mV; thus only the slow component is visible at that membrane potential. The slow component inverts at −80 mV. *B*, Reducing the temperature from 22° to 8° C produces a complete suppression of the late component (effect best observed at −60 mV) whereas the rapid component is considerably less affected. Calibration (see *B*): 5 mV; 5 s.

Although in these respects the dTC-resistant abdominal ganglion inhibition differs slightly from that of the pleural ganglion, both react identically to a reduction in external K^+ concentration, as can be seen in Fig. 3*C*. In normal seawater (K^+ = 10 mM), at −95 mV the early hyperpolarization is inverted, while the late hyperpolarization is not detectable. When the external K^+ concentration is reduced to 5 mM, both phases become marked hyperpolarizations.

We found that the effects of ouabain on the abdominal ganglion inhibition are similar to those on the pleural inhibition. Ouabain does not eliminate either the early or late dTC-resistant hyperpolarizations, but merely changes their inversion potentials (Fig. 4).

Thus certain evidence given by Pinsker and Kandel[21] in support of their hypothesis must be re-evaluated. We find first of all that it is possible to invert at least part if not all of the dTC-resistant hyperpolarization. Second, this hyperpolarization is sensitive to changes in external K^+, and, finally, ouabain does not eliminate it but merely changes the inversion potential.

Further similarities between the abdominal and pleural inhibitions became evident when we tested the effects of ACh on the abdominal neurones. It has been shown previously[27] that the response of pleural cells to an iontophoretic injection of ACh mimicks the inhibitory input. The ACh potential consists of two components: the first inverting at −60 mV; the second, at −80 mV (Fig. 2*A*). These two elements respond in the same way as those of the synaptic input to ionic and pharmacological manipulations[27–29]. ACh also provokes a two-component hyperpolarization on the abdominal cells[21]. When we analysed this response at different levels of membrane potential, we found it identical to that recorded from the pleural cells. The two components invert at −60 and −80 mV, respectively; the early phase is Cl^- dependent and is blocked by dTC, whereas the late phase is K^+ dependent and is blocked by methylxylocholine. Both phases are associated with changes in conductance.

Thus no distinction can be made between (*a*) the ACh potential of the abdominal neurones, (*b*) that of the pleural neurones, and (*c*) the synaptic inhibition of the pleural postsynaptic cells. The similar behaviour of these groups of responses contrasts with that of the synaptic response in the abdominal cells. As mentioned here, the dTC-sensitive component of this synaptic response inverts at approximately −65 mV; the dTC-resistant component is

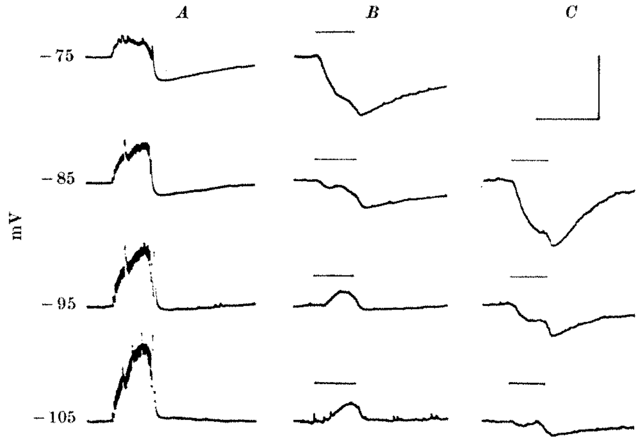


Fig. 3. Effects of a reduction in external K^+ on the dTC resistant synaptic inhibition of abdominal neurones. L10 was intracellularly stimulated so as to provoke a train of spikes. The duration of this train is indicated by the horizontal lines in *B* and *C*. *A*, These data correspond to those given by Pinsker and Kandel in Fig. 1*Ab*. No clear reversal of the "late IPSP" can be seen when the somatic membrane polarization is brought from −75 to −105 mV. *B*, After dTC chloride (10^{-3} g/ml.), the remaining response can be separated into two hyperpolarizing components. The first, seen during the period of presynaptic activity, inverts between −85 and −95 mV; the second appears after the train of presynaptic spikes has terminated and is no longer visible beyond −95 mV. *C*, Reduction of external K^+ from 10 to 5 mM causes a shift in the equilibrium potential of both phases of the dTC-resistant hyperpolarizations. These changes are most evident at −95 mV. Calibration: 5 mV; 10 s.

multiphasic (in contrast with the single phased response in the pleural cells—compare Figs. 1*A* and 3*B*), and its hyperpolarizing phases do not invert until at least −90 mV. Furthermore, whereas the combined application of dTC and methylxylocholine will completely eliminate the pleural synaptic responses and the ACh responses on both groups of cells, the abdominal synaptic response does not obey this rule. Although the rapid IPSP of the abdominal inhibition is indeed eliminated by dTC, and although part of the dTC-resistant hyperpolarization is blocked by methylxylocholine, there remains in the presence of both of these drugs a response to the activation of L10. It consists of an initial depolarization (which in many preparations is capable of triggering a spike in the postsynaptic cell) which is followed (usually at the termination of the presynaptic firing) by a hyperpolarization (Fig. 5). Although it is difficult to invert this later hyperpolarization it has been shown here that—like the early one—it is K^+ dependent and is not blocked by ouabain.

Thus it appears that the synaptic response in the abdominal cells is made up of (*a*) a two-component inhibition which differs from that studied in the pleural cells only with respect to the inversion potentials of the components (−65 mV versus −60 mV; 90 ± 5 versus −80 mV), and (*b*) a biphasic response which is not mimicked by ACh injection.

That the inversion potentials of both the Cl^- and K^+ dependent elements of the abdominal ganglion inhibition are more negative than those of the pleural inhibition can be understood if one assumes that the ACh liberated by the presynaptic terminals is acting on a distant or particularly fine part of the axon. The synapse of L10 on L3 and others has been, in fact, located at a great distance from the soma of the postsynaptic cell[31]. In experiments in which we entered both the soma and a distant region of the axon of L3, polarization of the soma to −100 mV polarized the axon to only −75 mV, and a similar attenuation was found in the other direction. That the polarization level recorded in the soma does not accurately represent that of the synaptic region is also suggested by experiments in which ACh was injected on the axonal region of the cell near the presumed synaptic region, while the recording and polarizing electrodes were in the soma. In these conditions, the early phase of the ACh response inverted at −65 mV rather than at −60 mV. (The same injection of ACh gave only a very small second phase, thus making it impossible to measure its potential of inversion which was, however, clearly more negative than −80 mV.)

If we then admit that the anomalous inversion potentials are due to the effect of a distant junction, it remains only to understand the biphasic synaptic potential which can still be observed after dTC and methylxylocholine have blocked the two components of the ACh-potential. While we cannot completely eliminate the hypothesis of a "chemical" synaptic effect (ACh acting on additional receptors that are not represented in the region of the soma, or the intervention of other transmitters) the fact that the hyperpolarizing component is never prominent as long as the presynaptic neurone is firing suggests that the biphasic potential is similar to that observed by Hughes and Tauc[32] and interpreted[33] as due to transmission of the presynaptic spike through an electrical junction which would favour the slow hyperpolarizing component (see also ref. 34).

The main conclusion from our experiments is that the synaptic activity previously attributed[21] to the stimulation of an electrogenic pump is in fact due to a change in K^+ permeability. The criteria used by Pinsker and Kandel[21] have been used for similar purposes in the interpretation of a number of hyperpolarizing potentials (salivary glands[13,14], salt glands[15], smooth muscle[16,17], sympathetic ganglion cells[18-20], molluscan neurones[22,23]) so it seems that these criteria must be carefully considered.

The interpretation of potential changes occurring without a change in membrane conductance and the interpretation of anomalous reversal potentials must be made cautiously if there is any possibility that the polarizing and recording electrodes might be distant from the membrane region where a conductance change could in fact be occurring. Diamond, for example[35], has shown that, when γ-aminobutyric acid is applied to the dendrites of Mauthner neurones, the conductance changes can be

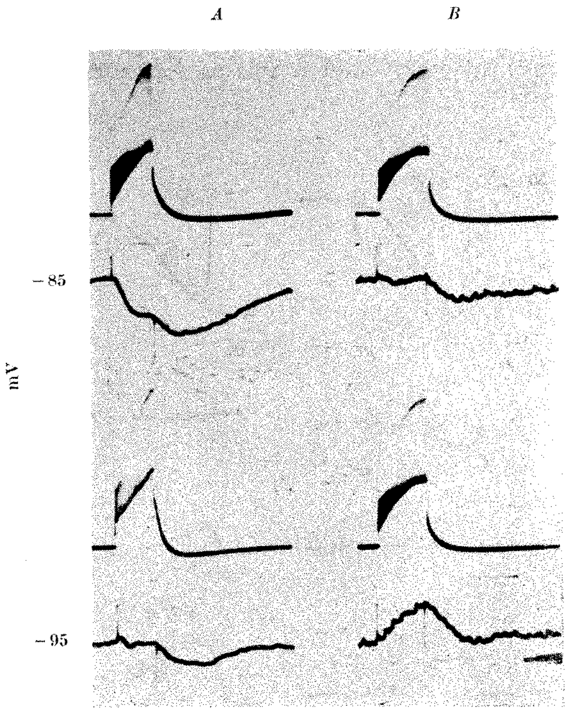


Fig. 4. Effects of ouabain (2×10^{-4} M) on the dTC-resistant hyperpolarization of the abdominal neurones. At each polarization level (−85 and −95 mV) both pre (L10) and post-synaptic (L5) records are presented. (Only the lower portion of the action potentials of L10 is visible.) The "early IPSPs" had been eliminated by dTC Cl (10^{-3} g/ml.), leaving a multiphased hyperpolarizing response. The earlier component inverted at −95 mV in the curarized preparation. When ouabain was added (*B*), its inversion potential was shifted 10 mV, as was that of the later hyperpolarization. Calibration: 5 mV; 10 s.

detected over only a few tens of microns, whereas there is very little decrement in potential changes over the same distance (see also ref. 36). Burke and Ginsborg[37], on the other hand, have shown how distance can introduce discrepancies between equilibrium potentials and reversal potentials measured with a single current microelectrode (see also ref. 38). Such effects of distance can be expected with axodendritic synapses (mammalian sympathetic ganglion cells) and with axo-axonic synapses (molluscan neurones, and possibly frog sympathetic ganglion cells), and similar difficulties arise in the case of "distributed innervation"[37] (smooth muscle, salt glands, salivary glands). Moreover, electrical junctions (known to occur in salivary glands, smooth muscle, and *Aplysia* neurones) will cause the same problems of measurement as are posed by distant junctions.

Ouabain can always be expected to reduce the ionic gradient for K^+, and any reduction of a synaptic potential must be evaluated in this respect. Such an evaluation might often be very difficult, however, for the effect of ouabain would be initially more pronounced in axonic or dendritic regions (where the surface/volume relation is high[39]) whereas measurement of the change in K^+ equilibrium potential is in general made in the tissue as a whole or in the cell soma. That a rapid fall in K^+ concentration in the synaptic region might occur without affecting the soma K^+ equilibrium potential is indeed suggested by another observation: an immediate depolarizing shift of the reversal potential of the late IPSP in the abdominal neurones can be observed following the triggering of only a few action potentials in the postsynaptic cells, and there is no corresponding shift for the reversal potential of the response to a somatically applied ACh injection.

K^+ free solutions and cooling stop the Na^+–K^+ pump, and can be expected to produce effects resembling those produced by ouabain. But the resulting shift of the K^+ equilibrium potential might be more difficult to detect. In K^+ free solutions, it will initially be masked by an inverse shift due to the immediate increase of the K^+ concentration gradient. This may account for the contradictory reports in K^+ effects on salivary potentials[40-42] or sympathetic ganglion potentials[43,44]. On the other hand, the effects of blocking the pump by cooling might be masked by direct suppressive effects such as those illustrated in Fig. 2. It is known that many "passive permeabilities" have a high temperature coefficient[45-48]; but the sensitivity of the K^+ potential illustrated in Fig. 2 appears exceptionally high and suggests an additional effect, which might occur in the sequence of events leading from the combination of ACh with its receptor to the change in K^+ permeability.

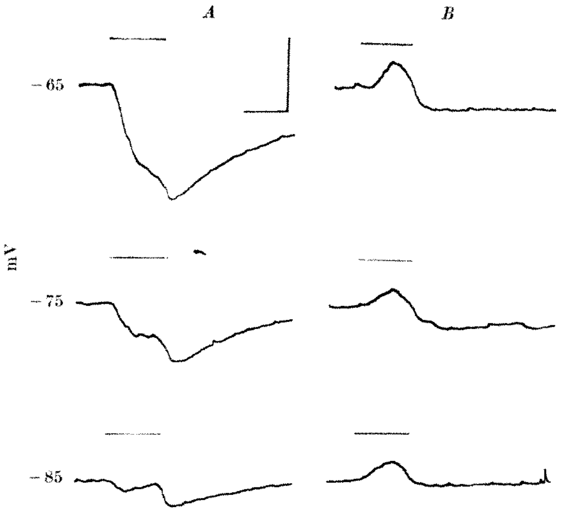


Fig. 5. Effects of methylxylocholine on the dTC-resistant components of the abdominal synaptic inhibition. *A*, As in Fig. 3*B*, the postsynaptic response has been recorded at different potential levels in a medium containing dTC chloride (10^{-3} g/ml.). It consists of two hyperpolarizing components (one starting during the presynaptic activity, the other after this activity terminates). *B*, Methylxylocholine (10^{-4} g/ml.) has been added to dTC seawater. This suppresses the first hyperpolarizing component (that which starts during the presynaptic activity) and unmasks a biphasic potential: the early component is depolarizing at all postsynaptic membrane potentials (and often triggers a spike at −50 mV); the late component is hyperpolarizing up to membrane potentials as high as −85 mV. In these pharmacological conditions, ACh injection will have no effect on the postsynaptic neurone. Calibration: 5 mV; 5 s.

Finally, we have observed on pleural neurones that Na^+ free solutions (Na^+ replaced by Li^+) had ouabain-like effects and progressively shifted the K^+ equilibrium potential.

Thus it seems that the criteria for establishing that a synaptic transmitter directly activates an electrogenic pump have in general not been used in such a way as to eliminate the hypothesis of a change in ionic permeability occurring at a distant junction and/or on the other side of an electrical synapse. On the other hand, there is no reason to doubt that the Na^+–K^+ pump might be activated after a synaptic potential, insofar as a change in cationic permeabilities will lead to a change in internal cation concentrations. It appears doubtful, however, that the minute changes involved in most synaptic potentials are sufficient to trigger a detectable "pump" potential change.

This work was supported by a grant from the US Public Health Service to Dr L. Tauc and Dr G. Horn, and a grant from the Tobacco Research Council to Dr J. F. Mitchell.

Received August 29, 1969.

[1] Post, R. J., and Jolly, P. C., *Biochim. Biophys. Acta*, **25**, 118 (1957).
[2] Kernan, R. P., *Nature*, **193**, 986 (1962).
[3] Cross, S. B., Keynes, R. D., and Rybova, R., *J. Physiol.*, **181**, 865 (1965).
[4] Adrian, R. H., and Slayman, C. L., *J. Physiol.*, **184**, 970 (1966).
[5] Keynes, R. D., *Quart. Rev. Biophys.*, **2**, 177 (1969).
[6] Frumento, A. S., *J. Theoret. Biol.*, **9**, 253 (1965).
[7] Connelly, C. M., *Rev. Mod. Phys.*, **31**, 475 (1959).
[8] Nakajima, S., and Takahashi, K., *J. Physiol.*, **187**, 105 (1966).
[9] Rang, H. P., and Ritchie, J. M., *J. Physiol.*, **196**, 183 (1968).
[10] Nicholls, J. G., and Baylor, D. A., *Science*, **162**, 279 (1968).
[11] Kerkut, G. A., and Thomas, R. C., *Comp. Biochem. Physiol.*, **14**, 167 (1965).
[12] Thomas, R. C., *J. Physiol.*, **201**, 495 (1969).
[13] Lundberg, A., *Acta Physiol. Scand.*, **40**, 35 (1955).
[14] Petersen, O. H., and Poulsen, J. H., *Acta Physiol. Scand.*, **71**, 194 (1967).
[15] Thesleff, S., and Schmidt-Nielsen, K., *Amer. J. Physiol.*, **202**, 597 (1962).
[16] Burnstock, G., *J. Physiol.*, **143**, 183 (1958).
[17] Bülbring, E., in *Ciba Foundation Symposium on Adrenergic Mechanisms*, 275 (Churchill, 1960).
[18] Nishi, S., and Koketsu, K., *Life Sci.*, **6**, 2049 (1967).
[19] *J. Neurophysiol.*, **31**, 717 (1968).
[20] Libet, B., and Kobayashi, H., *Science*, **164**, 1530 (1969).
[21] Pinsker, H., and Kandel, E. R., *Science*, **163**, 931 (1969).
[22] Ascher, P., *J. Physiol.*, **198**, 48 (1968).
[23] Kerkut, G. A., Brown, L. C., and Walker, R. J., *Life Sci.*, **8**, 297 (1969).
[24] *Nature*, **223**, 864 (1969).
[25] Fanestil, D. D., Porter, G. A., and Edelman, I. S., *Biochim. Biophys. Acta*, **135**, 74 (1967).
[26] Smith, T. G., Stell, W. K., Brown, J. E., Freeman, J. A., and Murray, G. C., *Science*, **162**, 456 (1968).
[27] Kehoe, J. S., *Nature*, **215**, 1503 (1967); *J. de Physiol., Paris*, **60**, suppl. 1, 266 (1968).
[28] Kehoe, J. S., *CR Acad. Sci. (Paris)*, **268**, 111 (1969).
[29] Kehoe, J. S., *J. Physiol.* (in the press).
[30] Kehoe, J. S., *Nature*, **221**, 866 (1969).
[31] Kandel, E. R., Frazier, W. T., Waziri, R., and Coggleshall, R. E., *J. Neurophysiol.*, **30**, 1352 (1967).
[32] Hughes, G. M., and Tauc, L., *J. Physiol.*, **197**, 511 (1968).
[33] Tauc, L., in *Progress in Brain Research*, **31**, 247 (Elsevier, 1969).
[34] Waziri, R., *Life Sci.*, **8**, 469 (1969).
[35] Diamond, J., *J. Physiol.*, **194**, 669 (1968).
[36] Rall, W., *Ann. NY Acad. Sci.*, **96**, 1071 (1962).
[37] Burke, W., and Ginsborg, B. L., *J. Physiol.*, **132**, 599 (1956); Ginsborg, B. L., *Pharmacol. Rev.*, **19**, 289 (1967).
[38] Calvin, W. H., *Exp. Neurol.*, **24**, 248 (1969).
[39] Duncan, G., and Bonting, S. L., *Science*, **164**, 1188 (1969).
[40] Lundberg, A., *Acta Physiol. Scand.*, **40**, 101 (1957).
[41] Petersen, O. H., and Poulsen, J. H., *Acta Physiol. Scand.*, **70**, 293 (1967).
[42] Yoshimura, H., and Imai, Y., *Jap. J. Physiol.*, **17**, 280 (1967).
[43] Kobayashi, H., and Libet, B., *Proc. US Nat. Acad. Sci.*, **60**, 1304 (1968).
[44] Kosterlitz, H. W., Lees, G. M., and Wallis, D. I., *J. Physiol.*, **195**, 39 (1968).
[45] Danielli, J. F., and Davson, H., *J. Cell. Comp. Physiol.*, **5**, 494 (1935).
[46] Glynn, I. M., *J. Physiol.*, **134**, 278 (1956).
[47] Passow, H., *Proc. XXIII Int. Cong. Physiol.*, Tokyo, 555 (1965).
[48] Willis, J. S., *J. Gen. Physiol.*, **49**, 1221 (1966).

Neurotrophic Control of Protein Synthesis in the Regenerating Limb of the Newt, *Triturus*

by
PAUL LEBOWITZ*
MARCUS SINGER
Developmental Biology Center,
and Department of Anatomy,
School of Medicine,
Case Western Reserve University,
Cleveland, Ohio

Evidence is presented for the stimulation of protein synthesis in newt limb buds by an agent produced by nerve fibres.

AMPHIBIAN limb regeneration is a nerve-dependent process[1,2]. Denervation of early regenerates leads to the complete cessation of growth and restoration of the nerve supply results in renewed capacity to grow. Limb regeneration is but one of a number of biological processes for which a neurotrophic influence is invoked[1]. The possibility has been suggested that nerve fibres emit from their distal processes a chemical substance with trophic properties[2]. The proof of this mechanism would require the extraction from neurones of one or more substances capable of substituting for innervation in a system clearly requiring the trophic action of the nerve. The studies that there have been in this direction offer no clear proof that the origin of the physiologically active extract is nervous or that the activity induced by the extract is normally nerve-dependent. Hommes *et al.*[7] extracted an inductor of cartilage formation from homogenates of combined notochord and spinal cord of chick embryos, Kanatani[8] prepared nerve extracts capable of stimulating meiosis and release of gametes in starfish. Lesh and Burnett[9] concentrated from the growth medium of *Hydra* a factor which induces and maintains polarity in this animal.

We have examined the chemical nature of the neurotrophic action in a system which is clearly nerve-dependent, the regenerating salamander limb. Previously we were unsuccessful in sustaining gross regeneration of the missing part by infusions of nerve extracts. We have now sought a more sensitive assay of the neurotrophic effect by assessing biochemical changes in the regenerate resulting from denervation because innervation has been shown to exert a significant role in the regulation of some biochemical processes. (For example, transient glycogen storage and diminution in lipid turnover occur following denervation of the brown fat pad in mice[3]; and the activity of muscle sole plate cholinesterase is dependent on the nerve ending[4-6].) Our object here was to test whether innervation is required for protein synthesis during the early blastema stage of limb regeneration at a time when the growth is most dependent on the nerve; and, if so, to see whether nerve homogenates could support protein synthesis in the denervated blastema.

Adult *Triturus viridescens*, native to western Massachusetts, were maintained—before and after operation—at 25° C. All operative procedures were carried out under light 'Chloretone' anaesthesia (30 per cent saturated aqueous solution). Bilateral forelimb amputations were performed just proximal to the elbow joint. Care was taken to establish a flat amputation surface to provide a transverse base from which the blastema could later be separated from adult stump tissues. When the regenerate reached the moderate early to early bud stage of regeneration[1] (10 to 14 days after amputation) the left experimental limb was denervated; simultaneously, an identical sham control operation was performed on the contralateral limb. This phase of growth, characterized by the rapid accumulation of blastemal cells, is most dramatically suppressed by denervation. The regenerate was denervated in the brachial plexus; unless otherwise stated, denervation was total and involved resection of at least 1 mm of the third and combined fourth and fifth spinal nerves. For partial denervation, single nerves or combinations of the three nerves were severed so as to produce regenerates innervated with 500, 800, 1,800, 2,100 and 2,600 nerve fibres[10]. The sympathetic nerves which follow the brachial artery into the forelimb were not disturbed.

Protein Synthesis by the Regenerate

Amino-acid uptake was studied in control and denervated blastemas 48 h after denervation in all except one series of animals in which the influence of the duration of denervation was investigated. DL-1-^{14}C-Leucine or uniformly ^{14}C-labelled L-leucine (New England Nuclear) was adjusted to final specific activities of 7·03 and 1·43 mCi/mmole, respectively, and a final concentration of 10 mM by dilution with distilled water. Each animal was injected intraperitoneally with 0·04 ml. without anaesthesia. At varying times following leucine injection the blastemas were separated from the original amputation

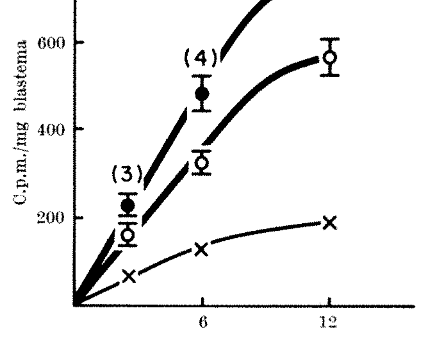


Fig. 1. Kinetics of appearance of leucine in innervated regenerates. Each animal received 0·40 μmoles of L-leucine (1·43 mCi/mmole) intraperitoneally at zero time. Blastemas were amputated for analysis of total radioactive leucine (●—●) and incorporated leucine (○—○) at the times indicated. Free radioactive leucine (×—×) was determined by subtraction. The bars correspond to *S.E.*; in parentheses are the number of regenerates in each series.

---

* Present address: Department of Internal Medicine, Yale Medical School, New Haven, Connecticut.

line with a sharp razor. Each blastema was blotted dry, weighed and immediately homogenized in 0·2 ml. of iced 5 per cent trichloroacetic acid (TCA). After transfer of the homogenate to another tube, the homogenizers were rinsed four times with a further 0·2 ml. of 5 per cent TCA and the washings combined with the homogenate. An 0·2 ml. aliquot of the TCA suspension was placed on a 'Millipore' filter (type *HA*, 0·8 μm porosity), washed with 10 ml. of iced 5 per cent TCA, and dried; radioactivity was counted either in a Nuclear Chicago proportional flow thin-window counter or in a Packard 'Tri-Carb' liquid scintillation spectrometer using a PPO–POPOP toluene scintillant. Neither additional washing with cold or hot TCA nor extraction of TCA precipitates with ethanol–ether–chloroform mixtures altered the counts significantly. In some cases, an 0·2 ml. aliquot of the TCA suspension was monitored directly without washing with cold TCA in order to obtain counts of the total leucine in the homogenate. An 0·4 ml. aliquot of each TCA suspension was centrifuged at 20,000*g* for 20 min, the precipitate taken up in 1M NaOH and its protein content determined by the method of Lowry *et al.*[11] using human serum albumin as a standard. The experimental and control blastemas of each animal were always processed simultaneously. Results are expressed either as c.p.m. of leucine incorporated per mg of blastemal protein; or in terms of percentage, based on specific activities, indicating incorporation in the denervated limb relative to 100 per cent incorporation in its contralateral control.

Preparation and Infusion of Nerve Homogenates

Newts whose forelimbs had been amputated 5 to 10 days previously served as a source of nerve. The animals were decapitated without anaesthesia, and placed on a bed of ice, and brachial nerves 3 and 4 were severed just distal to their spinal ganglia. The nerves and their branches into the upper arm were freed of perineurium, rapidly removed for weighing (weight was 1·5 to 1·7 mg), and then homogenized in a ground glass Potter–Elvehjem homogenizer at 0°–2° C containing 0·05 ml. of distilled water. The crude homogenates were either infused directly or as supernatants after centrifugation in a Sorvall 'RC2–B' for 1 h at 40,000*g*. The protein concentration of uncentrifuged homogenates was 2·8–3·1 mg/ml.; that of the centrifugation supernatant 0·8–0·9 mg/ml.[11]. Some homogenates were heated at 90° C for 10 min and were then infused to test for thermostability. Preparations were stored at –20° C for up to 24 h before use.

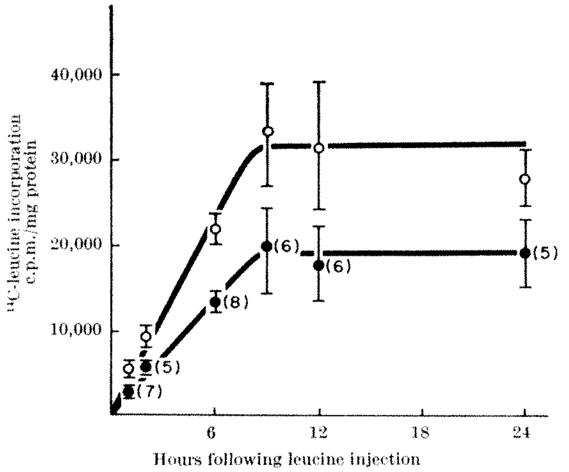


Fig. 2. Kinetics of incorporation of ^{14}C-leucine into protein of paired 48 h denervated (●—●) and contralateral sham operated control (○—○) regenerates. Each animal received 0·40 μmoles of leucine (7·03 mCi/mmole) at zero time intraperitoneally. Blastemas were amputated for incorporation analysis at the indicated times thereafter. The bars correspond to *S.E.* the mean; in parentheses are the number of animals in each series.

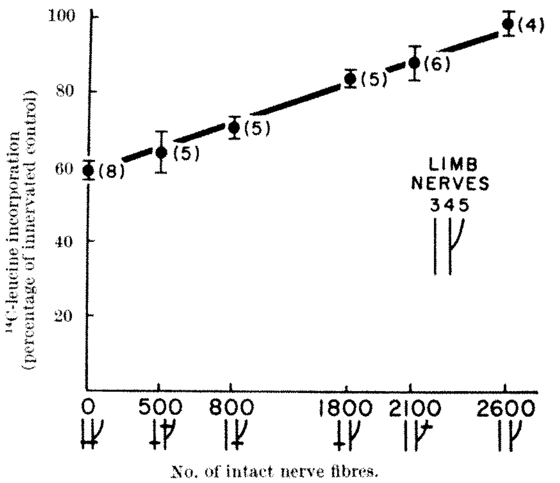


Fig. 3. Relationship between quantity of innervation and incorporation of ^{14}C-leucine into blastemal protein. Partial denervations were performed unilaterally as indicated; contralateral limbs were sham operated. Leucine was administered 48 h after denervation and blastemas removed 6 h later. The bars correspond to *S.E.*; in parentheses are the number of animals in each series.

Infusions of 0·005 ml. of homogenate were introduced directly into denervated blastemas with a Singer micro-infusion apparatus[12], 48 h after nerve transection. The infusion was always carried out for 1 h and was immediately preceded by an intraperitoneal injection of ^{14}C-leucine. Contralateral blastemas were infused with distilled water simultaneously or during the following hour. The tubing used in infusion of the homogenates was immersed in ice water.

Influence of Innervation and Denervation

Both innervated and denervated blastemas accumulate labelled leucine actively; although contributing only 0·04–0·07 per cent of the total body wet weight, each blastema accumulated at 6 and 12 h 0·1 per cent and 0·13–0·15 per cent, respectively, of the total administered leucine dose. Fig. 1 reveals that ^{14}C-leucine accumulates linearly in innervated blastemas for 8–10 h and then reaches a plateau. Similar kinetics of accumulation of total labelled leucine are seen in denervated regenerates (Fig. 2). In the innervated regenerate, approximately 70 per cent of the total label is in protein (Fig. 1), attesting to the vigour of protein synthesis in the growing regenerate.

Following a single dose of the labelled amino-acid, incorporation of leucine into blastemal protein (Fig. 2) increases linearly for approximately 8 to 9 h in innervated regenerates and in those denervated for 48 h; thereafter incorporation is maintained at a plateau for up to 24 h. Denervation has a profound effect on incorporation, reducing the rate and the final level attained by approximately 40 to 45 per cent. The parallel declines in accumulation of free radioactive leucine and in incorporation of leucine into protein in the innervated regenerate suggest that the inhibition of protein synthesis is a function of the decline in availability of unincorporated leucine. Further, the similarity of kinetics of accumulation of total labelled amino-acid in innervated and denervated regenerates indicates that the inhibition of protein synthesis by denervation does not arise from a diminution of available substrate.

To assess the specificity of neural control of protein synthesis, incorporation studies were undertaken in which the quantity of blastemal innervation and the duration of denervation were varied. As shown in Fig. 3 there is a linear inhibition of protein synthesis proportional to the extent of nerve destruction to a limiting value of 40 per cent inhibition attained in the fully denervated regenerate.

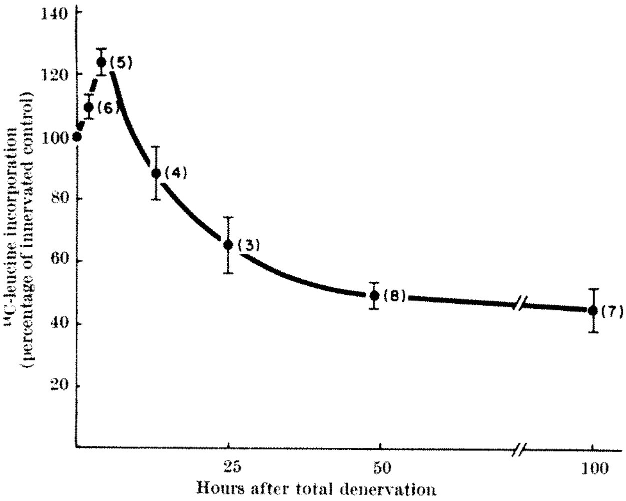


Fig. 4. Influence of duration of denervation on incorporation of ^{14}C-leucine into protein. Complete denervations were performed unilaterally and sham operations contralaterally. Leucine was administered at varying times. Blastemas were removed 1 h after leucine injection. For each series, points on the curve correspond to the time after denervation when leucine was administered plus the 1 h during which blastemas were exposed to the amino-acid. The bars indicate *S.E.*; in parentheses are the number of animals in each series.

The specificity of the neural effect was further explored by varying the time after complete denervation before leucine administration. Fig. 4 reveals that there is an initial transient stimulation of protein synthesis on transection of the nerves. A 10 per cent stimulation is evident by 2 h after denervation ($P < 0\cdot1$) and a 24 per cent stimulation by 4 h ($P < 0\cdot005$). Thereafter, incorporation recedes, falling below control levels by the tenth hour and reaching a baseline level of 45 to 50 per cent of control values about 50 h later. The burst of incorporation on nerve withdrawal is reminiscent of the initial heightened activity of muscle and other end organs which is observed after denervation—a phenomenon defined by Cannon's law of denervation[13].

Effect of Nerve Homogenates on Incorporation

The hypothesis that the neurotrophic influence resides in a substance extractable from nervous tissue was tested by assessing the ability of nerve homogenates to stimulate protein synthesis in regenerates denervated for 48 h. Fig. 5 demonstrates that the mean incorporation of leucine in denervated regenerates infused with nerve homogenate is 84·0 per cent that of paired contralateral control regenerates; that in similar non-infused or water-infused regenerates is only 62·0 and 58·2 per cent respectively, a result significant at $P < 0\cdot005$. Thus, on average, infusion of the homogenate abolishes about 60 per cent of the deficit in protein synthesis resulting from complete denervation. Further examination of the homogenate-infused series (Fig. 5) reveals that only three of thirteen regenerates overlap the range of incorporation of either non-infused or water-infused regenerates, while five of the thirteen regenerates synthesize protein at the normal control level. Clarified and non-clarified homogenates both stimulate leucine incorporation similarly.

Heating the nerve homogenate to 90° C for 10 min abolishes its capacity to stimulate protein synthesis, the mean incorporation in infused regenerates being 62·5 per cent of innervated control values, essentially the same as in non-infused or water-infused regenerates.

Partial Nerve Dependence of Protein Synthesis

We conclude that about 40 per cent of protein synthesis in the regenerating amphibian limb is nerve dependent. Protein synthesis, albeit depressed by denervation, does not cease. There are therefore other factors in addition to the neurotrophic one which also affect blastemal protein synthesis; presumably, total protein synthesis in the blastema is the sum of syntheses of many different proteins, each with its own control mechanism. Enzyme synthetic systems are known to be subject to hormonal regulation; and growth of the regenerate is influenced by the hormonal balance[14]. The blastema itself may exert control over the production of specific proteins; for example, after denervation a fibrocellular reaction develops with an increase in synthesis of collagen.

That control of protein synthesis is a function of neurotrophic action is indicated in our experiments by the proportionality between protein synthetic levels and extent of innervation and by the burst of protein synthesis which immediately follows denervation and which precedes the later decline. Autoradiographic findings that maximal incorporation of labelled methionine occurs in those cells of the regenerate in closest proximity to regenerating nerve fibres[15] further supports the specificity of the neural influence. The possibility that inhibition of protein synthesis might be a consequence of surgical trauma is ruled out by the sham operations on the control limb; nor is it the result of the immobility of the limb because significantly reduced protein synthesis is observed in partially denervated but fully motile limbs. Nor can interference with blastemal blood supply with consequent inequality of radioactive leucine pools be implicated, because autonomic nerves controlling blood flow in the newt do not travel with the brachial nerves and are not therefore severed; moreover, radioactive leucine pools do not differ significantly in innervated and denervated regenerates.

Our finding of a basal protein synthetic level not subject to neural control and above this a linear dependence of protein synthesis on the quantity of innervation resembles that of Guth *et al.*[4-6] on the muscle sole plate cholinesterase system of the rat.

The sensitivity of the response of the protein synthetic machinery in the regenerate to denervation is striking. Protein synthesis increases immediately, attains a peak by about 4 h, and thereafter declines, dropping below control levels by 10 h. By comparison, mitosis—also a nerve dependent function in the regenerate—exhibits a burst and reaches a peak within the first day but does not fall below control values for 3 to 5 days[16]. The rapid response of the protein synthetic machinery suggests that it may be a primary site of neurotrophic action on the amphibian limb regeneration system. But whereas protein synthesis is related linearly to the degree of innervation, gross

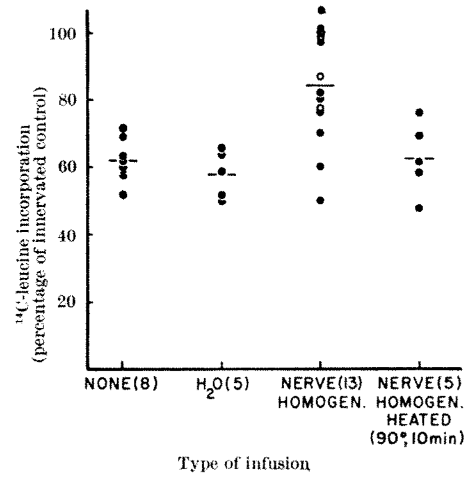


Fig. 5. Effect of infusion of water or nerve homogenates on incorporation of ^{14}C-leucine into protein of 48 h, fully denervated regenerates. Immediately following leucine injection, experimental regenerates were infused for 1 h with 0·005 ml. distilled water, a fresh nerve homogenate (○, unclarified; ●, 40,000*g* for 1 h) or a nerve homogenate heated to 90° C for 10 min; sham operated control regenerates were infused simultaneously or 1 h later with distilled water. Regenerates were removed for incorporation analysis 6 h after leucine administration. For comparison, incorporation in non-infused denervated regenerates is also presented. Cross bars denote the mean; in parentheses are the number of animals in each series.

regeneration is an all or none process requiring the presence of a threshold number of nerve fibres per unit area at the amputation surface. The explanation of this paradox may be that the initiation of gross regeneration requires the presence of certain minimal quantities of crucial proteins per unit area of the amputation surface, the syntheses of which are controlled in a linear manner by the quantity of innervation.

Physical Basis of the Regulatory Activity of Nerve Fibres

A second conclusion drawn from our experiments is that the neurotrophic influence is chemical in nature. This we conclude from the finding that nerve homogenates reverse about 60 per cent of denervation induced inhibition of protein synthesis. Indeed, in 40 per cent of the animals, the homogenates increased protein synthesis to control levels.

The agent of the nerve which stimulates protein synthesis in the regenerate may also reside in non-nervous tissues. Preliminary experiments with liver homogenates indicate the presence of a material, albeit less potent, which stimulates protein synthesis in the regenerate. The idea that the neurotrophic agent is common to other cells has already been suggested from experimental studies which showed that the extent of blastemal dependence on the nerve can be altered and also that limbs which develop in the embryo in the absence of the nerve can regenerate[1,17]. A similar widespread occurrence of an agent of growth has been demonstrated for the nerve growth factor which causes proliferation of sympathetic ganglion cells; it has been extracted from a variety of rat tissues—not merely from the salivary glands, its primary source[18].

Growth stimulating substances are diverse in nature: purified nerve growth promoting substance from rat salivary glands is a protein of medium size[18]; the meiosis stimulating and gamete shedding factor from starfish[8], isolated from neural tissue, and the hydra-polarization factor[9], presumably of nervous origin, seem to be small polypeptides; and the chondrogenic factor of chick embryo spinal cord and notochord contains nucleotide, amino-acid and sugar residues[7]. According to Brachet[19] the chondrogenic factor may be a glycoprotein. The thermolability of our neurotrophic agent rules out amino-acids, nucleotides and molecules such as histamine and acetylcholine, and suggests a polypeptide or protein. A nucleic acid, whose activity would be lost by heating, is another possibility. Indeed, the nerve cell body is a rich source of both protein and RNA: there is RNA in the axon[20], although its site of synthesis is not known; and it is widely accepted that proteins made in the cell body move down the axons to their endings[21-23] where they may be released to carry out metabolic functions.

One can only speculate on the mechanism by which the neural factor stimulates protein synthesis. It may act on the plasma membranes of the blastema cell; more probably, it may enter the cell and there affect the protein synthetic machinery.

During the completion of this manuscript a paper by Dresden[24] appeared in which denervation is shown to depress protein, RNA and DNA synthesis in the advanced regenerate (palette stage) at the time of tissue differentiation.

This work was supported by grants from the Multiple Sclerosis Society, the American Cancer Society and the National Institutes of Health.

Received September 8, 1969.

[1] Singer, M., *Quart. Rev. Biol.*, **27**, 169 (1952).

[2] Singer, M., in *Regeneration in Vertebrates* (edit. by Thornton, C. S.), 59 (University of Chicago Press, 1959).

[3] Sidman, R., and Fawcett, D. W., *Anat. Rec.*, **118**, 487 (1954).

[4] Guth, L., Albers, R. W., and Brown, W. C., *Exp. Neurol.*, **10**, 236 (1964).

[5] Guth, L., and Brown, W. C., *Exp. Neurol.*, **13**, 198 (1965).

[6] Guth, L., Zalewski, A. A., and Brown, W. C., *Exp. Neurol.*, **16**, 136 (1966).

[7] Hommes, F. A., van Leeuwen, G., and Zilliken, F., *Biochim. Biophys. Acta*, **56**, 320 (1962).

[8] Kanatani, H., *Science*, **146**, 1177 (1964).

[9] Lesh, G. E., and Burnett, A. L., *J. Exp. Zool.*, **163**, 55 (1966).

[10] Singer, M., *J. Exp. Zool.*, **101**, 221 (1946).

[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

[12] Singer, M., *Proc. Soc. Exp. Biol. and Med.*, **86**, 378 (1954).

[13] Cannon, W. B., and Rosenbleuth, A., *The Supersensitivity of Denervated Structures: A Law of Denervation* (Macmillan, New York, 1949).

[14] Thornton, C. S., *Advances in Morphogenesis* (edit. by Abercrombie, M., and Brachet, J.), 7, 205 (Academic Press, New York, 1968).

[15] Bodemer, C., and Everett, R., *Devel. Biol.*, **1**, 327 (1959).

[16] Singer, M., and Craven, L., *J. Exp. Zool.*, **108**, 279 (1948).

[17] Singer, M., in *Regeneration in Animals and Related Problems* (edit. by Kiortsis, V., and Trampusch, H. A. L.), 21 (North-Holland, Amsterdam, 1965).

[18] Cohen, S., *Proc. US Nat. Acad. Sci.*, **46**, 302 (1960).

[19] Brachet, J., *Israel J. Med. Sci.*, **2**, 371 (1966).

[20] Singer, M., and Green, M. R., *J. Morphol.*, **124**, 321 (1966).

[21] Taylor, A. C., and Weiss, P., *Proc. US Nat. Acad. Sci.*, **54**, 1521 (1965).

[22] Austin, L., Bray, J. J., and Young, R. J., *J. Neurochem.*, **13**, 1267 (1966).

[23] Schmitt, F. O., *Prog. Bull. Neurosci. Res.*, **5**, No. 4 (Mass. Inst. Tech. Press 1967).

[24] Dresden, M. H., *Devel. Biol.*, **19**, 311 (1969).

Characterization of the Germination Response to Temperature of Species and Ecotypes

by

P. A. THOMPSON

Jodrell Laboratory,
Royal Botanic Gardens, Kew

A new method of measurement gives more reliable data about the response of seeds to temperature in terms of their success in germination. This should help to answer many questions about plant distribution and seed performance.

During the course of evolution particular flowering plants have become adapted to grow in particular climatic conditions. One of the major factors influencing the establishment of a species in any locality is the season at which its seed germinates. This must occur at a time which is statistically favourable for the survival and eventual maturation of the seedlings, if the species is to maintain itself for any length of time. Each geographical area, and habitat, poses its own particular problems to plant survival and the flora of each area consists only of the species suitably adapted to survive these hazards. There have been many attempts to characterize the germination responses of seed to temperature, mostly based on trials in incubators set at particular temperatures. I have used a method which allows measurements to be made of germination responses for continuous ranges of temperature covering any desired span between 0° and 50° C.

Early work on seed germination responses was concerned very largely with the effects of temperature, and with ways of expressing the germination character of species or crop plants in relation to their behaviour at different temperatures. Sachs[1] first proposed the use of three cardinal points to describe the germination character, using the maximum and minimum temperatures at which any germination occurred, and the optimum temperature based on lengths of root or shoot growth within a particular period. His experiments were very short term and consequently masked the difficulties in definition of these cardinal points, which occur when more prolonged incubation periods are considered. Haberlandt[2,3] and deCandolle[4] investigated the effects of more prolonged incubation periods and their experiments provide early examples of the complexity of the germination responses of different species and the difficulties involved in them characterizing so as to permit simple comparisons one with another.

A review by Edwards[5] pointedly illustrates this problem. He cites, in a fairly detailed way, the results of many experiments done in incubators set to provide a range of temperatures. But, although the range covered is often wide, and the records made very detailed, it is practically impossible to coordinate the results to provide a simple basis for comparison of salient features of the germination characters of the species or crop plants concerned. The same criticism is applicable to more recent reviews on the effects of temperature on germination by Stokes[6] and Lang[7]. Neither of these reviews deals in any depth with the overall responses of different seeds to a range of temperatures. This omission can quite reasonably be considered a direct result of the problems inherent in such comparisons, which have stultified attempts to establish the germination character of different species to temperature, unless the criteria used are based on the broadest and most superficial interpretations of data.

Difficulty of Cardinal Points

The concept of three cardinal points seems to be simple and attractive. In practice it is complicated by variations in the approach of different workers (Edwards quotes seven different definitions of the optimum), and limited by dependence on the particular temperatures set up for the tests, and variations in the duration of incubation periods. These variations or inconsistencies of definition and conditions may be comparatively unimportant in artificial situations in which the prime consideration may be the provision of standard conditions at which to conduct routine tests of agricultural or horticultural seeds. In ecological studies, however, a sound method of comparing germination responses is essential before progress can be made in comparing responses of different species in relation to habitat or distribution. Such comparisons are essential for the study of the responses of populations or ecotypes, or for investigations on the adaptability of responses in relation to pressures exerted by climatic changes, or the consequences of introduction into cultivation by man. In these cases comparisons of germination responses depend on the definition of a germination character for each species or ecotype, which is as precise as possible, and which can be used to relate responses in different populations or species. As long as methods for the determination of cardinal points are linked closely to specific temperatures pre-set in incubators, and often almost arbitrarily selected, little progress can be expected in attempts to define germination responses.

Table 1 presents results obtained using a set of incubators in the traditional way to study germination responses of *Ajuga reptans* and *Fragaria vesca*. They are typical of many incubator experiments, and illustrate the difficulties of interpretation which may result from the use of such equipment. First, the range of temperatures provided failed to give any indication of maximum temperatures. Second, in spite of the provision of records made over a long term at weekly intervals optima are not defined and can be stated only in the most arbitrary terms. Third, although there is good reason in both species to suggest that 6° C is sub-minimal, no nearer estimation of the minimum temperatures for germination is possible. It seems to be somewhere near to 11° C for both species, but no closer estimate is possible and one can note only that the proportion of fruits germinating at 11° C is much higher for *F. vesca* than for *A. reptans*.

A more detailed comparison of germination responses is possible using thermo-gradient bars which can be set to provide controlled temperatures over a continuous range. Gradient bars constructed in this laboratory and described in greater detail elsewhere[8,9] have been used to examine temperature responses over the range 0° to 50° C. The bar consists of an aluminium alloy strip (60 cm long × 5 cm wide × 0·6 cm deep) heated at one end by a small ceramic electrical resistance, and cooled at the other end by a semi-conductor cooling module. This results in a continuous gradient of temperature from the hot end to the cold end of each bar, and the range and character of the gradient can be controlled by varying either the voltage supply to the ceramic resistance, or the d.c. current fed to the cooling module. These bars have been set up in units of six sharing common control and recording gear. Temperatures along the bars are monitored automatically using sets of matched thermistors inserted in each bar, and recorded at regular intervals on a chart recorder. Usually the bars are divided into 2·5 cm sections, the successive centre points of which differ from each other by from 1° to 2° C according to the overall temperature range. Fifty seeds sown in each section on pads of moist Whatman No. 3 *MM* filter paper overlying the bar are counted as a unit for recording purposes, so that detailed day to day germination response curves can be obtained for pre-set ranges of temperatures at intervals of 1° to 2° C. The results of an experiment using thermo-gradient bars to examine germination responses in the batches of *A. reptans* and *F. vesca* used previously are recorded in Fig. 1.

Table 1. COMPARISON OF PERCENTAGE GERMINATION OF *Fragaria vesca* AND *Ajuga reptans* AT DIFFERENT TEMPERATURES

Incubation period (weeks)	Temperature (°C) 26	21	16	11	6
(*a*) *Fragaria vesca*					
1	84	83	1	0	0
2	96	98	87	48	0
3	96	98	98	70	0
4	96	98	98	79	0
5	96	98	98	82	0
6	96	98	98	83	0
(*b*) *Ajuga reptans*					
1	61	53	16	3	0
2	73	77	38	11	0
3	74	80	50	13	0
4	75	81	65	15	0
5	75	81	77	16	0
6	75	81	77	17	0

Fruits were collected in early August from two populations growing close together at about 1,500 m.s.m. in the Alps at Serfaus in Austria. Tests were set up in incubators about 6 weeks after collection.

These curves provide a much more detailed and complete characterization of germination response than the incubator results in Table 1. In particular, they provide information about maximum and minimum responses unobtainable from the table and demonstrate that the transitions from temperatures which are favourable for germination and promote maximal levels, to unfavourable temperatures at which few if any fruits germinate, is abrupt and occurs within a range of only a few degrees centigrade. Fig. 1 also exemplifies the dilemma in quoting optimum temperatures, which can be resolved only by resorting to arbitrary criteria based on particular incubation periods or conditions. The graphs in Fig. 1 are too complex to provide a concise description of germination character suitable for simple comparisons between different species. The method adopted in this laboratory to simplify the complex of curves obtained in thermo-gradient bar experiments is to plot, for each successive day, the maximum and minimum temperatures at which a 50 per cent rate of germination is achieved. This resolves the curves into a single curve relating germination performance to both time and temperature. Curves obtained in this way for *F. vesca* and *A. reptans* plotted from Fig. 1 are shown in Fig. 2. These curves compare the germination character of the two species concerned, and reflect reasonably accurately, in view of the simplification involved, conclusions to be drawn from the complex of curves shown in Fig. 1. They also provide a full description of the positions of temperature maxima and minima, and pinpoint the differences in response between the two species. Although they are widely separated taxonomically, these species occupy geographical ranges and habitats which are rather similar, and often occur in association. It may not be surprising therefore that the germination characters of these two populations are, in general, rather similar, differing principally in the position of their minima for germination. Further comparisons, within genera, or between different species in particular habitats, are shown in Figs. 3 and 4. These figures illustrate ways in which the shape of the germination response curve can be modified to provide, for any particular sample of seed, a well defined curve in which particular salient features provide simple standards of comparison with the curves of other species. The major parameters responsible for the form of the curves can be defined as (*a*) the average time of incubation before germination—this may be relatively long as in the two *Primula* species (Fig. 3) or very short, for example, *Agrostemma githago* (Fig. 4); (*b*) the span of temperature within which germination occurs—this may be relatively narrow as in *Primula palinuri* (Fig. 3) and *Cistus creticus* (Fig. 4) or very wide, for example, *S. otites* and *S. nutans* (Fig. 3); (*c*) the position of temperature maxima and minima, for example, *Agrostemma githago* (Fig. 4) germinated to 50 per cent at temperatures down to 2° C, whereas *Primula farinosa* failed to germinate below about 11° C; (*d*) the range of temperature at which germination occurs—this naturally depends on the position of maxima and minima but its value as a character is well illustrated in a comparison of the curves for *P. palinuri* and *P. farinosa* (Fig. 3).

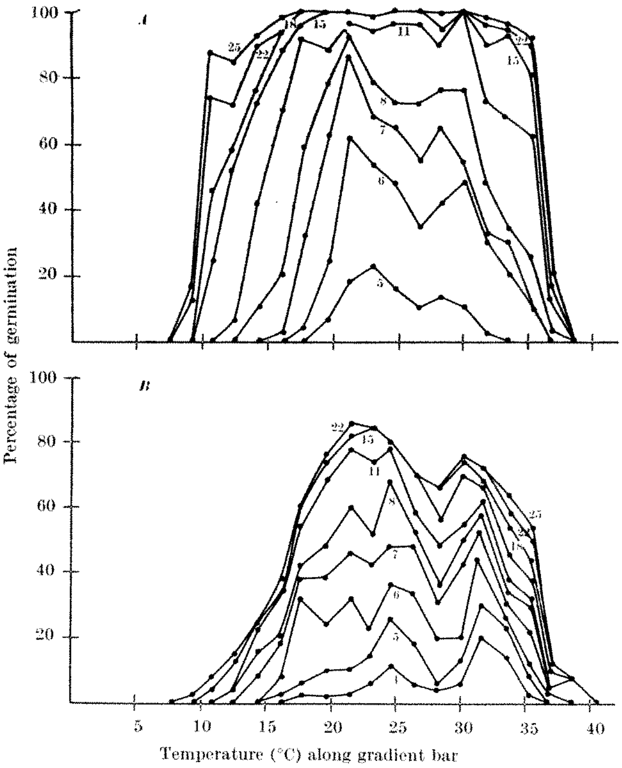


Fig. 1. Percentage germination at different temperatures of two species tested on a thermo-gradient bar. *A*, *Fragaria vesca* Linn. collected at about 1,500 m.s.m. in the Alps at Serfaus in Austria. *B*, *Ajuga reptans* Linn. collected in the same locality and in close proximity to the other species. Numbers on successive graphs indicate days of incubation before counting.

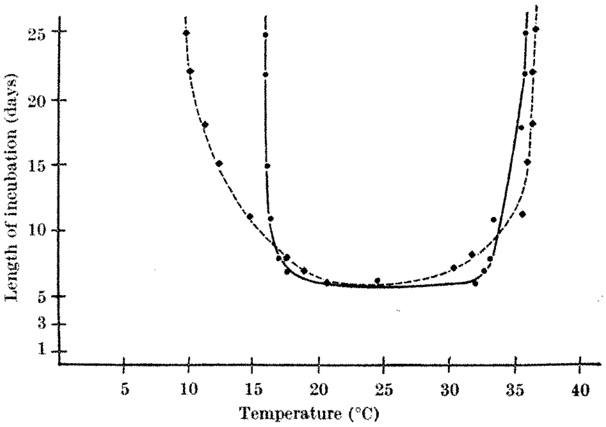


Fig. 2. Germination curves for *F. vesca* (◆- - -◆) and *A. reptans* (●——●), prepared from the graphs in Fig. 1 by plotting for successive days the maximum and minimum temperatures at which 50 per cent of maximum germination was reached.

S. otites and *S. nutans* (Fig. 3), with closely similar habitats and geographical ranges, were found to have markedly similar germination characters. Mediterranean species included in these tests all showed a marked adaptation to rapid germination at low temperatures and relatively low temperature maxima. These characteristics are apparent in a typical Mediterranean species, *Cistus creticus* (Fig. 4), in *P. palinuri* (Fig. 3) which is the only member of its genus occupying a truly Mediterranean habitat, and in a species of Mediterranean origin, *Agrostemma githago*, which has been widely dispersed as a weed of cultivation.

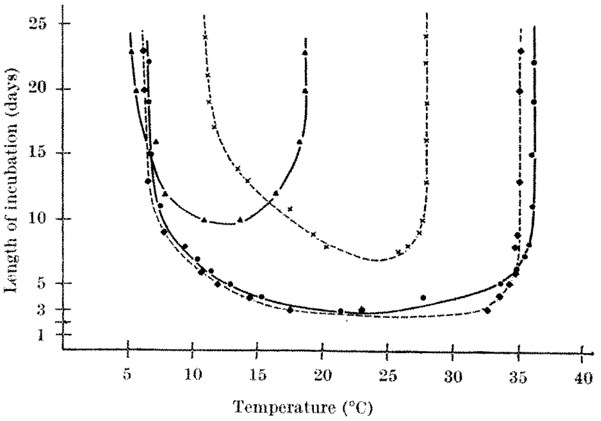


Fig. 3. Comparison of the germination responses of species from two genera; including two species of *Silene* with closely similar ranges and habitats, and two species of *Primula* with separate ranges and distinctively different habitat requirements. *a*, *Silene nutans* Linn. (●——●), collected near Dessau, German Democratic Republic; *b*, *S. otites* (Linn.) Wibel (◆- - -◆), also collected near Dessau; *c*, *Primula palinuri* Petagn (▲——▲), collected in Royal Botanic Gardens, Kew; *d*, *P. farinosa* Linn. (× - - - ×), also collected at Kew.

It is a characteristic of many species that germination of freshly shed seeds is restricted, and that the range of temperatures favouring germination becomes progressively wider as time from seed maturation increases. This phenomenon first noted by Harrington[10] and Munerati[11,12] has often been investigated, and Borriss[13] has proposed the term "relative dormancy" to describe the condition of restricted germination in freshly shed seed. It has been shown that after-ripening usually results in a progressive broadening of the span of temperatures within which germination occurs, accompanied by increased speed of germination and increases in percentage germination rates[14-16]. Seed collected from a stock of *Silene conoidea* (a Mediterranean species) was tested immediately after collection, 3 months and 1 yr later. The results plotted in Fig. 5 describe the nature of changes in response to temperature associated with after-ripening processes, and demonstrate the necessity of taking such changes into account when making comparisons between the germination characters of different species or populations.

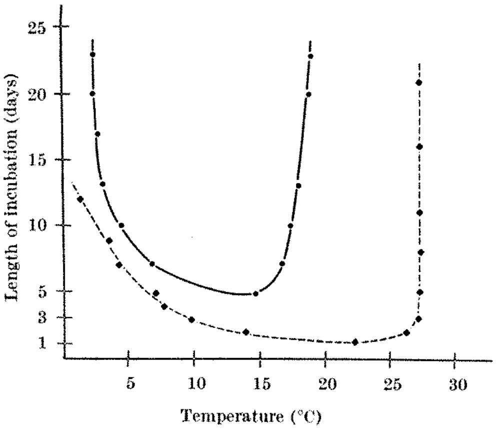


Fig. 4. Germination responses of two species of Eastern Mediterranean origin. *a*, *Cistus creticus* Linn. (●——●), using seed collected from plants growing in the Royal Botanic Gardens, Kew; *b*, *Agrostema githago* Linn. (◆– – –◆), using seed from a population growing as a weed species near Strykowo in Poland.

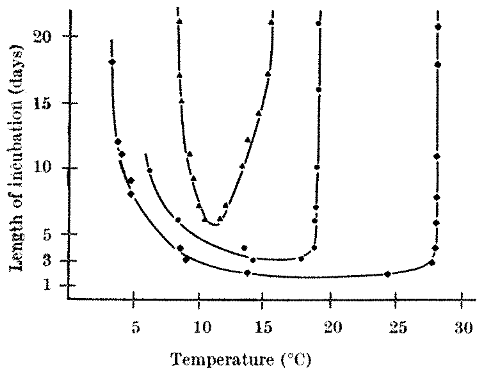


Fig. 5. Changes in germination responses of *Silene conoidea* Linn., occurring as a result of after-ripening in dry storage at laboratory temperatures. Seed collected from plants growing in the Royal Botanic Gardens, Kew. Tests were made (*a*) immediately (▲——▲); (*b*) 3 months (●——●); (*c*) 1 yr (◆——◆) after seed harvest.

Germination and Geography

Several of the early reports on seed germination[3,4,17,18] comment on the ability of seeds of some crop plants to germinate on blocks of ice or in ice stores at temperatures close to 0° C. Other reports[19,20] provide examples of seeds with rather high minimum requirements for germination sometimes in the neighbourhood of 20° C. Coffman[17] reported a wide variation in minimum germination temperatures for different crop plants, and it has been suggested[21] that the speed of germination at low temperatures, and the position of temperature minima for germination, may be correlated with the geographical distribution of different species of Caryophyllaceae. Germination tests on thermo-gradient bars were done with nine species of Caryophyllaceae using seed collected from wild plants in various parts of Europe. In Fig. 6 the results are plotted against a logarithmic time scale instead of the linear scale used before. This scale was adopted to separate more effectively relatively small differences occurring in incubation times before germination at median temperatures. Wide variations in the position of temperature minima for germination were found ranging from the three species with distributions in north-western and continental Europe, which failed to attain 50 per cent germination at temperatures below 12° to 15° C, to *Agrostemma githago* which germinated at a temperature of 0° C in less than 20 days; some of the seeds of the latter species were encased in ice by the time they germinated. The results confirmed previous observations relating germination behaviour at low temperatures to geographical distribution, showing that species with a more southern distribution germinated more rapidly and at lower temperatures than northern species. This result can be compared with Cooper's results on the effects of temperature on the induction of dormancy in various races of *Lolium perenne*[22]. The form of the curves was linear for a part of the temperature range of each species, but became markedly exponential as minima were approached. This exponential character would invalidate attempts, such as have been made[23], to determine minimum germination temperatures by extrapolation of results obtained at higher temperatures.

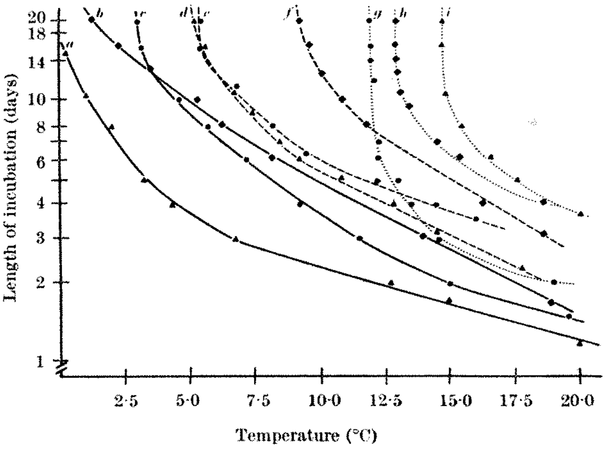


Fig. 6. Comparison, in the lower part of their ranges, of the temperature responses of species of Caryophyllaceae with different natural distributions in Europe. *a*, *Agrostemma githago* Linn., collected near Eisleben in the German Democratic Republic (▲——▲); *b*, *Silene coeli-rosea* (Linn.) Godron, collected from plants growing in the Royal Botanic Gardens, Kew (◆——◆); *c*, *S. secundiflora* Otth., collected from plants at Kew, raised from seed obtained near Almeria in south eastern Spain (●——●); *d*, *S. viscosa* (Linn.) Pers., collected near Budapest, Hungary (▲– – –▲); *e*, *S. nutans* Linn., collected near Feldkirch, Austria (●– – –●); *f*, *Lychnis viscaria* Linn., collected near Dessau in central Germany (◆– – –◆); *g*, *S. vulgaris* (Moechn) Garcke, collected near Bad Schwalbach in central Germany (● · · · · ●); *h*, *Lychnis flos-cuculi* Linn., collected in Øg, Sweden (◆ · · · · ◆); *i*, *S. alba* (Miller) E. H. L. Krause, collected near Sjklarska, Poland (▲ · · · · ▲).

Several studies have been published of attempts to interrelate the germination responses of populations of a particular species collected in different parts of its range. Haasis and Thrupp[24] and Stearns and Olson[25] working with coniferous species, and McNaughton[26] with *Typha* species all reported variations in germination of different ecotypes. Lauer[27], on the other hand, failed to distinguish notable differences between populations of *Agrostemma githago* and *Datura stramonium* collected in various locations in Europe. Results are shown here of experiments done on gradient bars with seed from three populations each of *S. otites* (Fig. 7) and *Lychnis flos-cuculi* (Fig. 8). Geographically the ranges of these two European species are predominantly quite similar, though *S. otites* has a significantly more continental and southern distribution than *L. flos-cuculi.* Habitat conditions, however, are quite different, for *S. otites* is a plant of dry sandy soils, requiring good drainage and relatively dry conditions in winter for survival in competitive conditions, whereas *L. flos-cuculi* is a plant of moist meadowland or even marshland. A comparison of Figs. 7 and 8 suggests that the principal differences in germination character between the two species derive from the much lower minima found for populations of *S. otites* and the more rapid overall germination of this species. The maxima for germination are broadly similar in both species with the exception of the population of *L. flos-cuculi* from Brno. Comparisons within and between Figs. 7 and 8 provide a broad indication of the level of similarities and differences between populations of the two species, and suggest that each has a dominant form of pattern, which may be modified in particular populations, but not to the extent of obscuring fundamental differences between the germination characters of the two species. The relationship between plant distribution and variation between populations of species of Caryophyllaceae is discussed in more detail elsewhere[8], and will not be elaborated here.

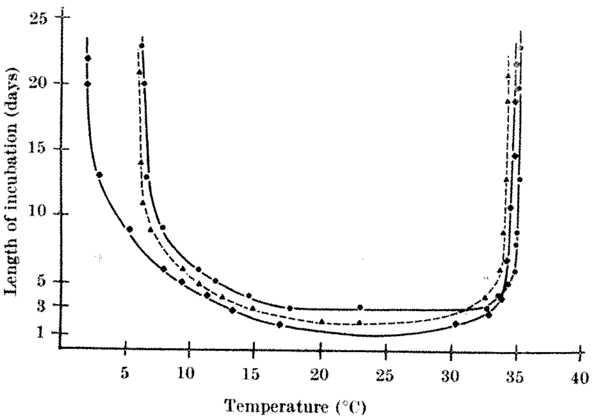


Fig. 7. Comparisons of the germination character of three populations of *S. otites* (Linn.) Wibel, collected in different parts of Europe. *a*, From Dessau, central Germany (●——●); *b*, from Budapest, Hungary (▲– – –▲); *c*, from Bouches du Rhone, France (◆——◆).

Went and Juhren[28,29,30] and Koller and Negbi[31] have presented results linking germination responses to temperature to particular ecological situations or seasons of germination, and on an *a priori* basis it is obvious that unless temperature responses are closely linked to the ecological requirements of a particular habitat a species has no prospect of establishing a persistent population. Harper[32] has already pointed out the vital and decisive importance of the season of germination as a factor dominating the prospects of the establishment of a species in an alien environment. But the germination character of a species would act as an impediment to the extension of its range only if it is a relatively fixed character, and therefore unable to adapt rapidly to the physical stimuli presented by a new or changing environment. There is a general impression, backed by little experimental evidence, that seed germination is highly adaptive as a character, and if this were so it would not be expected to act stringently as a curb to the extension of a plant's range of distribution. The question whether germination is a plastic, highly adaptive character, on the one hand, or a relatively fixed attribute characteristic of any particular species on the other, is still open, but vital to any understanding of the factors affecting plant distribution, and the extension or recession in range of particular species. Its solution depends first on a satisfactory method for defining the germination character of different species or ecotypes. It is believed that the method proposed here, and illustrated in Figs. 2 to 8, goes some way to solve problems inherent in making comparisons between different species or batches of seed tested in a wide range of different temperatures, in a way which allows a critical study of similarities and differences in germination character.

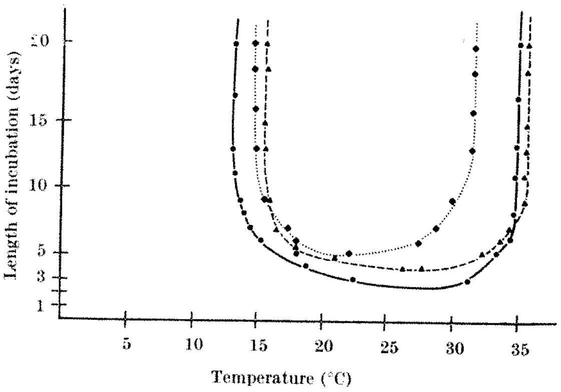


Fig. 8. Comparisons of the germination character of three populations of *L. flos-cuculi* Linn. collected in different parts of Europe. *a*, From Øg, Sweden (●——●); *b*, from near Brno, Czechoslovakia (◆ · · · · ◆); *c*, from Côte d'Or, France (▲– – –▲).

I thank Mr N. McAdam, Mrs D. Lower and Mrs P. Freeman for help with these experiments, and the directors and seed collectors of the following institutes for seed used in this work: Botanic Garden, University of Uppsala; Botanic Garden, Hungarian University, Budapest; Botanic Garden, University of Halle; Botanic Garden, Dijon University; Agricultural and Silvicultural University, Brno; Botanic Garden, University of Vienna; Botanic Garden, University of Liverpool.

Received October 22; revised December 15, 1969.

[1] Sachs, J., *Jb. Wiss. Bot.*, **2**, 338 (1860).

[2] Haberlandt, F., *Landwn. VersStnen.*, **17**, 104 (1874).

[3] Haberlandt, F., in *Wissenschaftlich-praktische, Untersuchungen auf dem Gebiete des Pflanzengause*, Vienna, **1**, 117 (1875).

[4] deCandolle, A. L. P. P., *Arch. Sci. Phys. Nat.*, **24**, 243 (1865).

[5] Edwards, T. I., *Quant. Rev. Biol.*, **7**, 428 (1932).

[6] Stokes, P., *Encyclopedia of Plant Physiology* (edit. by Ruhland, W.), **15** (2), 746 (Springer-Verlag, Berlin, 1965).

[7] Lang, A., *Encyclopaedia of Plant Physiology* (edit. by Ruhland, W.), **15** (2), 848 (Springer-Verlag, Berlin, 1965).

[8] Thompson, P. A., *Ann. Bot.* (in the press).

[9] Thompson, P. A., *Proc. Intern. Seed Test. Assoc.* (in the press).

[10] Harrington, G. T., *J. Agric. Res.*, **23**, 79 (1923).

[11] Munerati, O., *CR Acad. Sci.*, **181**, 1081 (1925).

[12] Munerati, O., *CR Acad. Sci.*, **182**, 535 (1926).

[13] Borriss, H., *Jb. Wiss. Bot.*, **89**, 255 (1940).

[14] Kearns, V. K., and Toole, E. H., *US Dept. Agric. Tech. Bull.*, No. 638 (1939).

[15] Toole, E. H., and Toole, V. K., *Proc. Intern. Seed Test. Assoc.*, **11**, 51 (1939).

[16] Hills, K. L., *Austral. CSIR J.*, **17**, 186 (1944).

[17] Coffman, F. A., *J. Amer. Soc. Agron.*, **15**, 257 (1923).

[18] Uloth, W., *Flora*, **29**, 185 (1875).

[19] Joseph, H. C., *Bot. Gaz.*, **87**, 127 (1929).

[20] Thompson, P. A., *J. Hort. Sci.*, 44 (1970).

[21] Thompson, P. A., *Nature*, **217**, 1156 (1968).

[22] Cooper, J. P., in *Climatic Response in Environmental Control of Plant Growth* (edit. by Evans, L. T.), 381 (Academic Press, New York and London, 1963).

[23] Went, F. W., in *The Experimental Control of Plant Growth* (Chronica Botanica, Waltham, Massachusetts, 1957).

[24] Haasis, F. W., and Thrupp, A. C., *Ecology*, **12**, 728 (1931).

[25] Stearns, F., and Olson, J., *Amer. J. Bot.*, **45**, 53 (1958).

[26] McNaughton, S. J., *Ecol. Monog.*, **36**, 297 (1966).

[27] Lauer, E., *Flora*, **140**, 551 (1953).

[28] Went, F. W., *Ecology*, **30**, 1 (1949).

[29] Went, F. W., Juhren, G., and Juhren, M. C., *Ecology*, **33**, 351 (1952).

[30] Went, F. W., and Westergaard, M., *Ecology*, **30**, 26 (1949).

[31] Koller, D., and Negbi, M., *Ecology*, **40**, 20 (1959).

[32] Harper, J. L., in *The Genetics of Colonising Species* (edit. by Baker, H. G., and Stebbins, G. L.) (Academic Press, London and New York, 1965).

LETTERS TO THE EDITOR

PHYSICAL SCIENCES

PK 0048–09: a Possible Radio Variable Galaxy

OBSERVATIONS of sixty Parkes Catalogue radio galaxies north of –20° declination have been made at a frequency of 8,000 MHz. Widely differing values of flux density have been measured on different dates for one of these galaxies, PK 0048–09, and it is believed to be varying.

The observations were made between November 1968 and November 1969 with the University of Michigan 85-foot radio telescope using a wide-band (1,000 MHz) tunnel diode radiometer[1] and a dual feed-horn system[2]. The technique was to scan the source in both right ascension and declination. All scans were one degree long; they were started 0·5° off source and continued for two minutes at a rate of 0·5° per minute. Data were integrated with a time constant of two seconds so that each scan consisted of a series of sixty intensity measurements. The low signal-to-noise ratio for PK 0048–09 required several scans to obtain a flux density measurement. Between thirty and fifty two-minute scans were made each time it was observed. The observing procedure, data reduction and calibration will be described in detail in a later article.

Fig. 1 shows the measured 8,000 MHz flux density of PK 0048–09 as a function of time along with the fluxes of three other galaxies in the programme (PK 1345+12, 3C 264, PK 0124+18) which showed no significant changes. Between November 1968 and February 1969 a decline of 17 per cent in the flux density of PK 0048–09 was noted. This was followed by an increase of 83 per cent between February and July. The flux then remained roughly constant until October, when it seems to have declined by almost 20 per cent in a period of about ten days. Since then no significant change has occurred.

The largest of these changes, the 83 per cent rise between February and July, was more than ten times greater than the mean errors (4–6 per cent) associated with the measurements. Many of the mean errors of the measurements of the three comparison sources in Fig. 1 are larger than those on the measurements of PK 0048–09. This is because more scans were taken per measurement on PK 0048–09 in an effort to reduce the uncertainty.

All three measurements of the flux density of the radio galaxy PK 0124+18 were made within three hours of measurements of the flux density of PK 0048–09. If instrumental effects were the cause of the changes in PK 0048–09, one would expect PK 0124+18 to have been similarly affected. Obviously this did not occur. The antenna gain was carefully monitored by observing a standard source immediately before and after observations of a source in the programme, and no change larger than a few per cent was recorded.

Fig. 2 shows the flux densities of five more galaxies in the programme (3C 227, 3C 444, 3C 136·1, 3C 71, PK 1313+07) during the time interval in question. Again, no significant changes are seen. The eight galaxies in Figs. 1 and 2 bracket the range of flux densities over which PK 0048–09 has been observed to vary and are located at declinations both north and south of that source. Variations between measurements made on different dates for all sixty galaxies in the programme are usually smaller than the mean errors of those measurements. It is therefore concluded that the latter are reliable indicators of observational uncertainty and that the changes recorded in the flux density of PK 0048–09 are probably intrinsic to the source.

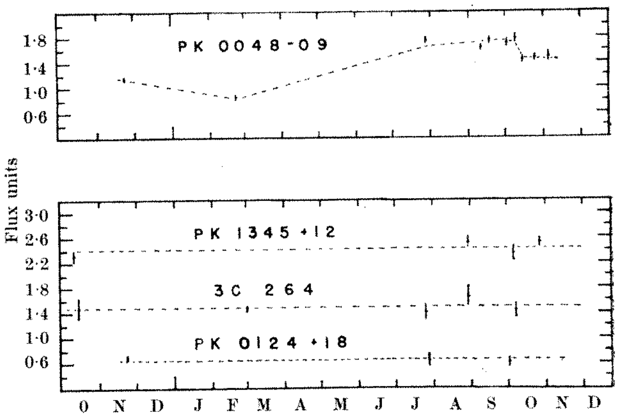


Fig. 1. Time variations in the flux density of PK 0048–09 and lack of similar behaviour in other galaxies. 1 flux unit = 10^{-26} W m^{-2} Hz^{-1}.

The radio spectrum of PK 0048–09 is shown in Fig. 3, where the range of variability recorded at 8,000 MHz is denoted by arrows. The points at 408 MHz, 1,410 MHz and 2,650 MHz have been taken from the Parkes Catalogue[3]. The point at 2695 MHz was measured by Kellermann, Pauliny-Toth and Tyler[4], while that at 5,000 MHz was recorded by Pauliny-Toth and Kellermann[5]. An attempt has been made to adjust all measurements to the same absolute calibration scale. The discrepancy between the flux densities at 2,650 MHz and 2,695 MHz may indicate that the source is also variable at those frequencies; differences of this size between the measurements of different observers are not uncommon, however.

The remarkable feature of the radio spectrum is the apparent lack of a low-frequency component. According to current ideas on the evolution of radio sources, this indicates that the radio source in PK 0048–09 is of recent origin and is considerably younger than those in most radio galaxies. It may be relevant that the extremely rapid radio variable VRO 42.22.01 (BL Lacertae)[6] has a radio spectrum similar to that of PK 0048–09 (ref. 7).

PK 0048–09 was identified as an eighteenth magnitude galaxy of indeterminate type in a small cluster of galaxies by Bolton and Ekers[8]. According to them it is much stronger on blue-sensitive plates than on red-sensitive plates. No optical spectrum has been reported.

Two other radio galaxies, 3C 84 (NGC 1275) and 3C 120, are definitely known to vary and both are classified as Seyfert galaxies. The variations in 3C 120 are not unlike those reported here for PK 0048–09. A decrease of 60 per cent in its 10,630 MHz flux between November

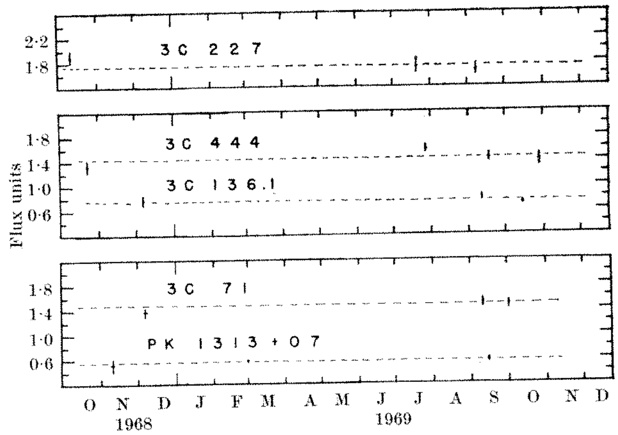


Fig. 2. Lack of significant time variations in the flux densities of five additional galaxies in the programme.

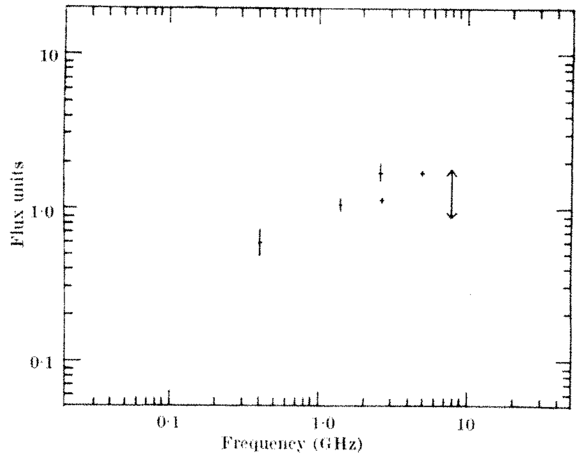


Fig. 3. The radio spectrum of PK 0048–09.

1966 and May 1967 was followed by an increase of 125 per cent between July and October 1967 (ref. 9). A similar outburst was observed at 8,000 MHz at the end of 1967 (ref. 10) and another occurred in the summer of 1968. On the other hand, 3C 84 has behaved more quietly. It showed a linear increase in flux density at 8,000 MHz of 10 per cent per year between August 1961 and January 1966 (ref. 11) and similarly at 10,630 MHz it increased by 7 per cent between July 1966 and April 1967 (ref. 9). McCullough and Waak[12] have reported a linear increase of 19 per cent at 19,300 MHz from 1966 to 1968 with the possibility that several small bursts have been superimposed on this rise not excluded. Most recently, however, an outburst at 90,000 MHz has increased the flux density of 3C 84 by 80 per cent between October and December 1969 (ref. 13). Both galaxies are also optical variables.

I thank Professor F. T. Haddock and Dr H. D. Aller for advice and criticism, and G. E. Latimer, Dr T. V. Seling and K. Holmes for designing and maintaining equipment. This work was supported in part by the US National Science Foundation and I was aided by a NASA traineeship.

Note added in proof. A new outburst has increased the 8,000 MHz flux density of PK 0048–09 from 1·41 ± 0·05 flux units on November 24, 1969, to 2·35 ± 0·08 flux units on January 31, 1970.

MARK A. STULL

Radio Astronomy Observatory,
University of Michigan.

Received January 29, 1970.

[1] Seling, T. V., *Proc. IEEE*, **52**, 423 (1964).
[2] Klein, M. J., and Seling, T. V., *Astrophys. J.*, **146**, 599 (1966).
[3] Ekers, J. A., *Austral. J. Phys., Astrophys. Suppl. No. 7* (1969).
[4] Kellermann, K. I., Pauliny-Toth, I. I. K., and Tyler, W. C., *Astron. J.*, **73**, 298 (1968).
[5] Pauliny-Toth, I. I. K., and Kellermann, K. I., *Astron. J.*, **73**, 953 (1968).
[6] Andrew, B. H., MacLeod, J. M., Locke, J. L., Medd., W. J., and Purton, C. R., *Nature*, **223**, 598 (1969).
[7] MacLeod, J. M., and Andrew, B. H., *Astrophys. Lett.*, **1**, 243 (1968).
[8] Bolton, J. G., and Ekers, J. A., *Austral. J. Phys.*, **20**, 109 (1967).
[9] Medd, W. J., Locke, J. L., Andrew, B. H., and van den Bergh, S., *Astron. J.*, **73**, 293 (1968).
[10] Aller, H. D., thesis, Univ. Michigan (1968).
[11] Dent, W. A., *Astrophys. J.*, **144**, 843 (1966).
[12] McCullough, T. P., and Waak, J. A., *Astrophys. J.*, **158**, 849 (1969).
[13] Epstein, E. E., Dworetsky, M. M., and Montgomery, J. W., *IAU Circ. No. 2195* (1969).

An Explanation for the Broad Supernova Bands

SPECTRA of type I supernovae show broad emission bands, none of which has been satisfactorily explained[1]. Seddon[2] has proposed that the structure in the visible spectrum be regarded as broad absorption bands on a more intense continuum. The absorption bands could be produced by clouds of dust surrounding the star. On this viewpoint several supernovae absorption features fall near some of the much narrower unidentified absorption bands arising in the interstellar medium. This article reports an application of this new interstellar band explanation to supernovae spectra interpreted in terms of absorption features. The more intense background used to obtain the supernovae absorption will be discussed after a brief presentation of the proposed absorption band origin.

My explanation for the most intense interstellar bands is that they are due to iron ions in crystals such as garnet silicates (unpublished). Because of the strong electric fields of neighbouring O^{2-} ions in the crystal, the absorption bands due to iron ions are very different from the absorption bands of free iron ions. At low crystal temperatures, bands with widths of about 30 Å can occur, while at room temperature and above, band widths are typically several hundred Å. Garnets are a type of silicate of variable composition indicated by

$$\overset{(8)}{P_3}\overset{(6)}{Q_2}(\overset{(4)}{Si}O_4)_3$$

where P may be Ca^{2+}, Mn^{2+}, Fe^{2+}, Mg^{2+}, and other ions, Q may be Fe^{3+}, Al^{3+}, Cr^{3+}, and other ions, and Si may be replaced by Fe^{3+}. The numbers in parentheses indicate that P is surrounded by eight negative ions, Q by six ions (octahedrally) and Si by four ions (tetrahedrally). Iron ions can occupy all three different positive ion sites giving rise to several different crystal field bands in each site. Energies of these transitions do not vary greatly from one composition of garnet to another because negative ion configurations are the same and distances are comparable. Fig. 1*b* shows the bands due to iron ions in garnets taken from room temperature optical absorption measurements. The solid curves are all taken from data on laboratory grown yttrium iron garnet (YIG) crystals[3], chemical composition $Y_3Fe_2(FeO_4)_3$, whose optical properties have been more extensively studied than any naturally occurring garnets. In YIG, Fe^{3+} ions occupy the octahedral Q sites (6) and the tetrahedral Si sites (4). To complete the sketch of all iron bands in garnets, bands due to Fe^{2+} in the P sites are shown in the dotted curves as taken from the natural almandine garnet spectrum in Fig. 1*c* (ref. 4). It is worth noting that iron impurities occupying the octahedral sites (6) give rise to bands in Fig. 1*c* near the same wavelengths as in YIG. The bands near 4400 Å and 6100 Å are absent in the mineral, however, because iron rarely replaces silicon.

In all garnet crystals there is a rising absorption in the ultraviolet, with shape and position which depend on the exact chemical composition. The background has been subtracted to display the bands of Fig. 1*b*, but such an absorption edge is seen in Fig. 1*c*. If the absorption bands in supernovae spectra are due to surrounding clouds of such dust, the ultraviolet intensity transmitted through the dust should be very low. A suggestion of this effect may be seen in the decrease of the short wavelength intensities as the absorption bands of the supernova increase with time[5]. In the absence of knowledge about the original light spectrum incident on the dust, I have assumed that it follows an infinite temperature Planck distribution. The supernova spectrum has been subtracted from this assumed continuum and plotted in Fig. 1*a*. Data from the supernova in NGC 4496 studied by Bloch, Chalonge and Dufay[5] have been used because of the wide spectral range covered and because the authors have attempted to correct for instrumental errors to present true intensities. It may be noted, however, that the averaged spectrum used by Seddon[2], and the spectra of other type I supernovae[6,7], show similar bands. Only one spectrum has been used rather than an average of all spectra in order to display the largest possible wavelength range. The break in the curves near 6000 Å indicates the addition of longer wavelength data from the

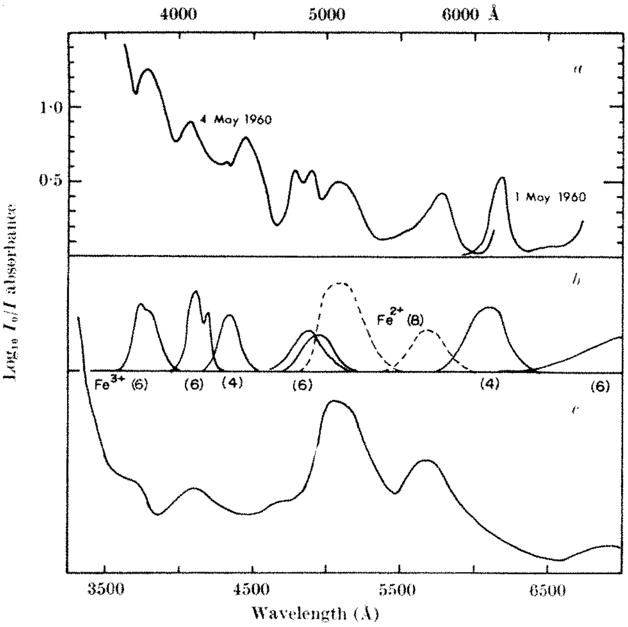


Fig. 1. *a*, Data from the supernova in NGC 4496. *b*, Bands due to iron ions in garnets taken from optical absorption measurements. *c*, Spectrum of almandine garnet $Fe_3Al_2(SiO_4)_3$.

spectrum of May 1 to the spectrum of May 4 in order to further extend the wavelength range. Due to the use of only one spectrum, the band positions are uncertain to perhaps 50 Å and are somewhat uncertain in shape.

Comparison of Fig. 1*a* and Fig. 1*b* shows that seven bands due to iron ions in garnet crystals correlate in wavelength and approximate width within the supposed uncertainty. These bands are centred at wavelengths of about 6100, 5700, 5100, 4900, 4400, 4100 and 3800 Å. The band at 4900 Å in the supernova spectrum even seems to show a two-fold splitting corresponding to the laboratory spectrum, and the increase of the long wavelength limit of the supernova curve corresponds to the edge of the 7000 Å garnet band. One also notices the rising background in the ultraviolet produced by the form of the continuum used. The similarity of this absorption to that in garnets may offer some vindication for use of the high temperature continuum background. In view of the fact that only the bands near 5100 and 5700 Å are due to Fe^{2+} (8), there may be significance in the fact that these two bands seem to grow faster in the later stages of the supernova in NGC 4496 (ref. 5). The two bands also appear comparatively weaker in spectra from supernovae in NGC 2713 (ref. 6) and NGC 3389 (ref. 7). A very rough estimate of the amount of garnet dust necessary to produce the observed bands can be obtained by using the absorption coefficients of almandine. The estimated quantity of dust in a unit cross-section in the line of sight, if compacted into a solid mass, would be equivalent to a thickness of 0·01 to 0·1 cm.

If the present interpretation is correct, several important facts concerning supernovae are implied: (1) The widths of the features in the supernovae spectra are chiefly due to inherent broadness of the optical absorption bands in the solids. The large widths should not then be associated with large velocities. (2) The measured blue and ultraviolet intensities which reach us may bear little resemblance to the initial intensities. (3) The supernova dust cloud which gives rise to the broad absorption bands, when cooled down to the low temperature of interstellar dust, could give rise to a number of the much narrower bands observed to arise in the interstellar medium.

DONALD R. HUFFMAN

Department of Physics,
University of Arizona,
Tucson, Arizona.

Received January 8, 1970.

[1] Zwicky, F., *Stellar Structure* (edit. by Aller, L. H., and McLaughlin, D. B.), 367 (Univ. Chicago Press, 1965).
[2] Seddon, H., *Nature*, **222**, 757 (1969).
[3] Wood, D. L., and Remeika, J. P., *J. Appl. Phys.*, **38**, 1038 (1967).
[4] Clark, S. P., *Amer. Mineralogist*, **42**, 732 (1957).
[5] Bloch, M., Chalonge, D., and Dufay, J., *Ann. d'Astrophys.*, **24**, 315 (1964).
[6] Ford, W. K., and Rubin, V. C., *Pub. Astron. Soc. Pacific*, **80**, 466 (1968).
[7] Rubin, V. C., and Ford, W. K., *Pub. Astron. Soc. Pacific*, **79**, 322 (1967).

Scattering of X-rays from Pulsar NP 0532 by Interstellar Grains

SLYSH[1] recently considered the scattering of soft X-rays from the Crab Nebula, and suggested that although all the observed X-rays from the Crab Nebula could have originated as pulsed X-rays from the pulsar NP 0532 at the centre, only ~10 per cent unscattered by interstellar grains are recorded as pulsed X-rays in the 1–10 keV region[2–4]. The remaining 90 per cent are scattered, appearing as a diffuse source about 2′ wide[5,6] in which the pulses are smeared out. In this report we show that the optical depth for scattering by interstellar grains is small in the 1–10 keV region, and that the pulsed component is reduced by ~20 per cent at the most. Consequently, the observed spectrum of the pulsed component in the 1–100 keV region[2–4,7,8] should closely correspond to the spectrum of the pulsar at the source, which would not have been the case from the analysis of Slysh. The optical depth for scattering is quite insensitive to the exact model of interstellar grains.

We have assumed that interstellar grains are single smooth spheres, that single scattering holds, and that the incident and scattered radiations have the same wavelength. The optical depth due to extinction (scattering and absorption) by the grains along the line of sight is defined by

$$\tau(\lambda) = n_g R \pi a^2 Q_{\mathrm{ext}}(\rho) \tag{1}$$

where n_g and a are the number density and radius of the grains, R the distance of the object and Q_{ext} is the extinction efficiency (suffix x is used later to indicate X-ray quantities). ρ is an important physical variable defined by

$$\rho = 2x(m-1) \tag{2}$$

where

$$x = 2\pi a/\lambda \tag{3}$$

and $m = m' - m''$ is the complex refractive index of the grain. Re{ρ} represents the additional phase difference introduced by the grain for the wave passing across its diameter. For X-rays, m is typically close to but less than 1.

$$m_{\mathrm{x}} - 1 = -\frac{N_e r_e \lambda_{\mathrm{x}}^2}{2\pi} - i\frac{\mu\lambda_{\mathrm{x}}}{4\pi} \tag{4}$$

where r_e is the classical electron radius ($2{\cdot}818 \times 10^{-13}$ cm), μ is the linear attenuation coefficient of the grain material, λ_{x} is the wavelength of the X-radiation, and N_e is the number density of both bound and free electrons in the grain

$$N_e = N_0 \left\langle s\,\frac{Z}{M} \right\rangle \tag{5}$$

Here s is the density of the grain material (g cm^{-3}), N_0 is Avogadro's number, M is the gram molecular weight and Z is the number of electrons per molecule. The factor $\left\langle s\,\frac{Z}{M} \right\rangle$ should be taken as a weighted mean over the molecular species constituting the grain. For typical values of $a \approx 0{\cdot}1$–1 μm, $s \approx 1$, and $\lambda_{\mathrm{x}} \approx 1$–$10$ Å, we find

$$0 > m_{\mathrm{x}} - 1 \gg -1,\quad x \gg 1,\quad 0{\cdot}02 < \rho_{\mathrm{x}} < 2$$

Because x can be as large as 10^4 it is not practicable to use the rigorous Mie theory[9] of scattering by spheres. We may, however, take advantage of the fact that

$m_x \approx 1$ and m''/m' is very small ($\sim 3 \times 10^{-2}$–3×10^{-3}), that is, absorption is negligible. Taking only the real part of ρ_x

$$\rho_x = 0{\cdot}34 a \lambda_x \left\langle s \frac{Z}{M} \right\rangle \tag{6}$$

Depending on the values of m_x, x and ρ_x, appropriate approximations can be used to calculate Q_{ext} (ref. 9). With $0 > m_x - 1 \gg -1$ and $x \gg 1$, Rayleigh–Gans scattering can be used for $\rho < 1$ and the anomalous diffraction approximation for $\rho \gtrsim 1$. In the latter case

$$Q_x = 2 - \frac{4}{\rho_x} \sin \rho_x + \frac{4}{\rho_x^2}(1 - \cos \rho_x) \tag{7}$$

For very soft X-rays $\rho_x \gg 1$ and Q_x approaches 2·0, the asymptotic limit. For hard X-rays $\rho_x < 1$ and

$$Q_x \approx \frac{1}{2} |\rho_x|^2 - \frac{8x}{3} Im(m-1)$$

Dielectric grains (dirty ice) of radius $a = 0{\cdot}25$ µm, density $s = 1{\cdot}05$ and $m = 1{\cdot}33 - i\ 0{\cdot}05$ give a good fit to the observed optical and near-infrared extinction in the region 0·33 to 2·13 µm, as shown in Fig. 1, based on Mie theory calculations. In the visual region $\lambda = 0{\cdot}54$ µm, $\rho = 1{\cdot}92$ and $Q_{ext} = 1{\cdot}78$. From equation (7) it can be seen that the same extinction occurs for $\rho_x = 2{\cdot}15$. Then, putting $\left\langle s \frac{Z}{M} \right\rangle = 0{\cdot}55$ for dielectric grains we obtain $\lambda_x = 43{\cdot}5$ Å. Similarly in the infrared, $\lambda = 1$ µm, $\rho = 1{\cdot}04$ and $Q_{ext} = 0{\cdot}58$. The same extinction occurs in the X-ray region for $\lambda_x = 22{\cdot}5$ Å. It is thus seen that the optical depth in the visual and infrared is also reflected in the X-ray region as a consequence of the refractive index as defined in equation (4). It may be noted that whereas in the optical region $\rho \propto a/\lambda$, in the X-ray region $\rho_x \propto a \lambda_x$. It is not obvious from this, however, if similar wavelength dependence would occur for Q_{ext} and Q_x.

In the direction of the Crab Nebula, the extinction in the visual is 1·08 magnitudes kpc^{-1} (ref. 12). This corresponds to an optical depth of 1·0 kpc^{-1}. Taking a distance of $R = 2$ kpc for the Crab Nebula[13], we obtain $\tau(0{\cdot}54\ \mu m) = 2{\cdot}0$. In Fig. 2, we have shown the optical depth for scattering of X-rays from the Crab Nebula as a function of wavelength (curve 1) by setting $\tau(43{\cdot}5\ \text{Å}) = 2{\cdot}0$. It should be noted that it is not necessary to know the values of $N_g = n_g R$ to calculate τ_x. But substituting for τ and Q_{ext} at 0·54 µm in equation (1) we deduce the column density of grains as $N_g = 5{\cdot}7 \times 10^8$ grains cm^{-2}. For $R = 2$ kpc, $n_g = 9{\cdot}3 \times 10^{-14}$ grains cm^{-3}. Calculations show that the extinction in the visual region for pure ice ($m'' = 0$) differs from that of dirty ice only by ~ 1 per cent.

Ice grains, pure or dirty, do not adequately account for the observed extinction in the ultraviolet and infrared.

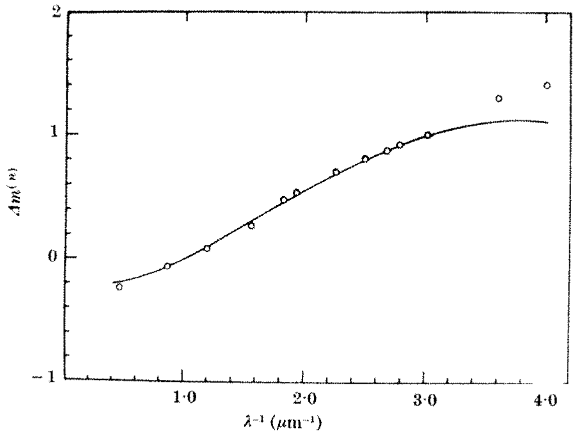


Fig. 1. Theoretical normalized extinction curve in the visual and near-infrared, for dielectric spheres of radius $a = 0{\cdot}25$ µm and refractive index $m = 1{\cdot}33 - i\ 0{\cdot}05$. The circles are experimental observations of Boggess and Borgman[10] and Stecher[11]. $\Delta m^{(n)} = \dfrac{\Delta m(\lambda^{-1}) - \Delta m(1)}{\Delta m(3) - \Delta m(1)}$

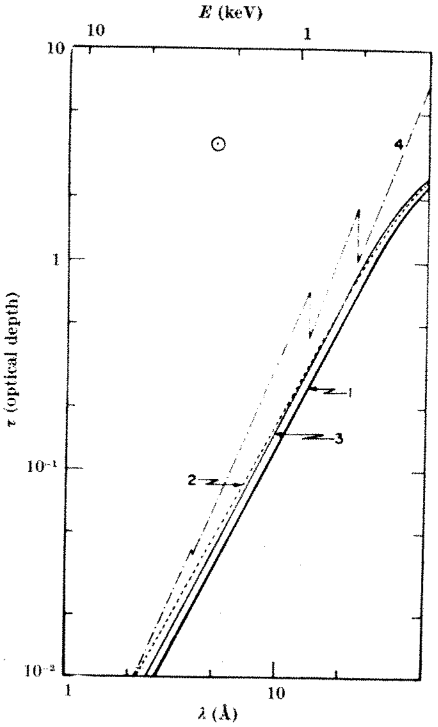


Fig. 2. Optical depth against wavelength of X-rays in the direction of Crab Nebula. Curves 1, 2, 3 are for scattering by interstellar grains. (1) Dirty-ice grains of uniform size, $a = 0{\cdot}25$ µm and $m = 1{\cdot}33 - i\ 0{\cdot}05$; (2) same as 1 but with a size distribution function (see equation 8); (3) composite grains with graphite core of radius 0·054 µm, and ice mantle of radius 0·16 µm. Curve 4 is the optical depth for photoelectric absorption by interstellar gas. The circle is the optical depth due to scattering given by Slysh[1] at 5 Å.

Composite grains with graphite cores of radius 0·054 µm and ice mantles of radius 0·16 µm have been proposed for explaining the ultraviolet and infrared observations[14,15]. This model of grains also successfully accounts for the observed results on polarization, albedo (the fraction that is scattered) and back-scattering[14]. For graphite core–ice mantle grains, Q_{ext} ($\lambda = 0{\cdot}54$ µm) $= 1{\cdot}26$ (ref. 15). The same extinction occurs in the X-ray region at $\lambda_x = 37{\cdot}5$ Å. Curve 3 in Fig. 2 shows the optical depths against λ_x for graphite–ice grains. It is similar to the curve for dirty ice.

So far we have assumed all the grains to be of the same size. We have calculated the optical depth in the X-ray region for ice grains using a size distribution

$$n(a)\text{d}a = n(0)\text{e}^{-5(a/a_0)^3}\text{d}a \tag{8}$$

This functional form suggested by Greenberg[16] is a very good representation of the original Oort–Van de Hulst distribution derived physically[17]. Following Greenberg[16] we have chosen $a_0 = 0{\cdot}5$ µm as the best fit for the optical observations of extinction. The corresponding optical depth in X-rays is shown as curve 2 in Fig. 2. To draw this curve we have chosen n_g such that the total mass of the grains is the same as for the case of the uniform size model. The distribution function slightly alters the shape of the curve. Comparing curves 1, 2 and 3 we note that the optical depth is insensitive to the model of the grains. Also shown is the optical depth obtained by Slysh[1] which is too large by a factor of ~ 80 at 5 Å.

The optical depth for photoelectric absorption in the interstellar gas is also shown (curve 4) using the cross-sections given by Bell and Kingston[18], and assuming a column density of $1{\cdot}6 \times 10^{21}$ H atoms cm^{-2} (ref. 19 and unpublished work of Grader *et al.*). At $\lambda_x = 20$ Å the optical depth for absorption is 1, and at all wavelengths the interstellar gas absorption dominates over scattering by interstellar grains.

Table 1 shows the rocket and balloon results on F_{obs}, the fraction of pulsed X-rays from the pulsar NP 0532. In the last column we have given F_s, the pulsed fraction

Table 1. ROCKET AND BALLOON RESULTS

Authors	Energy interval E_1, E_2 (keV)	Wavelength interval λ_1, λ_2 (Å)	F_{obs} (%)	F_s (%)
Fritz *et al.*[2]	0·8– 4	3 –15	$5·0 \pm 0·6$*	6 ± 1
Bradt *et al.*[3]	1·5– 8	1·5 –8	$8·4 \pm 0·3$*	$9·0 \pm 0·5$
Boldt *et al.*[4]	2 – 10	1·2 –6	15 ± 3	16 ± 4
Floyd *et al.*[7]	25 –100	0·125–0·5	15 ± 5	15 ± 5
Fishman *et al.*[8]	45 – 70	0·18 –0·28	18 ± 5	18 ± 5
	70 –120	0·10 –0·18	18 ± 5	18 ± 5
	120 –200	0·06 –0·1	18 ± 5	18 ± 5

* Errors were estimated by us from the data.

at the source, after correcting for scattering by the interstellar grains. The pulsed component observed in hard X-rays (> 20 keV) is unaffected by grains, and in soft X-rays the correction is 10–20 per cent. It is therefore clear that the observed spectrum of the pulsar in the 1–100 keV region should correspond closely to the spectrum at the source. The absorption by interstellar gas depletes the steady as well as the pulsed components by the same factor, so that F_{obs} is independent of photoelectric absorption. It should be emphasized that the conclusions remain unchanged even if the optical depth is altered by a factor two either way. Such a large uncertainty in optical depth is unlikely, however, because of the constraints placed by experimental observations of optical extinction on grain models.

In conclusion, the spectrum of the pulsed X-rays from NP 0532 (in the 1–100 keV region) is affected only very slightly by scattering by interstellar grains. The major part of the X-ray flux—80 to 95 per cent depending on the energy—arises as unpulsed X-rays from the pulsar source and/or the nebula, although the contribution from the former is likely to be quite small. It is worth noting that optical observations of NP 0532 (ref. 20) show that the unpulsed radiation from the pulsar itself is very small.

We thank Professor B. V. Sreekantan and Dr M. V. Krishna Apparao for comments.

S. NARANAN
G. A. SHAH

Tata Institute of Fundamental Research,
Homi Bhabha Road,
Bombay–5.

Received December 12, 1969.

[1] Slysh, V. I., *Nature*, **224**, 159 (1969).

[2] Fritz, G., Henry, R. C., Meekins, J. F., Chubb, T. A., and Friedman, H., *Science*, **164**, 709 (1969).

[3] Bradt, H., Rappaport, S., Mayer, W., Nather, R. R., Warner, B., MacFarlane, M., and Kristian, J., *Nature*, **222**, 728 (1969).

[4] Boldt, E. A., Desai, U. D., Holt, S. S., Serlemitsos, P. J., and Silverberg, R. F. *Nature*, **223**, 280 (1969).

[5] Bowyer, S., Byram, E. T., Chubb, T. A., and Friedman, H., *Science*, **146**, 912 (1964).

[6] Oda, M., Bradt, H., Garmire, G., Spada, G., Sreekantan, B. V., Gursky, H., Giacconi, R., Gorenstein, P., and Waters, J. R., *Astrophys. J. Lett.*, **148**, L5 (1967).

[7] Floyd, F. W., Glass, I. S., and Schnopper, H. W., *Nature*, **224**, 50 (1969).

[8] Fishman, G. J., Harnden, jun., F. R., Johnson, W. N., and Haymes, R. C., *Astrophys. J. Lett.*, **158**, L161 (1969).

[9] Hulst, H. C. van de, *Light Scattering by Small Particles* (Wiley, New York, 1957).

[10] Boggess, A., and Borgman, J., *Astrophys. J.*, **140**, 1636 (1964).

[11] Stecher, T. P., *Astrophys. J.*, **142**, 1683 (1965).

[12] O'Dell, C. R., *Astrophys. J.*, **136**, 809 (1962).

[13] Trimble, V., *Astron. J.*, **73**, 535 (1968).

[14] Wickramasinghe, N. C., and Krishnaswamy, K. S., *Nature*, **213**, 895 (1967).

[15] Greenberg, J. M., and Shah, G. A., *Physica*, **41**, 92 (1969).

[16] Greenberg, J. M., *Nebulae and Interstellar Matter* (edit. by Middlehurst, B. M., and Aller, L. H.), **7**, 221 (Univ. Chicago Press, 1968).

[17] Oort, J. H., and Hulst, H. C. van de, *Bull. Astron. Inst. Netherlands*, **10**, 187 (1946).

[18] Bell, K. L., and Kingston, A. E., *Mon. Not. Roy. Astron. Soc.*, **136**, 241 (1967).

[19] Clark, B. G., *Astrophys. J.*, **142**, 1398 (1965).

[20] Cocke, W. J., Disney, M. J., and Taylor, D. J., *Nature*, **221**, 525 (1969).

Pulsars and the Universal X-ray Background

IT is now established that there is a universal X-ray background with an energy flux of about 10^{-7} erg cm^{-2} sr^{-1} s^{-1} (M. Oda, at the thirty-seventh IAU symposium, Rome, 1969). One possibility is that the background is a superposition of X-ray emission by all the galaxies in the universe, but the value obtained by taking the average X-ray emission per galaxy as that estimated for our galaxy is not enough[1]. Silk[2] has taken the evolution of the universe into account using observations on radio sources as a guide to raise the intensity to the required level. The second explanation is the Compton scattering of the universal microwave background photons by intergalactic electrons[3]. This seems to be inadequate using earlier estimates of the intensity of intergalactic electrons, and an appeal to cosmological evolution did not help[4]. Recently, however, Brecher and Morrison[5] using shorter lifetimes for escape of electrons from galaxies, and also the observations on radio sources, find the Compton process capable of explaining the X-ray background. Both explanations involve important assumptions, however. Silk estimates the average galactic emission by assuming that the galaxy contains a hundred sources like the strongest observed source Sco X–1. Brecher and Morrison assume that the lifetime of electrons in all galaxies is less than a million years and that the electrons escape into intergalactic space without being trapped in galactic haloes.

In this report, I suggest that the X-ray background arises as a superposition of sources, but that the sources are nascent pulsars in various galaxies. I use as a guide the pulsar NP 0532 in the Crab nebula, the strongest pulsar observed so far. The X-ray energy output[6] from NP 0532 is about 10^{36} ergs s^{-1}; the optical output[6] is about 10^{34} ergs s^{-1} and the radio radiation[7] is a few times 10^{31} ergs s^{-1}. The total energy dissipation is estimated[8] to be $2–4 \times 10^{37}$ ergs s^{-1}. Thus the X-ray emission of the pulsar is 2–5 per cent of the total energy dissipation. A newborn pulsar is estimated to have a rotational energy content[8] of 10^{52} ergs. The rate of energy dissipation and its half-life can be estimated using certain assumptions, but it turns out that this is not necessary for the present work.

The X-ray background intensity I from the superposition of galaxies can be written as

$$I = \frac{1}{4\pi}\,\frac{\rho\,.\,L\,.\,S\,.\,\tau}{T} \text{ ergs cm}^{-2}\text{ sr}^{-1}\text{ s}^{-1}$$

where ρ is the density of galaxies, L is the dimension of the universe, T is the period for the birth of a pulsar (supposed to occur in a supernova explosion), S is the rate of X-ray emission of the pulsar and τ is the time for which the pulsar emits X-rays at that average rate. It is sufficient to know the product $S\tau$ and not S and τ separately. The product $S\tau$, which is the total energy emitted by the pulsar in X-rays, is taken to be about one per cent of the total energy, taking NP 0532 as a guide. In fact when the pulsar is born, magnetic fields associated with the supernova remnant are higher and it is likely that intensities are higher. Thus $S\tau$ is greater than 10^{50} ergs. I use $\rho \simeq 3 \times 10^{-75}$ galaxies per cm^3, $L = 10^{28}$ cm and $T = 100$ yr. Then:

$$I = \frac{1}{4\pi}\,\frac{3 \times 10^{-75}\,.\,10^{28}\,.\,10^{50}}{3 \times 10^{9}}$$

$$\simeq 10^{-7} \text{ ergs cm}^{-2}\text{ sr}^{-1}\text{ s}^{-1}$$

Thus it seems the universal background can arise as a superposition of radiation from pulsars in various galaxies. It may be argued that every supernova explosion may not be associated with a pulsar and the average time T for the birth of a pulsar might be larger than 100 yr. Even in such a case the product $S\tau$ plausibly can be taken higher by a factor of ten than the value which I have used. Effects due to the evolution of the universe, which I have not dealt with here, should also enhance the intensity of the background[2].

It is important that the superposition of the radio radiation from these pulsars should not be larger than the observed extragalactic radio radiation. This is possible if the pulsar radio radiation is less than the X-radiation by a factor of 10^4. This is true for NP 0532 and is even more likely to hold in the earlier life of a pulsar when the magnetic fields are higher.

The X-ray energy spectrum due to the superposition cannot be predicted—it would involve assumptions on the nature of the energy spectrum of pulsars during their early life. But as the radiation escapes from the pulsars into intergalactic space, absorptive effects caused by gas within the galaxies should leave an impression on the spectrum.

I thank my colleagues at the Tata Institute of Fundamental Research for criticism and discussion.

M. V. Krishna Apparao
Tata Institute of Fundamental Research,
Homi Bhabha Road,
Bombay-5, India.

Received December 8, 1969.

[1] Gould, R. J., and Burbidge, G. R., *Astrophys. J.*, **138**, 969 (1963).
[2] Silk, J, *Astrophys. J. Lett.*, **151**, L19 (1968).
[3] Felten, J. E., and Morrison, P., *Astrophys. J.*, **146**, 686 (1966).
[4] Brecher, K., and Morrison, P., *Astrophys. J. Lett.*, **150**, L61 (1967).
[5] Brecher, K., and Morrison, P., *Phys. Rev. Lett.*, **23**, 802 (1969).
[6] Bradt, H. L., Rappaport, S., Mayer, W., Nather, R. E., Warner, B., Macfarlane, M., and Kristian, J., *Nature*, **222**, 728 (1969).
[7] Comella, J. M., Craft, jun., H. D., Lovelace, R. V. E., Sutton, J. M., and Tayler, G. L., *Nature*, **221**, 453 (1969).
[8] Bertotti, B., Cavaliere, A., and Pacini, F., *Nature*, **221**, 624 (1969).

Secondary Periodicities in Pulsars

PERIODIC intensity fluctuations in pulsars, first noted by Conklin *et al.*[1] in CP 1919, have now been reported by a number of observers[2-9]. The present data support the earlier results. They also reveal a very pronounced modulation for CP 0834 which cannot be explained readily by current theories and give an upper limit for the stability of the secondary periodicity in the case of CP 1919.

This report describes measurements of five pulsars using the 4-acre, 81·5 MHz phased array at the Mullard Radio Astronomy Observatory. The aerial is used as a transit instrument and allows approximately two minutes' observation of each source per day. Successive pulse amplitudes were used to obtain the autocorrelation function of pulse amplitude in the range 0 to 30 pulse periods. The power spectrum was obtained by Fourier transformation of the observed autocorrelation function.

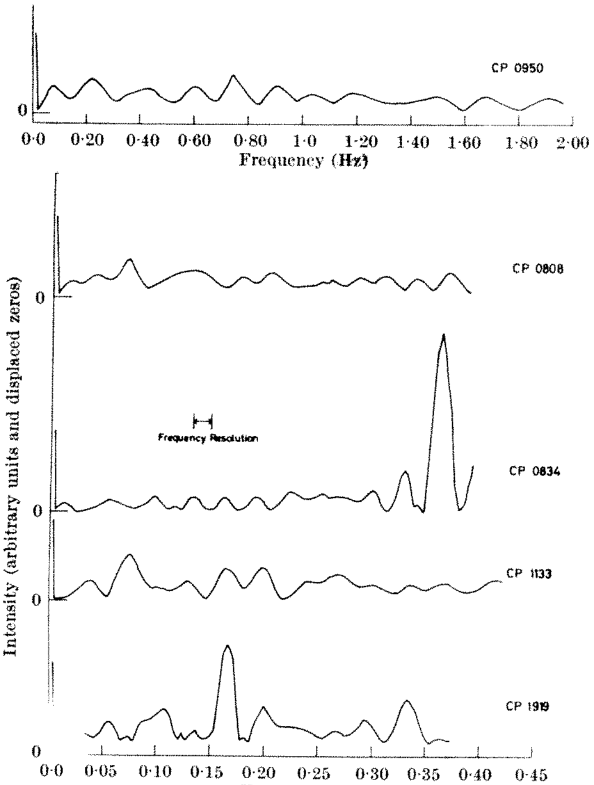


Fig. 1. Power spectra of the pulse-to-pulse intensity variations for five pulsars. Intensity scale has arbitrary units and displaced zeros.

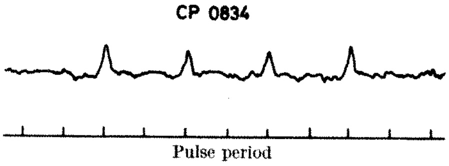


Fig. 2. Record of CP 0834 showing alternate pulses only.

Table 1. SUMMARY OF THE POWER SPECTRA DATA FOR FIVE PULSARS

Pulsar	Frequency of second periodicity (Hz)	Percentage modulation
CP 0808	0·069	$M \sim 5\%$
CP 0834	0·363	$33\% \leq M \leq 100\%$
CP 0950	—	—
CP 1133	0·075	$M \sim 5$–10%
CP 1919	0·167	$25\% \leq M \leq 50\%$

Fig. 1 shows the derived power spectra for five pulsars, and the significant features are summarized in Table 1. For the pulsar CP 0950 no significant feature has been found. In the case of CP 0808, however, a possible feature near 0·07 Hz coincides with a more marked feature noted by Taylor *et al.*[5]. A similar feature for CP 1133 at ~0·075 Hz may also be significant. The pulsars CP 0834 and CP 1919 both show prominent spectral components, corresponding to modulation periods of 2·16 times and 4·39 times the pulse period respectively. The spectrum of CP 1919 shows a clear feature at 0·167 ± 0·008 Hz which is only partially obscured by the effects of the large random pulse-to-pulse variations found in this source. Taylor *et al.*[5] have suggested that their failure to observe this feature is due to the smallness of their data sample of 1,344 pulses. In our data, at a somewhat lower frequency, the feature could sometimes be observed in samples of only 100 pulses, however. It is therefore possible that this modulation is not a constant feature of the source. The results for CP 0834 are particularly interesting because of the very strong modulation effect observed. At 81·5 MHz the percentage modulation (defined as $[(I_{\max}-I_{\min})/(I_{\max}+I_{\min})\times 100$ per cent$]$) is rarely found to be less than 33 per cent and can approach 100 per cent. The very pronounced character of the amplitude variations at this frequency is consistent with the frequency dependence reported by Taylor *et al.*[5], who found little modulation for frequencies above about 200 MHz. A section of record with 100 per cent modulation (that is, every second pulse missing) is shown in Fig. 2.

It is important to investigate the constancy of the observed secondary periodicities. For both CP 0834 and CP 1919 the spectral features are unresolved, implying a Q value greater than 100. In order to look for long-term changes, a power spectrum analysis of CP 1919 has been carried out using data obtained in December 1967. No detectable difference exists between the two sets of data, implying an upper limit of 5 per cent for the change in period over the time interval of 1·4 yr. This result, together with the measurements of other observers[3,5], seems to conflict with the change of period reported by Slee[4] for this source, which implied a variation in period of the order of 2·5 per cent per month.

The origin of the secondary periodicities is uncertain. For CP 1919 Drake and Craft[2] have reported sub-pulse structure of ~15 ms period which, being incommensurate with the main pulse period, will produce a drifting pattern with respect to the long main pulses 35 ms. The observed peak in the power spectrum can then be explained in terms of aliasing. For the case of CP 0834, however, where the modulation reaches 100 per cent, such an explanation seems unlikely because, in order to obtain such a large modulation, the sub-pulse period would have to be of the same order as the basic pulsar period. A phase scatter similar to that noted by Drake and Craft[2] in the sub-pulses of CP 1919 and CP 2015 would also be inconsistent with the observed modulation. Similarly, the model of Vila[10] which explains the sub-pulse in terms of a rotation and the primary pulsar periodicity as due to precession is also inappropriate in a case where the two periods are comparable in value.

For CP 0834, at least, some mechanism apart from sub-pulse structure seems to be necessary.

I thank Drs P. F. Scott and A. Hewish for discussions.

K. H. Hesse

Mullard Radio Astronomy Observatory,
Cavendish Laboratory,
University of Cambridge.

Received February 9, 1970.

[1] Conklin, E. K., Howard, H. T., Craft, jun., H. D., and Comella, J. M., *Nature*, **219**, 1238 (1968).
[2] Drake, F. D., and Craft, jun., H. D., *Nature*, **220**, 231 (1968).
[3] Lovelace, R. V. E., and Craft, jun., H. D., *Nature*, **220**, 875 (1968).
[4] Slee, O. B., *Proc. Astron. Soc. Austral.*, **1**, 222 (1969).
[5] Taylor, J. H., Jura, M., and Huguenin, G. R., *Nature*, **223**, 797 (1969).
[6] Lang, K. R., *Astrophys. J. Lett.*, **158**, L172 (1969).
[7] Staelin, D. H., Ewing, M. S., Price, R. M., and Sutton, J. M., *IAU Circ. No. 2196*.
[8] Vitkevich, V. V., and Shitov, Yu. P., *Nature*, **225**, 248 (1970).
[9] Jones, B., and Wielebinski, R., *Astrophys. Lett.*, **5**, 17 (1970).
[10] Vila, S. C., *Nature*, **224**, 157 (1969).

Pulsar Wobble and Neutron Starquakes

Two types of non-secular changes in pulsar frequency f have been reported. One is a sudden small frequency jump Δf in both NP 0532 (the Crab pulsar)[1,2] and PSR 0833 (the Vela pulsar)[3,4]. The other is a slow, small amplitude "wobble" of frequency ν in the Crab pulsar frequency[5]. A number of explanations for the jumps have been put forward, based on the model of a pulsar as a rotating neutron star: (1) the near passage of a planet in an orbit of high eccentricity (unpublished work of Michel); (2) a fluid instability caused by the crust of the neutron star rotating more slowly than its core[6]; (3) a sudden relaxation of strains which have built up in the stellar crust (starquake)[7,8]. Suggested mechanisms for the wobble include: (a) induced motion by yet another planet[5]; (b) free precession of a non-spherical star (unpublished work of Chiuderi and Occhionero); (c) normal mode vibrations of quantized vortex lines within the rotating superfluid core[9].

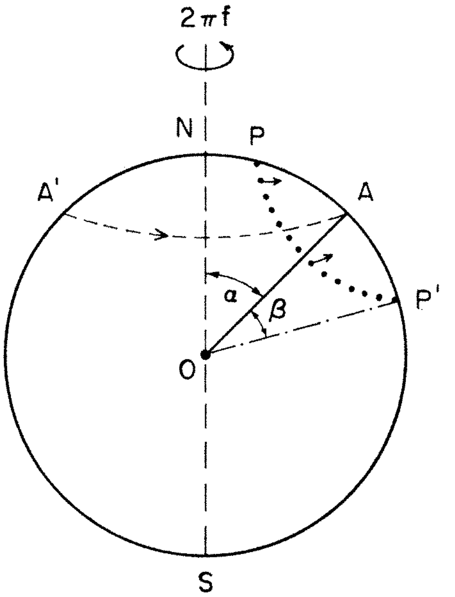


Fig. 1. Reference figure for motion of points on the surface of a precessing oblate spheroid.

The various models for the frequency jumps have different consequences for the subsequent behaviour of the wobble frequency. If explanations (3) and (b) hold (free precession and starquake frequency jumps), there should be a considerable and predictable post-jump decrease in the Crab wobble frequency. Such a model, together with the similarity between the Crab pulsar wobble frequency (Rome pulsar conference) $\nu = 1{\cdot}6 \times 10^{-7}$ Hz, and its residual jump after a few weeks $\Delta f_p = 4{\cdot}3 \times 10^{-8}$ Hz, supports the notion of a relatively weak crust strength in the Crab which relaxes every few years.

For simplicity I consider a rapidly spinning, axially symmetric, slightly oblate spheroid whose paraxial moment of inertia I exceeds its perpendicular moment of inertia by δI. It has a Poinsot precessional motion when its spin angular momentum is not exactly along a principal axis of inertia. The motion of characteristic points on the surface of such a spheroid is sketched in Fig. 1. The fixed angular momentum direction is $\overline{\mathrm{ON}}$; the line $\overline{\mathrm{OA}}$ is the axis of symmetry of the rotating spheroid. The instantaneous spin direction deviates from OA by such a small angle ($\sim \delta I/I$) that it is ignored in the figure. The point A moves along the line of constant latitude (relative to ON) AA′ at constant angular frequency f. Any other point P in addition rotates with precessional frequency ν_p about the OA axis with

$$\nu_p = \frac{\delta I}{I} f \cos \alpha \tag{1}$$

It therefore moves along constant latitude lines, but the latitude adiabatically varies up and down. As the point A disappears over the horizon, the point P on various revolutions has moved from P to P′ and then back to P over the horizon. In the absence of a more definitive understanding of the pulsar signal it is not clear how to associate such geometrical motions with the period of the pulsar clock. I assume that it is related to the time some special point on the neutron star surface comes over the horizon or passes any other fixed longitude determined by the fixed axis $\overline{\mathrm{ON}}$ and the direction to the observer. For this motion, the pulsar clock phase φ is

$$\varphi = 2\pi\nu_p t + \tilde{\varphi} \tag{2}$$

with the wobble in phase, $\tilde{\varphi}$, determined by

$$\cos \tilde{\varphi} = \frac{(\cos 2\pi\nu_p t) \sin \alpha}{[1 - (\cos \alpha \cos \beta + \sin \alpha \sin \beta \sin 2\pi\nu_p t)^2]^{\frac{1}{2}}} \tag{3}$$

The wobble is not sinusoidal except in the two limiting cases where the point P lies near the pole A or near the equator. For $\beta \ll 1$, $\beta < \alpha$

$$\tilde{\varphi} \sim \frac{\beta}{\sin \alpha} \cos 2\pi\nu_p t \tag{4}$$

For P on the spheroid equator $\beta = \pi/2$. Then when $\alpha \ll 1$

$$\tilde{\varphi} \sim \nu t + \frac{\alpha^2}{4} \sin 4\pi\nu_p t \tag{5}$$

In these limits the wobble frequency therefore can be ν_p or $2\nu_p$. The reported wobble phase amplitude $\sim 0{\cdot}06$. From equation (1) the reported 72 day wobble period implies

$$\frac{\delta I}{I} \cos \alpha \sim 2{\cdot}6 \times 10^{-9} \text{ or } 5{\cdot}3 \times 10^{-9} \tag{6}$$

The frequency jump in the Crab pulsar was initially (September 28 ± 1, 1969) $2{\cdot}6 \times 10^{-7}$ Hz. After about a week there had been a relaxation to a "permanent" $\Delta f = 4{\cdot}3 \times 10^{-8}$ Hz. In the starquake interpretation of such events, the initial jump was associated with a sudden relaxation in shape of the slightly over-oblate crust toward the oblateness appropriate to its present rotation. The interval of a week was the characteristic relaxation time for the crust to communicate its changed angular speed to the deep stellar interior[10]. Then the permanent decrease in stellar moment of inertia was

$$\frac{\Delta I}{I} = -1{\cdot}4\times10^{-9} \tag{7}$$

Because the crust–core relaxation time of about a week is so much less than the precession period, the I and δI of equation (6) presumably also refer to the entire star and not just the deformed crust. From equations (1), (4), (5), (6) and (7) it follows that after the starquake the wobble period would be expected to increase from 72 ± 3 days to either 95 or 170 days (as long as α remains small or does not change greatly). For a non-axially symmetric star δI and ΔI would be the geometric mean of the perpendicular moments of inertia, but there would be no qualitative change in interpretation.

For a rapidly spinning elastic or fluid spheroid, only residual deformation not directly caused by its present rotation contributes to the δI in equation (1) (refs. 11 and 12). The relatively large centrifugal oblateness of the rotating neutron star ($\sim10^{-4}$ for the Crab) is neglected because it adjusts to any change in the rotation axis. The much smaller fixed residual oblateness from crust rigidity or perhaps the magnetic field (private communication from J. Ostriker), which would remain if $\nu\to0$, gives δI. The long wobble period in the Crab therefore implies a shape which has almost entirely relaxed to the hydrodynamic one even before the small quake. This is qualitatively different from the state of the Vela crust. The Vela frequency jump $\Delta\nu/\nu\sim2\times10^{-6}$ implied a crust shear strain $\sim10^{-2}$, of order of the maximum strain which crust matter could sustain[7]. The Crab crust, on the other hand, should be highly faulted so that it relaxes only after a strain $\sim10^{-5}$. Such a small strain and another small starquake should build up from stresses associated with the slowing down of the Crab in a very few years (private communication from D. Pines and G. Baym)

$$(\sim10^{-5}(GMR^{-2})(4\pi^2\nu\dot{\nu}R)^{-1})$$

I thank Drs C. Chiuderi and F. Occhionero for interesting conversations. This research was supported in part by the US National Science Foundation.

M. RUDERMAN

Columbia University, New York.

Received January 30, 1970.

[1] Boynton, P., Groth, E., Partridge, R., and Wilkinson, D., *IAU Circ. No. 2179* (1969).

[2] Richards, D., Pettengill, G., Roberts, J., Councelman, C., and Rankin, J., *IAU Circ. No. 2181* (1969).

[3] Reichley, P., and Downs, G., *Nature*, **222**, 299 (1969).

[4] Radhakrishnan, V., and Manchester, P., *Nature*, **222**, 298 (1969).

[5] Richards, D., Pettengill, G., Councelman, C., and Rankin, J., *IAU Circ. No. 2178* (1969).

[6] Greenstein, G., and Cameron, A., *Nature*, **222**, 862 (1969).

[7] Ruderman, M., *Nature*, **223**, 597 (1969).

[8] Dyson, F., *Nature*, **223**, 486 (1969).

[9] Ruderman, M., *Nature*, **225**, 619 (1970).

[10] Baym, G., Pethick, C., Pines, D., and Ruderman, M., *Nature*, **222**, 872 (1969).

[11] Munk, W., and MacDonald, G., *The Rotation of the Earth* (Cambridge Univ. Press, New York, 1960).

[12] Goldreich, P., and Toomre, A., *J. Geophys. Res.*, **74**, 2555 (1969).

Search for High Energy Gamma Rays from Four Pulsars

IT has been suggested[1,2] that high energy cosmic rays originate in pulsars. If this is true, it is conceivable that the pulsars might also be emitting ultra high energy, pulsed gamma rays with the same periodicities as observed at radio frequencies. These gamma rays might be the result of either the decay of π^0-mesons or the bremsstrahlung of electrons in the regions surrounding the pulsars. There is no precise theory yet on this aspect. There have already been several searches for high energy gamma rays from pulsars[3-5].

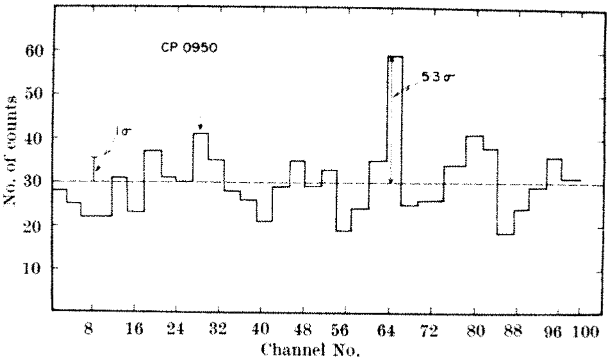


Fig. 1. Phase histogram obtained for CP 0950. The pulsar period is divided into 100 phase channels which are grouped in threes in the figure. The position of the interpulse as observed by Rickett and Lyne[7] at 408 MHz is shown by the arrow approximately at the 29th channel. The average of the histogram is shown by the dashed line.

During the latter part of April 1969 we tried to detect the possible emission of high energy pulsed gamma rays ($\gtrsim10^{13}$ eV) from four pulsars: CP 0950, CP 1133, AP 1541 and PSR 1642. Detectable Čerenkov light flashes are produced in the atmosphere by the relativistic electrons of cascade showers induced by gamma rays and charged cosmic rays of high energy ($\gtrsim10^{12}$ eV). The latter constitute the main background of noise limiting the minimum measurable gamma ray flux. The method in our experiment, as in the others, is based on looking for these nanosecond duration Čerenkov light signals in excess of the background from the direction of pulsar under investigation. If pulsars emit gamma rays in a pulsed mode with the same periodicity and similar pulse widths as in the case of the radio signals, time averaging by superposition of the gamma ray signals over a large number of time periods can reveal such a gamma ray flux even if it is much weaker than the background.

The Čerenkov light is detected by two large searchlight mirrors (90 cm diameter and 41 cm focal length) and focused onto two fast photomultipliers (56 AVP) placed at their foci. The mirrors track each pulsar for about an hour when it is close to meridian transit. Whenever a coincidence occurs within the resolving time of 5 ns between the two photomultipliers, the time as shown by a crystal controlled digital clock is recorded to a precision of 5·1 ms. The short term drift (typically over a few hours) of the crystal oscillator is found to be less than one part in 10^6. The full field of view of each mirror is 3° and the ratio of genuine Čerenkov light signals (which are mostly due to showers induced by cosmic rays) to chance coincidences is about 1 : 2. From the background Čerenkov signal rate, the known aperture of the field of view and the approximate area of collection of high energy gamma rays of 3×10^4 m^2 (see ref. 6, for example), the threshold energy of detection in our experiment is estimated to be $\approx10^{13}$ eV. Observations were made on clear nights during the period April 16–28, 1969, at Mukurthi located at an altitude of 7,200 feet above sea-level in the Nilgiri Hills in South India.

Time averaged phase histograms were made for each pulsar, with the recorded Čerenkov coincidence times for

Table 1. GAMMA RAY FLUXES FROM PULSARS

Object	O'Mongain *et al.* Threshold* (eV)	O'Mongain *et al.* Flux (cm^{-2} s^{-1})	Charman *et al.* Threshold* (eV)	Charman *et al.* Flux (cm^{-2} s^{-1})	Fazio *et al.* Threshold* (eV)	Fazio *et al.* Flux (cm^{-2} s^{-1})	This experiment Threshold* (eV)	This experiment Flux (cm^{-2} s^{-1})
CP 0950	—	—	—	—	4×10^{12}	$\lesssim2\times10^{-11}$	10^{13}	$\approx4\times10^{-11}$
CP 1133	3×10^{12}	5×10^{-11}	7×10^{13}	$\lesssim1{\cdot}7\times10^{-12}$	2×10^{12}	$\lesssim2\times10^{-11}$	10^{13}	$\lesssim2\times10^{-11}$
AP 1541	—	—	—	—	—	—	10^{13}	$\lesssim1\times10^{-11}$
PSR 1642	—	—	—	—	—	—	10^{13}	$\lesssim1{\cdot}6\times10^{-11}$

* The threshold energies are to be trusted only to within a factor of 3.

the corresponding geocentric period of the epoch, using a computer. A calibration procedure and measured value of the clock drift permitted us to establish that the clock was accurate within ± 50 parts in 10^6. The phase histograms were therefore made for a scan of the clock calibration extending over this range of uncertainty. The scan resolution used of 2 parts in 10^6 was determined by the duration of the run and an assumed minimum pulse width of about 1 per cent of the corresponding pulsar period for the pulsed gamma ray emission.

Of the four pulsars, only CP 0950 showed a significant peak in one of the channels, of 5·3 standard deviations above the average (Fig. 1). Fig. 1 also shows the position where Rickett and Lyne[7] found the interpulse at a frequency of 408 MHz. They estimate that the energy in the interpulse is about 1·8 per cent of that in the main pulse[7].

A χ^2 test on the histogram, excluding the channel in which the peak appeared, shows that $P(\chi^2) = 0{\cdot}38$ for the assumption that the distribution is Poissonian. The probability of statistical fluctuations accounting for the peak observed in Fig. 1 is deduced to be $2{\cdot}5 \times 10^{-3}$. This value was arrived at as follows. (i) The Poisson probability of obtaining ≥ 59 counts when the expected average is 30 is $1{\cdot}88 \times 10^{-6}$. (ii) The number of ways of grouping into three adjacent bins, when the total number of bins is 100, is = 100. (iii) The number of trial clock periods is 50, and the number of adjacent trial periods in which the same peak has appeared with appropriate phase shift is 4.

The expectation value is therefore

$$1{\cdot}88 \times 10^{-6} \times 100 \times \frac{50}{4} \sim 2{\cdot}5 \times 10^{-3}$$

We also made a total of ten attempts on the other pulsars which showed no significant peaks. Depending on the caution one wishes to exercise, one might say that the statistical chance of observing a peak which is $\geq 5{\cdot}3$ standard deviations above the average is $2{\cdot}5 \times 10^{-3}$ for that particular run on CP 0950 or $2{\cdot}5 \times 10^{-3} \times 11 \sim 3 \times 10^{-2}$ for the whole experiment. A second observation on CP 0950 would have established unambiguously whether it is steadily pulsating in gamma rays or not. This was not feasible because the experiments were started late in April. The number of clear nights were few and when the atmospheric conditions were good the Moon interfered with the observations on CP 0950. Further observations are feasible on this pulsar only after December 1969.

In the case of the other pulsars, CP 1133, AP 1541 and PSR 1642, at least two observations were made on each and no significant peaks ($\geq 5\sigma$) were observed. Only upper limits could therefore be deduced for their gamma ray fluxes. Our results along with those of others[3-5] are listed in Table 1. The upper limits given by us are such that, if the true gamma ray fluxes were higher than those given, we would have seen them as five standard deviations or higher from the average. The threshold energies quoted can be trusted only to a factor of ~3. This uncertainty is due to assumptions of the lateral and angular spread, time distribution and atmospheric absorption and so on of Čerenkov light.

It is unlikely that CP 1133, AP 1541 and PSR 1642 are emitters of pulsed gamma rays resulting in intensities higher than the limits given by us. CP 0950 seems to be a good candidate for further observations. It may be significant that CP 0950 is also probably the nearest pulsar, considering that it has the lowest dispersion measure[8] ($\int N_e ds = 2{\cdot}94$ electrons cm^{-3} pc).

We thank Miss K. B. Vijayakar and Messrs A. R. Apte, N. V. Gopalakrishnan and S. G. Khairatkar for their help.

B. K. CHATTERJEE
G. T. MURTHY
P. V. RAMANA MURTHY
B. V. SREEKANTAN
S. C. TONWAR

Tata Institute of Fundamental Research,
Bombay-5, India.

Received August 20; revised November 17, 1969.

[1] Gold, T., *Nature*, **218**, 721 (1968); *ibid.*, **221**, 25 (1969).
[2] Gunn, J. E., and Ostriker, J. P., *Phys. Rev. Lett.*, **22**, 728 (1968).
[3] O'Mongain, E. P., Porter, N. A., White, J., Fegan, D. J., Jennings, D. M., and Lawless, B. G., *Nature*, **219**, 1348 (1968).
[4] Charman, W. N., Jelley, J. V., Orman, P. R., Drever, R. W. P., and McBreen, B., *Nature*, **220**, 565 (1968).
[5] Fazio, G. G., Helmken, H. F., Rieke, G. H., and Weekes, T. C., *Nature*, **220**, 892 (1968).
[6] Jelley, J. V., *Prog. Elementary Particle and Cosmic Ray Phys.*, **9** (edit. by Wilson, J. G., and Wouthuysen, S. A.) (North-Holland Pub. Co., 1967).
[7] Rickett, B. J., and Lyne, A. G., *Nature*, **218**, 934 (1968).
[8] Lyne, A. G., and Rickett, B. J., *Nature*, **218**, 326 (1968).

Dynamic Relaxation of Planetary Systems and Bode's Law

BODE's law, which states that the semi-major axis r_i of the ith planet from the Sun is given by

$$r_i = r_1 a^{(i-1)} \tag{1}$$

where a is a constant, is obeyed by the inner satellites of Jupiter, Saturn and Uranus as well as the planets[1]. Because the initial conditions in these satellite systems are not likely to have been the same as in the planetary system, this suggests to me that Bode's law may have resulted from a process of dynamical relaxation. That an epoch characterized by strong dynamical encounters occurred before the planets relaxed into stable orbits is also suggested by the surprisingly large differences between the inclinations of their angular momentum vectors of rotation and those of orbital revolution.

To test this hypothesis eleven planetary systems each with a central star of 1 $M_\odot$ but with widely different planetary mass functions were evolved from random initial orbits on an IBM 360/67 computer by numerically integrating the equations of motion. The integrations were done in two dimensions to save computer time. They were performed in double precision using a fourth-order Adams–Moulton routine with the length of the time steps being controlled by the truncation errors. Special precautions were taken to reduce these errors during close encounters. The median cumulative error, ΔE, in the energy E_0 of a system was $\Delta E/E_0 \sim 10^{-5}$.

Despite large differences in planetary mass functions and initial conditions the various systems tended towards stationary states having a number of common characteristics, the principal one being a tendency for the periods of adjacent orbits to be small integer fractions of each other, that is, they show the commensurabilities of the mean motions of classical celestial mechanics (see ref. 2). This

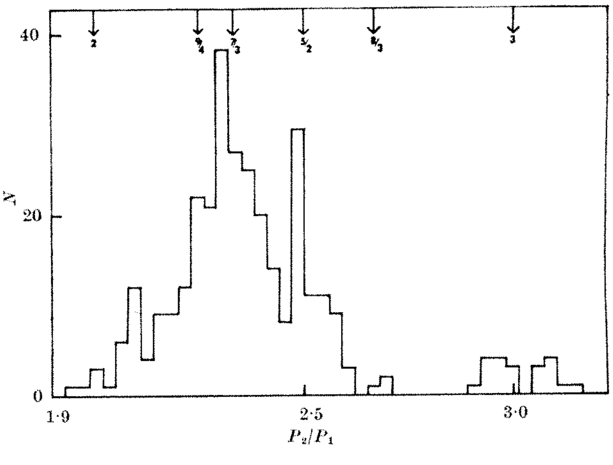


Fig. 1. Frequency of the ratio of the periods of planets 2 and 4 in system 5. This was evaluated at intervals of ten years starting 1,000 yr after the beginning of the planetary evolution.

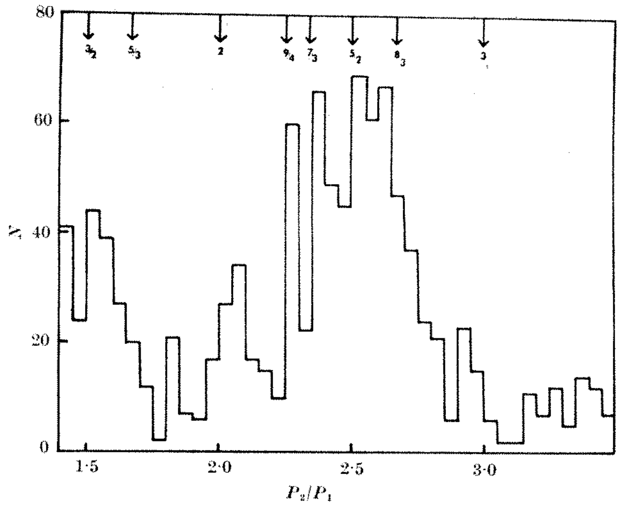


Fig. 2. Frequency of the ratio of the periods of the two outermost planets in system 11 except when the ratio is less than 1·4, in which case the third outermost planet is used in place of the second. The ratio was evaluated at intervals of ten years starting 1,000 yr after the beginning of the planetary evolution.

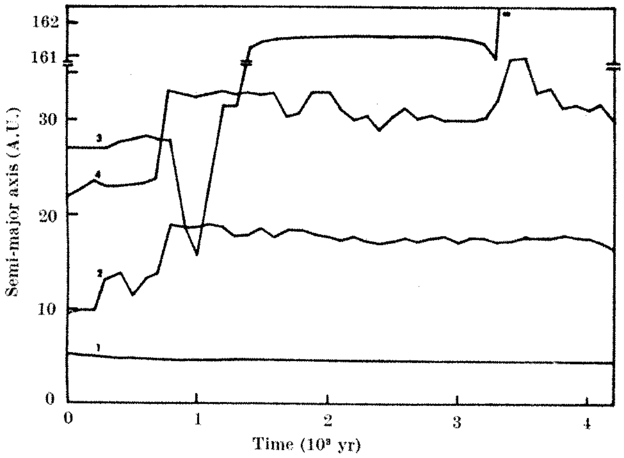


Fig. 3. Time variation of the semi-major axes of the planets in system 5.

is illustrated by Figs. 1 and 2, which give the frequency of period ratios of planets in systems 5 and 11, the two systems which were evolved for the longest periods of time. Figs. 3 and 4 give the time variations of the semi-major axes of the planets in these two systems while Fig. 5 shows these for system 9, the only one in which two planets were in stationary orbits from the beginning of the integrations. These two planets were accidentally placed into nearly commensurate orbits with a period ratio of (13/6). The tendency towards commensurate orbits is also evident in Figs. 1 and 2 despite the two systems having at best reached only quasi-stationary states.

A quantitative test of the correlation between the observed frequency pattern of the period ratios, R_i, shown in Figs. 1 and 2 and the commensurate points is difficult to construct because any given R_i is, in general, not independent of the R_is which proceeded it. If the distribution of R_is tends to be Gaussian around the commensurabilities and if the commensurabilities are equally probable the function

$$F(\Delta)=\sum_{i=1}^{N}\sum_{j=1}^{8}\exp\{-[R_i-(C_j+\Delta)]^2/\sigma\} \qquad (2)$$

where the C_js are the commensurabilities 3/2, 5/3, 2, . . . 3 will have a maximum near $\Delta=0$. In Fig. 6 we have plotted this function for the R_is of system 5 with $\sigma=0{\cdot}025$. This shows a maximum near $\Delta=-0{\cdot}01$, which is less than 0·05 of the average spacing between commensurabilities. This suggests a high correlation between the frequency pattern of the R_is and the commensurabilities.

The three systems which have been discussed cover a wide spectrum of initial conditions and planetary mass functions. In system 5 each of the four planets has ten times the mass of one of the Jovian planets while in system 11 each of the six planets has a mass of $5\times10^{-4}\,M_\odot$. The initial semi-major axes and eccentricities of the planetary orbits in these two systems were chosen at random, although, due to an early misjudgment about conditions in the solar nebula, the initial semi-major axes in system 11 were only allowed to lie in a narrow range between 6 and 8 A.U. In system 9 the masses of the planets are $8\times10^{-4}\,M_\odot$, $3\times10^{-4}\,M_\odot$ and $1\times10^{-7}\,M_\odot$. As such widely different systems could evolve towards stationary states characterized by commensurate orbits, it seems likely that nearly all planetary systems will evolve towards these stationary states. The initial conditions and planetary mass functions seem only to determine the time necessary to reach such a state.

If the dynamical evolution of the semi-major axes of the planets in a system can be approximated by a succession of random walks for which the length of each step is directly proportional to the mass of the perturbing planet, the fact that system 5, which has planets ten times as massive as the Jovian planets, reached a quasi-stationary state in about 2×10^3 yr suggests that the Jovian planets reached such a state in about 2×10^5 yr. Considering the large uncertainty in this calculation, a reasonable estimate of the time necessary for the Jovian planets to evolve to their present orbits after their formation is 10^5–10^6 yr.

Recent investigations[3–6] have shown that commensurate orbits are more frequent in the solar system, particularly among the Jovian planets and among their satellite

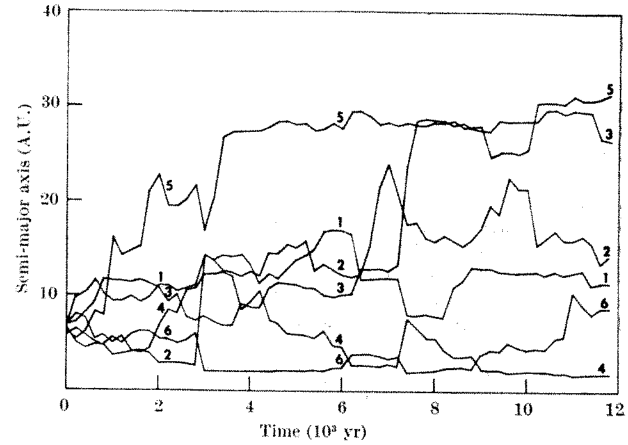


Fig. 4. Time variation of the semi-major axes of the planets in system 11.

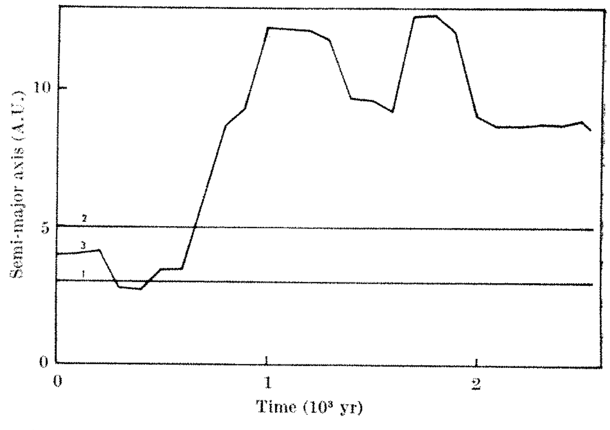


Fig. 5. Time variation of the semi-major axes of the planets in system 9.

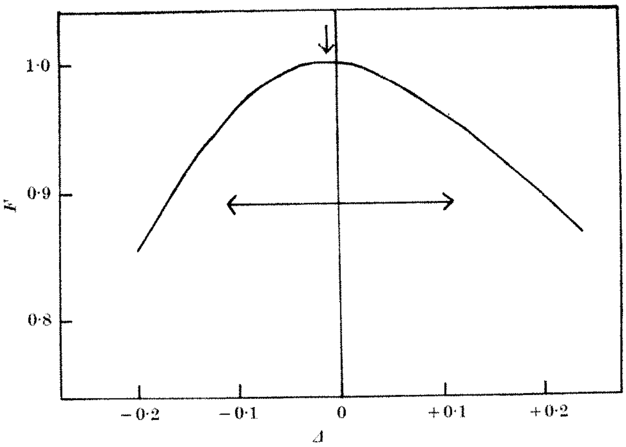


Fig. 6. Correlation between the frequency pattern of the period ratios of system 11 shown in Fig. 2 and the commensurate points. The arrow indicates the position of the peak of the curve, and the horizontal bar shows the average spacing between commensurabilities.

systems, than is expected from chance alone. For those commensurate pairs of orbits which have been studied in detail it has been found that the commensurability condition leads to resonant perturbations of large amplitude, but these cause only periodic changes in the orbital elements[7]. It thus seems likely that commensurate orbits are the final signatures of a system of strongly interacting planets with commensurability being the stablest possible arrangement of their semi-major axes.

Bode's law results from the fact that some commensurabilities are more favoured than others. We see in Figs. 1 and 2 that most commensurabilities lie in a narrow range between (9/4) and (8/3); this is true for the other computer simulated systems as well. Because of this small spread in the period ratios, the period of the ith planet from the Sun is given closely by the relation

$$P_i = P_1 A^{(i-1)} \tag{3}$$

where A is constant, but this is just Bode's law as it can be trivially converted to equation (1) by Kepler's third law. Pluto is usually cited as being the most flagrant violator of Bode's law. This results from the approximate character of the law. The orbit of Pluto is actually highly commensurate with the orbit of Neptune, the period ratio being nearly (3/2). We see in Fig. 2 that the second largest clumping of commensurate points is near (3/2), but these commensurabilities are considerably less frequent than those in the range (9/4)–(8/3). The smaller commensurabilities are much more frequent in the satellite systems than in the solar planetary system and in the computer simulated planetary systems. The reason for this will be discussed in a later paper.

The implications of these results on theories of the origin of planetary and satellite systems as well as a more detailed report on the analysis of the numerical experiments will also be given in a later paper.

I thank Professor F. T. Haddock and Dr R. L. Sears for helpful discussions.

J. G. Hills

Department of Astronomy,
University of Michigan,
Ann Arbor, Michigan.

Received October 16, 1969; revised January 13, 1970.

[1] Brandt, J. G., and Hodge, P., *Solar System Astrophysics* (McGraw-Hill, New York, 1964).

[2] Brouwer, D., and Clemence, G. M., in *Planets and Satellites* (edit. by Kuiper, G. P., and Middlehurst, M.) (Univ. Chicago Press, 1961).

[3] Roy, A. E., and Ovenden, M. W., *Mon. Not. Roy. Astron. Soc.*, **114**, 232 (1954).

[4] Roy, A. E., and Ovenden, M. W., *Mon. Not. Roy. Astron. Soc.*, **115**, 296 (1955).

[5] Goldreich, P., *Mon. Not. Roy. Astron. Soc.*, **130**, 159 (1965).

[6] Dermott, S. F., *Mon. Not. Roy. Astron. Soc.*, **141**, 349 (1968).

[7] Hagihara, Y., in *Planets and Satellites* (edit. by Kuiper, G. P., and Middlehurst, M.) (Univ. Chicago Press, 1961).

Lunar Continental Migration and Maria Spreading

Contemporary ideas about the broad features of continental migration and ocean floor spreading are largely independent of whether or not there is any sea present. This report considers the possibility that the lunar maria were formed by internal geological processes similar to those which formed the ocean floors of the Earth.

Evidence that the maria are younger than elsewhere comes from the density of cratering[1]. But if the rate of bombardment has remained more or less constant and the maria, though "new", are still quite old, why are they not more heavily cratered ? I believe we have no alternative but to abandon the uniform bombardment rate and accept that the rate of cratering diminished rapidly during the first 10^9 yr of the existence of the Moon. The mean density of cratering allows us to place the maria in an "age" sequence. For example, Mare Nubium should be older than Mare Crisium. We are thus led to the view that some of the maria surfaces are newer than others.

In order to understand the role of maria spreading I have constructed a simple laboratory model. This is a layer of hot viscous fluid, which can be cooled from above. A layer of aluminium powder of thickness about 0·1 mm is spread on the upper surface to represent a patch of crustal material. Fig. 1 shows the ensuing distribution of crustal material, which should be compared with the view of the Moon in Fig. 2.

The vigour of the motion, in a sufficiently viscous material, is determined by the Rayleigh number $A = \gamma g \Delta T R^3/\kappa\nu$, where γ is the coefficient of cubical expansion, g the acceleration of gravity, ΔT the temperature drop across the layer, R the depth of the layer, κ the thermal diffusivity, and ν the kinematic viscosity of the fluid (refs. 2 and 3). If $A \gg 10^3$, after a short interval of time (of order $A^{-2/3}$), convective motion is vigorous and extends throughout the layer. Close inspection of the flow reveals a remarkable situation[3] in which small-scale eddying motions develop near the cooled upper surface in a thin layer, called the thermal sublayer, of thickness $\delta \sim A^{-1/3}$ (Fig. 3).

As they grow in amplitude, the rising hotter fluid, seen from above, takes the form of upwelling blobs, while at the margin between these blobs narrow sheets of colder fluid plunge downwards. These sheets stir the deeper interior to the overall appearance of a motion made up of large eddies extending right across the layer. Once the blob has expended its thermal energy it rapidly dies away. But other sublayer eddies are continuously being formed to create an ever changing pattern of rapid small-scale motions in the sublayer and slower larger motions at depth. I want to emphasize that this structure is quite different from convection at low Rayleigh numbers ($A \sim 10^3$) where a regular structure of convective cells more or less fixed in space occurs.

The role of a surface layer, simulating old crust, is partially to inhibit the motion in the sublayer beneath it by lowering the cooling rate. If the surface layer is sufficiently thick the outgoing horizontal viscous stress during the upwelling phase of blob production may not persist for sufficient time to rupture the surface layer. Once the surface layer has been ruptured, however, there is a tendency for the crust immediately surrounding the mare to be stretched by a surface flow predominantly outward so that new blobs can more readily rupture the mare surroundings.

The model reveals an important aspect of the process of production of new mare. All but the smallest mare are produced not by the action of a single buoyant blob but by many. As each blob approaches the surface of the lithosphere the outgoing horizontal viscous stresses push outward the old crust. The net effect of the mutual action of many blobs is to expand progressively a mare by sweeping away old crust at the margin. Each blob rising near a mare margin thereby produces a "bay" in the old crust.

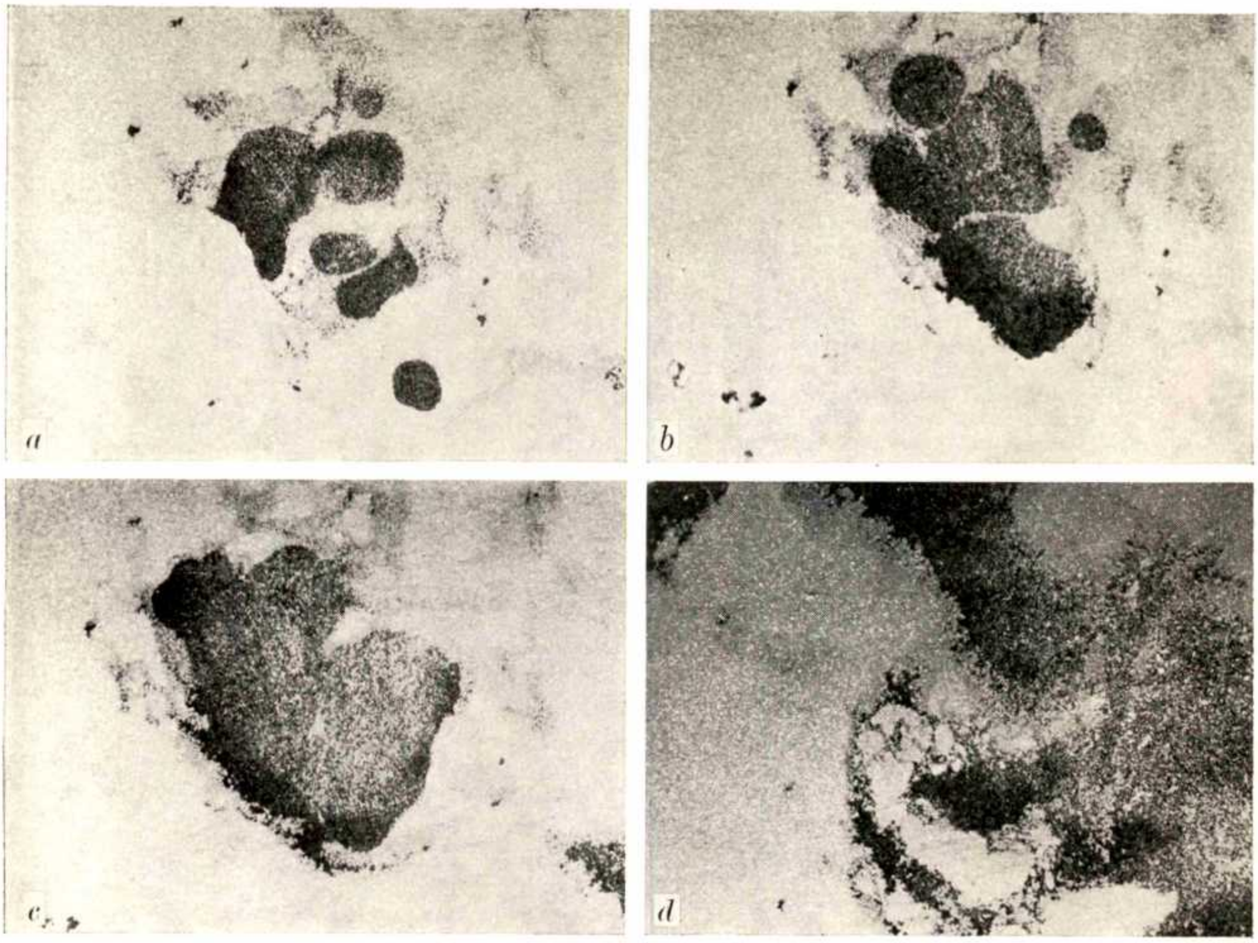


Fig. 1. Laboratory simulation of mare formation. Photographs of the upper surface of a layer of vigorously convecting viscous fluid cooled from above which has a thin surface layer of white powder. Photographs taken at times from the onset of cooling equivalent to, in units of 10^9 yr: *a*, 0·3; *b*, 0·4; *c*, 0·5; *d*, 1·0. In this particular simulation *d* shows an extreme stage, the complete disruption of the surface layer into two distinct parts.

To demonstrate that blobs are also rising continuously beneath the entire area of the mare, Fig. 4 is a photograph of a large mare on the surface of which I have sprinkled some extra aluminium powder. The blob structure is revealed by the horizontal displacement of this material to the regions between rising blobs. Here I want to emphasize that the size of the buoyant elements is reflected not in the size of the mare (which is determined by the persistence of upwelling) but in the size of the embayments at the margin of the mare. The embayments have widths of about 5°, and taking the width as about twice the depth of the sublayer in which the blobs develop, we have $\delta/R \approx 1/40$. This requires a Rayleigh number of order 10^7.

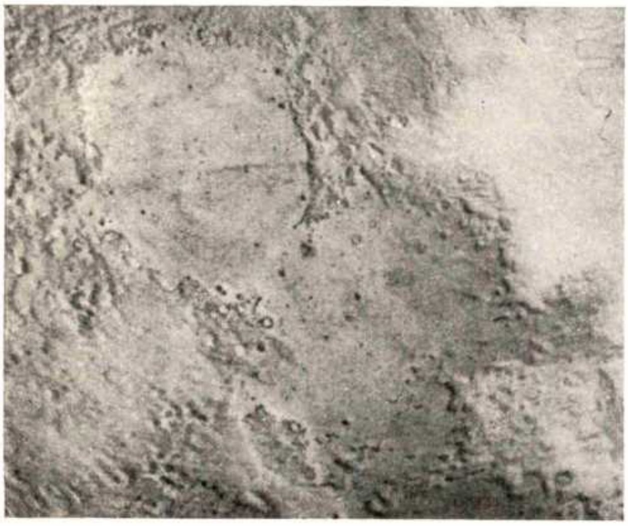


Fig. 2. A view of part of the lunar surface centred on Mare Tranquillitatis—an attempt to emphasize the distinction between mare and continent by printing simultaneously a positive and negative photograph with a small relative displacement.

Certain lunar structures provide some supporting evidence. Domes occur almost exclusively in the maria and may be analogous to the volcanic domes on terrestrial ocean floors. This type of volcanism does not occur on continental areas. Rilles, ridges and wrinkle ridges are evidence of vigorous displacements in the mantle beneath the maria. They can be identified with similar ridge systems on the ocean floors of the Earth and are analogous to the structures shown within the mare of Fig. 4. Mountain chains outside the heavily cratered areas near the south pole take the form of isolated or connected chains usually marginal to the maria. I suggest that these systems are analogous to the so-called island–arc structures on Earth. Where mantle convection has been locally persistent, fragments of continental type material have been swept together and possibly augmented by discharge from the mantle itself. This process is seen in the model, for example, the strip of crustal material stretching from the right across the middle of the mare of Fig. 1*b*.

In broad terms the maria occupy a single connected area outside which continental material extends continuously from the southern portion of the front face across the rear face except for isolated maria such as Mare Muscoviense, At the margin of a mare the predominant tendency is not only a progressive growth of the mare by embayment but an outward surface stress. The overall effect of this stress is to push continental material away from the mare. This is a version of continental drift produced by "mare floor spreading". Hence the surface area which is predominantly mare will expand and tend to push all the continental material together.

The occasionally more vigorous blob may penetrate the continental material (Mare Muscoviense) and if the supply of internal energy is not yet depleted this new mare will expand, possibly to the point of breaking up the continental mass once more. On Earth, one of the puzzling features of what is known about continental migration is that apparently an original Gondwanaland began to break up about 10^8 yr ago, with the implication that nothing much had happened to Gondwanaland before that time. To my mind it is difficult to accept that continental disruption and migration have only occurred in the recent past. But there then remains the question of how a Gondwanaland was formed at all. I would suggest that the discussion of the distribution of the lunar mare can be applied to the Earth and that Gondwanaland was simply one of a sequence of continental aggregations of which there have been many.

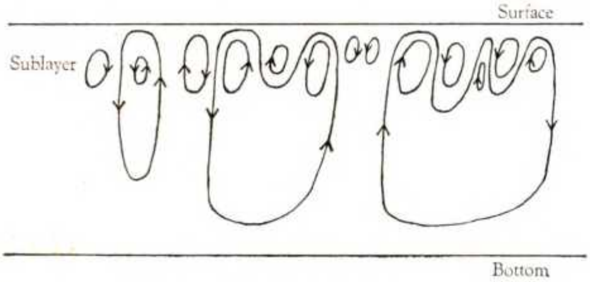


Fig. 3. Schema of the motions in a fluid layer vigorously cooling from above.

The relative rates on the Moon and the Earth at which the processes of continental migration and ocean floor spreading occur can be estimated if we assume that the dominant process is free convection. The ratio of cooling times[4] is

$$\sigma = \tau_{\text{Moon}}/\tau_{\text{Earth}} = (R_{\text{Moon}}/R_{\text{Earth}})\ \ (g_{\text{Earth}}/g_{\text{Moon}})^{1/3} \doteq 0{\cdot}5$$

In other words, lunar processes run down about twice as fast as those in the Earth. If the age of the Sea of Tranquillity is about $3{\cdot}5 \times 10^9$ yr and assuming a solar system age of about 5×10^9 yr we have a crude estimate of the rate ratio $\sigma \approx 1{\cdot}5/5 \approx 0{\cdot}3$.

This report has emphasized the role of mare spreading, with continental migration playing a neutral and secondary part. Previously[5] I have suggested that on the Earth, at present, migration through continental self-propulsion could play a primary part. Taken together with computed thermal histories[4] these two ideas suggest the following. The early stages of the cooling of all bodies of planetary size is the same, provided the initial temperatures and the rock types are similar. Vigorous convection is confined largely to a thin sublayer near the surface and the interior of the body is little affected. The lunar evidence suggests that mare production arising from vigorous penetrative convection is the dominant process at this stage. Gradually the internal energy of the body becomes depleted and if the body is sufficiently small, as seems to be the case with the Moon, convective processes rapidly lose their vigour and the mare structure is "frozen". On the other hand, with a somewhat larger body, as seems to be the case with the Earth, the less vigorous processes responsible for continental self-propulsion progressively become relatively more important as the dominance of penetrative convection diminishes. There is already rather good evidence for the dominance of penetrative convective processes in the early stages of the Earth from studies of the extensive areas of dome-like structures[6] in shield areas of Archaean age (3×10^9 yr BP).

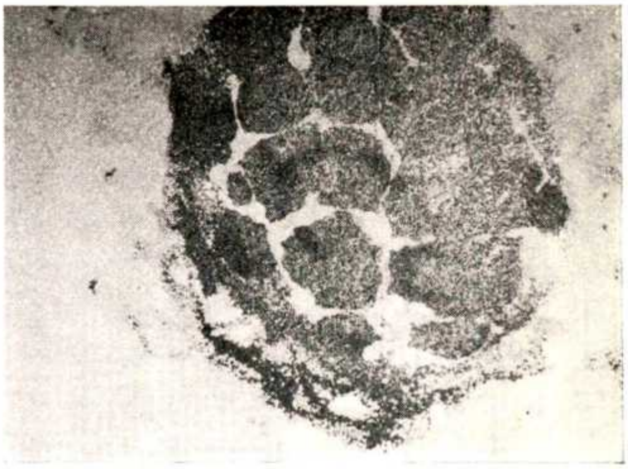


Fig. 4. Demonstration of the convective structure within the mare area, at an equivalent time of $0{\cdot}7 \times 10^9$ yr. Some extra powder was sprinkled on the surface just before the photograph was taken.

J. W. ELDER

Churchill College,
University of Cambridge.

Received September 10; revised November 19, 1969.

[1] Shoemaker, E. M., in *Physics and Astronomy of the Moon* (edit. by Kopal, Z.) (Academic Press, New York, 1962).
[2] Elder, J. W., in *Mantles of the Earth and Terrestrial Planets* (edit. by Runcorn, S. K.) (Interscience, London, 1967).
[3] Elder, J. W., *J. Fluid Mech.*, **32**, 69 (1968).
[4] Elder, J. W., *Sci. Prog. Oxford*, **56**, 1 (1968).
[5] Elder, J. W., *Nature*, **214**, 657 (1967).
[6] Salop, L. I., and Scheinmann, Tu. M., *Tectonophys.*, **7**, 567 (1969).

Symmetry of Crustal Earthquakes above Benioff Zones

INTERMEDIATE and deep earthquakes have received much attention since the establishment of the World Wide Standard Seismographic Network (WWSSN) seismograph stations and the introduction of the concepts of the "new global tectonics". The WWSSN is not suitable for studying the distribution of shallow earthquakes and the morphology of such earthquakes can best be defined with local networks. The excellence of the New Zealand local network and its position with respect to the seismicity of the Hikurangi active margin[1,2] allow shallow (and deeper) earthquakes to be located with considerable accuracy.

Fig. 1 shows the positions of seismograph stations in the northern half of New Zealand together with the epicentres of well determined shallow earthquakes (in which Pg or Sg is recognized) between 37° S and 34° S for the period January 1, 1963, to April 30, 1966. Most earthquakes occur within the seismograph network. The axis of the negative isostatic gravity anomalies which characterize the Hikurangi margin of New Zealand[3] is also shown in Fig. 1.

Fig. 2 gives the depths of all well determined[2] earthquakes for the same period and region. Crustal earthquakes are restricted to one of two depths: 12 km or 33 km. All shocks in which P^* or S^* phases are identified (but not Pg or Sg phases) are restricted to 33 km depth; because focal depth is an adjusted parameter in the

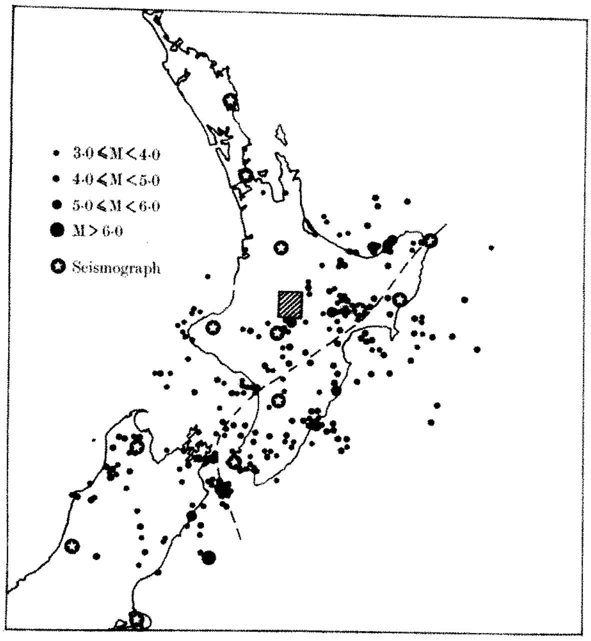


Fig. 1. New Zealand Seismograph Stations, shallow earthquakes from January 1, 1963, to April 30, 1966, between latitudes 37° and 43° S and axis of negative isostatic gravity anomalies (dashed line). Shaded area indicates many epicentres of Taupo earthquake swarm, 1964–5.

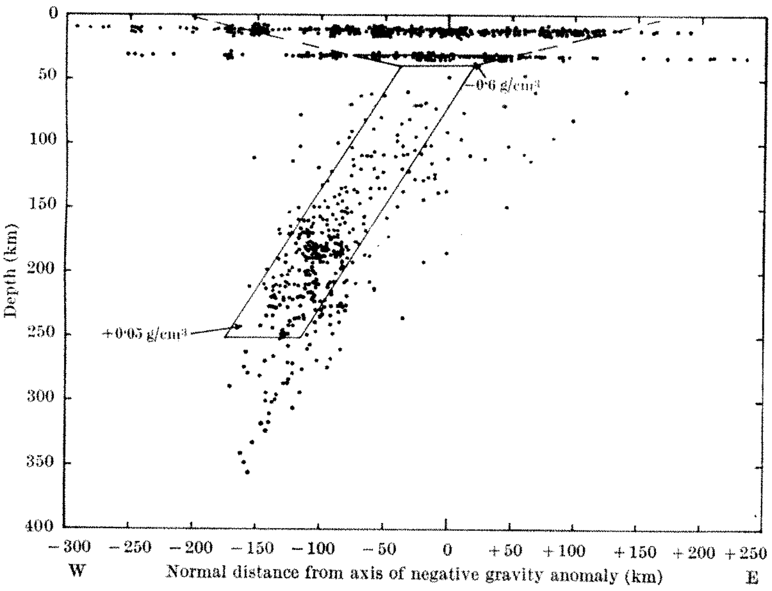


Fig. 2. Section of seismicity across Hikurangi Margin normalized to axis of negative gravity anomalies. Crustal downwarp model which fits gravity anomalies is superimposed on section. The Benioff zone is taken to have a density contrast of $+0\cdot05$ g/cm^3 with the mantle and the crust a density contrast of $-0\cdot6$ g/cm^3.

determination of the hypocentres, those shocks which do not have identified P^* or S^* phases but which have adjusted focal depths of less than 33 km are restricted to 33 km depth. Shocks below the crust are plotted at their computed depths.

The section has been produced by taking the normal distance of all epicentres from the axis of the negative gravity anomaly shown in Fig. 1. One of the models developed to explain the gravity anomalies of the Hikurangi Margin is superimposed on the section. These models have in common a high density region coincident with the Benioff zone to produce the positive anomaly[4], but low density regions can be disposed in several ways to produce the negative anomaly. The model in Fig. 1 is the "crustal downwarp" analogue in which the depth of the base of the crust is depressed by 8 km[1].

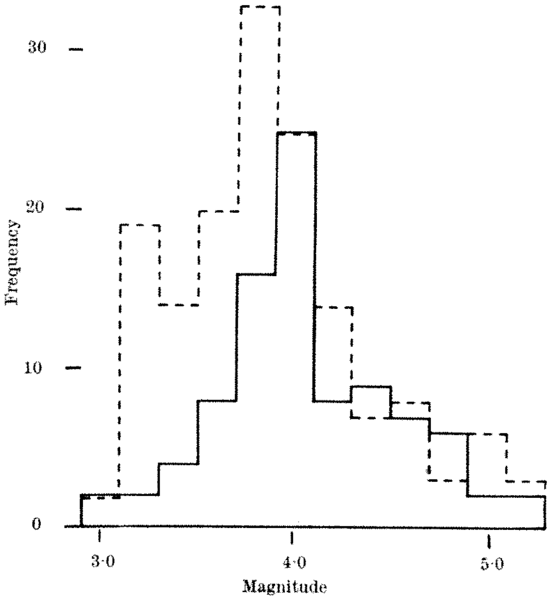


Fig. 3. Frequency histogram of magnitudes east of the gravity anomaly axis (full line) and west of the axis (dashed line). Shocks of Taupo earthquake swarm not included.

Crustal seismicity is roughly symmetrical about the negative gravity anomaly axis. More accurately, the axis of crustal seismicity coincides with the centre of the crustal downwarp or low density zone or, equally, with the centre of the upper surface of the high density zone. Moreover, the arms of the crustal downwarp envelop most of the crustal earthquakes, the spread of the 12 km earthquakes being greater than that of the 33 km earthquakes. It must also be borne in mind that 12 km and 33 km are maximum depths for these earthquakes.

Fig. 3 shows a histogram of magnitude frequencies of shallow shocks east and west of the negative gravity anomaly axis. Above magnitude $M=4\cdot0$ there is no discernible difference between the two sides. A much greater number of shocks of $M<4\cdot0$ occurs on the western side of the gravity axis, but this only indicates population distribution because the New Zealand Seismological Reports "contain *all* shocks of magnitude 4·0 and above . . . together with those shocks of lower magnitude . . . that have been reported felt".

The crustal seismicity of the Hikurangi margin is therefore essentially symmetrical, in contrast to the mantle seismicity. Such symmetry is perhaps unexpected if the cause of the earthquakes is sought in terms of overthrusting of lithospheric plates[5]. Rather, crustal earthquakes may be caused by what is effectively a gravitational loading of the crust.

TREVOR HATHERTON

DSIR, Geophysics Division,
Wellington,
New Zealand.

Received October 6, 1969; revised January 5, 1970.

[1] Hatherton, T., *J. Geophys. Res.*, **75** (in the press).
[2] Hatherton, T., *N.Z. J. Geol. Geophys.*, **13** (in the press).
[3] Reilly, W. I., *Gravity Map of New Zealand 1 : 4,000,000* (NZ DSIR, Wellington, 1965).
[4] Hatherton, T., *Nature*, **221**, 353 (1969).
[5] Isacks, B., and Molnar, P., *Nature*, **223**, 1121 (1969).

Molecular Oxygen as an Electron Trap in the Thermoluminescence of Saturated Hydrocarbons

MOLECULAR oxygen participates in organic thermoluminescence both as an electron trap and as a luminescence quencher[1–4]. Although the mechanisms of luminescence quenching have been discussed[5], the trapping process is only poorly understood. This communication reports new results which throw further light on the problem in saturated hydrocarbon matrices.

It was shown by Charlesby and Partridge[1–4] that when molecular oxygen is present in polyethylene, the thermoluminescence glow curve produced by warming samples previously subjected to low temperature gamma radiolysis exhibited an additional glow peak which disappeared when molecular oxygen was removed. This suggested that the peak originated in electron trapping on the oxygen molecules. The results presented here cover not only a wide range of both low and high density polyethylenes but also a series of saturated hydrocarbons having between five and twenty main chain carbon atoms.

The measurements were made using a technique which has already been described in an earlier report[6]. The essential features are a controlled slow rate of warming of 3 K per minute and a careful measurement of the sample temperature. Polyethylene samples, in the form of thin sheets 1/16 inch thick, were found to contain sufficient luminescent impurity introduced during the manufacturing process to provide an adequate number of luminescence centres. On the other hand, the paraffin hydrocarbons, obtained from Koch Light Laboratories, were purified in the laboratory using the method of Potts[7] and trace quantities of anthracene were added to provide the necessary luminescence centres.

Table 1. TEMPERATURES OF GLOW MAXIMA IN THE THERMOLUMINESCENCE GLOW CURVES OF THE LOW MOLECULAR WEIGHT *n*-ALKANES DOPED WITH ANTHRACENE

Sample	Molecular formula	Peak temperatures (K)
n-pentane	C_5H_{12}	120, 138
n-hexane	C_6H_{14}	102, 113, 160
n-heptane	C_7H_{16}	90, 108, 144, 180
n-octane	C_8H_{18}	94, 111, 186*, 215
n-nonane	C_9H_{20}	91, 108, 130, 170, 196, 208
n-decane	$C_{10}H_{22}$	93, 110, 135, 175*, 209
n-undecane	$C_{11}H_{24}$	91, 140, 190
n-dodecane	$C_{12}H_{26}$	98, 109, 163*, 215, 260
n-tridecane	$C_{13}H_{28}$	87, 92, 125, 135, 172
n-tetradecane	$C_{14}H_{30}$	90, 108, 160*, 176, 203, 269
n-pentadecane	$C_{15}H_{32}$	94, 190, 218, 233
n-hexadecane	$C_{16}H_{34}$	96, 102, 155*, 267
n-octadecane	$C_{18}H_{38}$	97, 105, 154*, 252
n-eicosane	$C_{20}H_{42}$	98, 107, 145*, 160, 204

Oxygen peaks are denoted by an asterisk.

All samples of polyethylene showed the oxygen glow peak at 145 ± 4 K. This was found to be independent of polymer molecular weight, degree of branching and consequently degree of crystallinity. The glow curves of the low molecular weight paraffins showed a number of glow peaks and the peak temperatures are summarized in Table 1. The oxygen concentration in these samples was reduced by bubbling the samples with oxygen free nitrogen gas for prolonged periods before irradiation. While not completely eliminating molecular oxygen, such treatment does reduce the oxygen concentration by displacement and so reduces the intensity of any glow peak caused by electron trapping on molecular oxygen. The attenuated peaks are indicated in Table 1 by an asterisk.

From Table 1 two features are immediately apparent. No oxygen peak is observed in any of the paraffins having an odd number of main chain carbon atoms. Also, the temperature at which the oxygen peak occurs in the even paraffins decreases with increasing molecular weight. This distinction between the even and odd paraffins is reflected in a number of the other physical properties of these compounds; for example, melting temperature[8] and mechanical relaxation mechanisms[9]. This effect is thought to originate in the different crystalline structures of the even and odd paraffins; the even paraffins have triclinic crystal symmetry while the odd paraffins are orthorhombic[10].

If the differences between the thermoluminescence properties of the even and odd paraffins are in fact due to the different crystalline structures, then it is expected that the temperatures of the thermoluminescence glow maxima of the oxygen peaks might follow the same type of molecular weight dependence as the other properties which behave in a similar manner. Broadhurst[8], for example, has examined the melting characteristics of these compounds and has shown that the melting temperature, T, follows a dependence on the number of main chain carbon atoms, n, of the form

$$T = T_0 \cdot \frac{n+a}{n+b}$$

where T_0, a and b are constants. Similarly Sinnott[11] has shown that the temperatures of occurrence of the mechanical relaxations in single crystals of polyethylene can be regarded as the extrapolation to infinite chain length of the relaxation temperatures observed in the low molecular weight paraffins observed by Illers[9] and obey a law of the same form as the equation above. Using such a law, the results for the thermoluminescence glow peak temperatures of the oxygen peak are plotted in Fig. 1 together with a theoretical plot of the equation with $T_0 = 145$, $a = -4{\cdot}66$ and $b = -5{\cdot}46$. The agreement is good, suggesting that this form explains the observed variation in the oxygen peak temperatures.

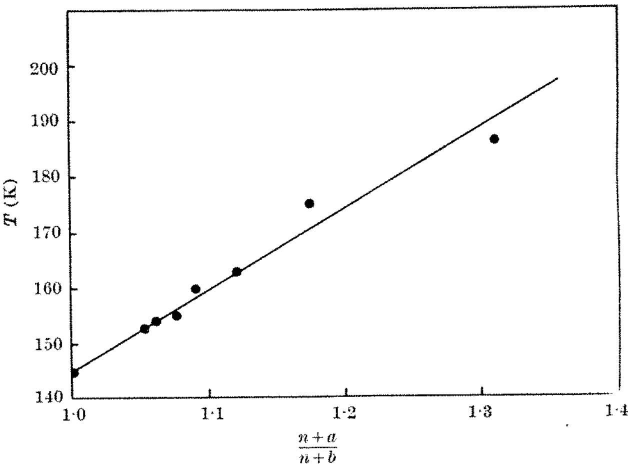


Fig. 1. Plot of oxygen peak temperature as a function of $(n+a)/(n+b)$, where n is the number of main chain carbon atoms, $a = -4{\cdot}66$ and $b = -5{\cdot}46$. Polyethylene is represented by putting $n = \infty$.

To find the physical meaning of this law it is necessary to consider the way in which molecular oxygen acts as an electron trap. The simplest form of such trapping which has been proposed[2] is the direct attachment of an electron to the oxygen molecule to form the negative ion, O_2^-. It is expected that the temperature of liberation of the attached electron will depend on the electron affinity of molecular oxygen. Vedeneyev[12] quotes a value of 0·87 ± 0·13 eV for the electron affinity by comparing several sets of data. When the approximation of Curie[13] is used for the untrapping of electrons from traps of fixed depth, this suggests that the molecular oxygen glow peak ought to occur in the temperature range 370 K to 500 K, which is considerably higher than any of the experimentally observed values. The experimental values therefore imply that the trap depth of the molecular oxygen traps in saturated hydrocarbon matrices is reduced from the electron affinity of oxygen to the observed value by an interaction between the matrix and the oxygen molecules. If such an interaction were dependent on molecular weight, then the variation would be expected to be greater at low molecular weights as the relative change produced by the addition of an extra main chain carbon is proportionally greater in the low molecular weight range. This is indeed observed.

Because of the similarity of the interaction with the variation in crystalline structure, it is clear that at some chain length the properties of the even and odd paraffins must converge. In terms of crystallinity this occurs at about twenty carbon atoms[8]. To test whether the oxygen peak also appears in the odd paraffins of similar chain length, a sample of highly purified $C_{35}H_{72}$ was tested and a weak oxygen peak was found at about 152 K. This showed that oxygen trapping does occur in odd paraffins and that the above law is obeyed when the crystal structures of the even and odd paraffins are similar. These results are therefore consistent with the view that electron trapping occurs on molecular oxygen by the formation of the negative ion O_2^- and that the trap depth is given by the electron affinity of molecular oxygen reduced by a factor determined by the matrix–oxygen interaction energy, which is dependent on molecular weight.

The exact nature of the interaction forces is, however, more difficult to ascertain, but some qualitative deductions can still be made. The oxygen may interact with either the $—CH_2—$ groups or the $—CH_3$ end groups. If the interaction is with the latter, then as the chain length increases the proportion of end groups relative to the remainder of the chain will decrease and the interaction will decrease. In fact the reverse is observed, indicating an interaction with the $—CH_2—$ groups.

Of the possible interaction forces available, only van der Waals and hydrogen bond forces are of the correct order of magnitude. Muller[14] has considered the van der Waals forces existing in the n-alkane chains; it has been found that such forces have an interaction energy of the order of 0·1 eV, which is somewhat below the value required to give the observed reduction in the oxygen trapping energy. Furthermore, such forces fall off according to an inverse sixth power law, which implies that the interaction energy would vary with molecular weight only to an appreciable extent in the very low molecular weight range. The results indicate, however, that although the depression of the trapping energy is proportionally less in the middle range of chain lengths it is easily measurable. Hydrogen bonding forces, on the other hand, which are caused by electrostatic polarization[15], fall off according to an inverse square law, and this coupled with the fact that such forces can be greater than the van der Waals forces suggests the origin of the observed interactions. Further work is at present being carried out with a view to ascertaining the exact nature of these interaction forces.

I. BOUSTEAD

Department of Physics,
Royal Military College of Science,
Shrivenham,
Near Swindon,
Wiltshire.

Received November 7, 1969; revised January 7, 1970.

[1] Charlesby, A., and Partridge, R. H., *Proc. Roy. Soc.*, A, **271**, 170 (1963).
[2] Charlesby, A., and Partridge, R. H., *Proc. Roy. Soc.*, A, **271**, 188 (1963).
[3] Charlesby, A., and Partridge, R. H., *Proc. Roy. Soc.*, A, **283**, 312 (1965).
[4] Charlesby, A., and Partridge, R. H., *Proc. Roy. Soc.*, A, **283**, 329 (1965).
[5] Kawaoka, K., Khan, A. U., and Kearns, D. R., *J. Chem. Phys.*, **46**, 1842 (1967).
[6] Boustead, I., and Charlesby, A., *Europ. Polymer J.*, **3**, 459 (1967).
[7] Potts, W. J., *J. Chem. Phys.*, **20**, 809 (1952).
[8] Broadhurst, M. G., *J. Chem. Phys.*, **36**, 2578 (1962).
[9] Illers, K. H., *Rheologica Acta*, **3**, 194 (1964).
[10] McClure, D. W., *J. Chem. Phys.*, **49**, 1830 (1968).
[11] Sinnott, K. M., *J. Appl. Phys.*, **37**, 3385 (1966).
[12] Vedeneyev, V. I. (editor), *Bond Energies, Ionization Potentials and Electron Affinities* (Arnold, 1966).
[13] Curie, D., *Luminescence in Crystals* (Methuen, 1960).
[14] Muller, A., *Proc. Roy. Soc.*, A**54**, 624 (1936).
[15] Coulson, C. A., *Valence* (Oxford University Press, 1956).

Electron Channelling Patterns from Small (10 μm) Selected Areas in the Scanning Electron Microscope

THE generation of electron channelling patterns in the scanning electron microscope (SEM) (refs. 1, 2) presents a unique method for obtaining crystallographic information from bulk crystals while viewing directly in the SEM. A technique for generating patterns from small selected areas has been described[3] and developed[4] to give electron channelling patterns from regions 50 to 100 μm in diameter. In this double deflexion rocking beam method the double deflexion scan system in the 'Stereoscan' SEM was modified by attenuating the current in the lower scanning coils so that the scanning crossover point, which normally occurs approximately in the plane of the final aperture (Fig. 1*a*), was lowered into the specimen chamber (Fig. 1*b*). A specimen could then be brought into coincidence with the lowered crossover point, and the variations in direction of incidence necessary to generate patterns occurred about a fixed point on the specimen surface.

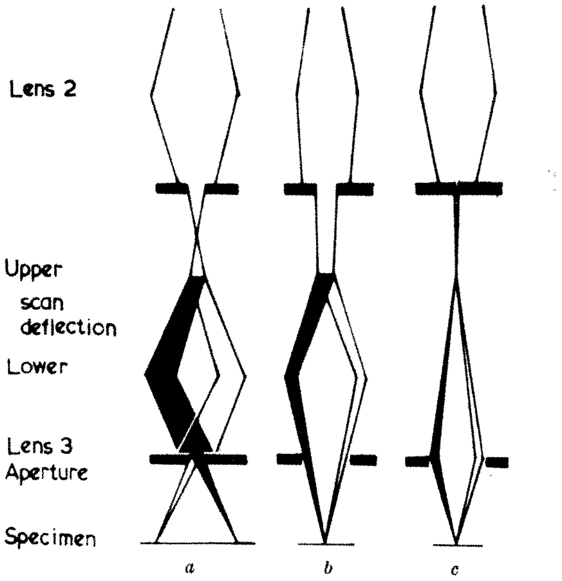


Fig. 1. Schematic beam diagrams of (*a*) standard scanning; (*b*) double deflexion rocking beam technique (method 1); and (*c*) deflexion focusing rocking beam technique (method 2).

A detailed analysis of the factors limiting the size of the selected area (to be published separately) showed that the double deflexion system behaved very much as a lens, possessing defects analogous to spherical aberration and astigmatism. These enlarge the crossover from a "point" the size of the probe to a "circle of confusion" of much larger diameter. By appropriately trimming the deflexion coils, the "astigmatism" type of defect can be reduced to negligible proportions; the "spherical aberration" type of defect can be reduced by superimposing a small correction signal on the main scan currents activating the coils.

By making these corrections, the circle of confusion was reduced to about 10 μm in diameter. Further reductions should be possible with better corrections. To illustrate the present state of development of the double deflexion technique, an electron channelling pattern was generated from a twin ~10 μm wide in an annealed copper polycrystal. The micrograph and pattern are shown in Fig. 2. Grain contrast in the micrograph is a direct result of the electron channelling mechanism, each grain essentially showing part of an electron channelling pattern.

Another method for obtaining a rocking beam has been developed, and can also be used to generate channelling patterns from selected areas 10 μm in diameter. Instead of using both upper and lower deflexions of the scanning system, a single deflexion is used in conjunction with the final lens. This lens "focuses" the scan and brings the beam back to a point on the optic axis of the SEM. We call this technique the deflexion focusing method, and it is illustrated schematically in Fig. 1*c*. Here the divergence of the beam, which must be kept low to display channelling patterns[3], is defined by means of an aperture replacing an existing splash aperture.

The deflexion focusing method gives similar results to the fully corrected double deflexion method: patterns can be obtained from grains 10 μm wide. The principal factor determining the size of the circle of confusion is the spherical aberration of the final lens. This could be overcome by means of correction waveforms applied to an auxiliary set of beam deflexion coils, and it should be possible to reduce the diameter of the selected area to 2–3 μm.

The operation of either method is very simple: the sample is examined under standard scan (corresponding to Fig. 1*a*) and the area of interest is brought to a predetermined spot on the screen. Switching to rocking beam (corresponding to Fig. 1*b* or *c*) causes a channelling pattern from the area of interest to appear on the screen.

The patterns are limited in angular width to about 10° by the dictates of microscope geometry. This is sufficient for a unique identification of the crystal orientation, which can either be performed analytically[5] or more easily by comparison with a map of channelling patterns of all possible orientations. The crystallographic orientation (relative to the optic axis of the SEM) of any grain, subgrain, precipitate, particle and so on, down to 10 μm in diameter on the surface of a solid specimen, can thus be determined to an accuracy of better than 10^{-2} radian.

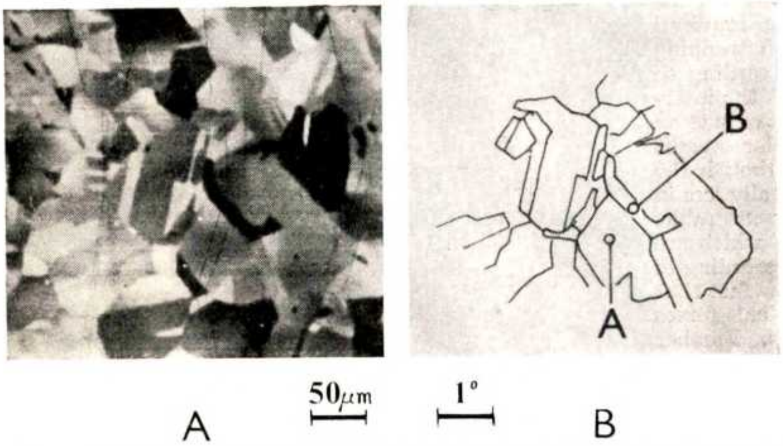


Fig. 2. Electron channelling pattern from a small twin in an annealed copper polycrystalline specimen using method 1. (Beam current was ~10^{-7} A, divergence ~5×10^{-3} radians, and diameter ~5 μm.

We thank Dr G. R. Booker and Mr D. C. Joy for valuable discussions.

C. G. VAN ESSEN
E. M. SCHULSON
R. H. DONAGHAY

Department of Metallurgy,
University of Oxford.

Received January 14, 1970.

[1] Coates, D. G., *Phil. Mag.*, **16**, 1179 (1967).

[2] Booker, G. R., Shaw, A. M. B., Whelan, M. J., and Hirsch, P. B., *Phil. Mag.*, **16**, 1185 (1967).

[3] Schulson, E. M., and van Essen, C. G., *J. Sci. Instrum.*, **2**, Ser 2, 247 (1969).

[4] van Essen, C. G., and Schulson, E. M., *J. Materials Sci.*, **4**, 336 (1969).

[5] Schulson, E. M., *J. Sci. Instrum.*, **2**, Ser 2, 361 (1969).

Migration of a Neutrally Buoyant Particle in Poiseuille Flow: a Possible Explanation

MANY theoretical and experimental investigations have been made into particle migration in laminar tube flow. Segré and Silberberg[1,2] noted that all the particles in a neutrally buoyant suspension of rigid spheres flowing down a vertical tube congregate in about 0·6 of the tube radius, and Maude and Yearn[3] reported precise experimental evidence for another high concentration zone near to the tube wall, which they term the "wall peak". Rubinow and Keller[4] explained the occurrence of high concentration zones by deriving the transverse force on a spinning sphere. It was subsequently shown by Saffman[5] that the lift force on a freely rotating particle caused by rotation is insignificant, and the radial velocity of the particle is given by

$$\frac{V_r}{U_0} = -6\cdot1\left(\frac{a}{R}\right) Re_{\mathrm{T}}^{\frac{1}{2}} \left(\frac{r}{R}\right)^{\frac{1}{2}}\left(\frac{\Delta V_z}{U_0}\right) \quad (1)$$

where U_0 is the maximum fluid velocity, a the particle radius, R the tube radius, Re_{T} the Reynolds number $(=U_0R/\nu$, ν is the kinematic viscosity), r the radial position of the particle and ΔV_z the axial relative velocity. If the particle lags behind the fluid, $\Delta V_z>0$ and the radial migration is towards the tube axis. The relative velocity ΔV_z cannot directly be applied for this purpose, but Repetti and Leonard[6] have introduced the effective fluid velocity caused by the presence of the particle, and they explained the Segré–Silberberg effect, using the axial particle velocity obtained experimentally by Goldsmith and Mason[7].

This communication provides a possible expression for the relative velocity, ΔV_z, and explains the occurrence of the high concentration zone in Poiseuille flow of a neutrally buoyant suspension. It is assumed that the particle tends to minimize disturbances in the flow tube caused by its own presence.

According to Vand[8], the velocity gradient G of the fluid is disturbed by interaction between a sphere and a rigid wall, and the effective velocity gradient G' at the centre of the sphere becomes

$$G' = G(1 - \varphi) \qquad (2)$$

where

$$\varphi \doteqdot \frac{5}{16}\left(\frac{a}{R-r}\right)^3 - \frac{1}{8}\left(\frac{a}{R-r}\right)^5$$

and Goldsmith and Mason[7] have observed that the rate of rotation of single spheres is approximately $G'/2$ in place of $G/2$. Because the gradient G' acts on the particle rotation, we postulate that the remainder $(G - G')$ acts on the migration, and the particle should move with a velocity frame

$$V_z = U + \int (G - G')\mathrm{d}r + C \qquad (3)$$

where

$$U = U_0\left[1 - \left(\frac{r}{R}\right)^2\right]$$

$$G - G' = G\varphi = -\frac{2U_0 r}{R^2}\varphi$$

U is the fluid velocity and C the constant of integration. To minimize the disturbance for the parabolic velocity profile of the fluid, the following relation is assumed for the velocity frame

$$\int_0^R V_z\,\mathrm{d}r \left(= \int_0^{R-a} V_z\,\mathrm{d}r + V_z \Big|_{r=R-a} \times a \right) = \int_0^R U\mathrm{d}r \qquad (4)$$

The velocity frame, V_z, can be obtained from equations (3) and (4), but, to avoid duplication, it is not shown here.

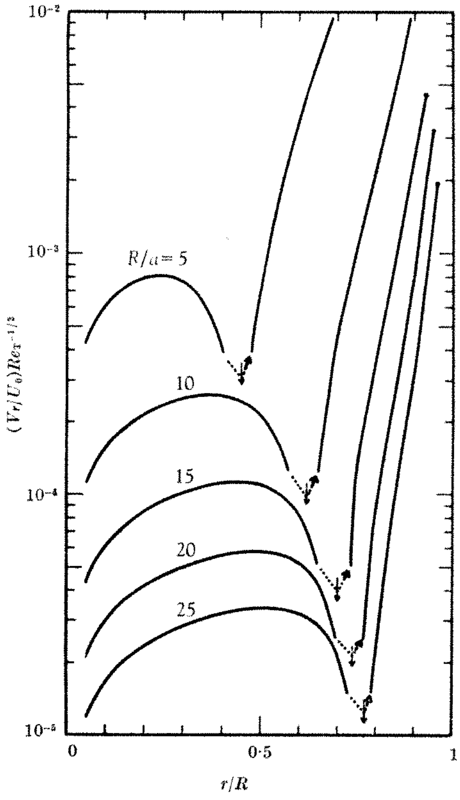


Fig. 1. Radial velocity of a neutrally buoyant particle. The right-hand side of the broken arrows is the negative velocity region and the left-hand side (convex curves) is positive.

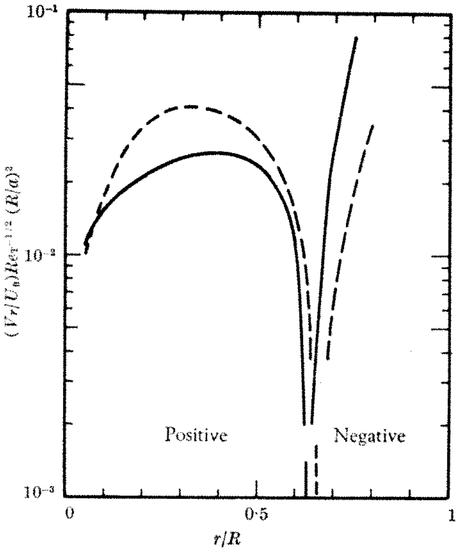


Fig. 2. Comparison of the calculated result (full curve) with the data line of Jeffrey and Pearson (broken curve). $R/a = 11{\cdot}1$.

In the absence of particle and wall interactions, a freely suspended particle lags behind the fluid by $(2/3)U_0(a/R)^2$. This slip velocity is caused by the particle being unable to accommodate itself perfectly to the curvature of the Poiseuille field[9-11]. Accordingly, the fluid velocity effectively acting on the particle is

$$U_{\text{eff}} = U - \frac{2}{3}U_0\left(\frac{a}{R}\right)^2 \qquad (5)$$

The effective relative velocity therefore becomes

$$\frac{\Delta V_z}{U_0} = \frac{U_{\text{eff}} - V_z}{U_0} = -\frac{5P^3}{8}\left(\frac{1}{1-r/R}\right)^3 + \frac{5P^5}{16}\left(\frac{1}{1-r/R}\right)^2$$
$$+ \frac{P^5}{12}\left(\frac{1}{1-r/R}\right)^3 - \frac{P^5}{16}\left(\frac{1}{1-r/R}\right)^4 - \frac{5P^2}{24} \qquad (6)$$
$$+ \frac{7P^3}{48} - \frac{5P^3}{8}\ln P + \frac{P^5}{48}$$

where $P = a/R$.

The particle entering a mouth of the flow tube congregates at a radial point where $\Delta V_z = 0$ (see Table 1). The results calculated from equation (6) agree well with the experiments of Segré and Silberberg[1,2] ($R/a \doteqdot 6 \sim 15$), Oliver[12] ($R/a \doteqdot 4$), and Jeffrey and Pearson[13] ($R/a \doteqdot 11 \sim 22$). The stable point shifts towards the tube wall as R/a increases. The radial particle velocity can be derived by substituting equation (6) into (1), as shown in Fig. 1. The radial velocity becomes zero at the tube axis and at a point listed in Table 1. The tube axis is the unstable equilibrium point at which no particle congregates. Despite including conditions to which Saffman's equation does not apply, Fig. 2 agrees well with the radial velocity calculated from equation (1), and the data line of Jeffrey and Pearson[13]. Although equation (6) includes the above assumption, it explains the migratory phenomena of the particle fairly well.

Table 1. STABLE POINT WHERE $\Delta V_z = 0$

R/a	4	5	10	15	20	22	25
r/R	0·40	0·45	0·62	0·70	0·74	0·75	0·77

For small particle and/or low Reynolds number, the particle migration becomes slow, and they will not congregate at a point in a tube of finite length. When the

Reynolds number is large and the particle is small, it is probable that only particles placed near the tube wall migrate prominently. For example, in the experiment of Maude and Yearn[3], in which $Re_T \doteqdot 600$ and $R/a \doteqdot 20$, the negative maximum velocity is fifty-five times as large as the positive one (see Fig. 1). Even if the outward migration is insignificant, it is therefore probable that the particle placed near the tube wall migrates inwardly by a short distance, and the prominent migration ceases in a short time. This will cause the "wall peak".

The above analysis gives the relative velocity effectively acting on a neutrally buoyant particle in Poiseuille flow and, applying it to Saffman's equation[5], the radial particle velocity can be calculated. The result explains the occurrence of the high concentration zone fairly well and the wall peak seems to be a phenomenon similar to the Segré–Silberberg effect.

I thank Mr Hikaru Nagashima for help with the early stage of this work.

KEISHI GOTOH

Department of Chemical Process Engineering,
Hokkaido University,
Sapporo, Japan.

Received June 30; revised November 28, 1969.

[1] Segré, G., and Silberberg, A., *Nature*, **189**, 209 (1961).

[2] Segré, G., and Silberberg, A., *J. Fluid Mech.*, **14**, 115 (1962).

[3] Maude, A. D., and Yearn, J. A., *J. Fluid Mech.*, **30**, 601 (1967).

[4] Rubinow, S. I., and Keller, J. B., *J. Fluid Mech.*, **11**, 447 (1961).

[5] Saffman, P. G., *J. Fluid Mech.*, **22**, 385 (1965).

[6] Repetti, R. V., and Leonard, E. F., *Nature*, **203**, 1346 (1964).

[7] Goldsmith, H. L., and Mason, S. G., *J. Colloid Sci.*, **17**, 448 (1962).

[8] Vand, V., *J. Phys. and Colloid Chem.*, **52**, 277 (1948).

[9] Simha, R., *Kolloid-Z.*, **76**, 16 (1936).

[10] Happel, J., and Byrne, B. J., *Ind. Eng. Chem.*, **46**, 1181 (1954).

[11] Greenstein, T., and Happel, J., *J. Fluid Mech.*, **34**, 705 (1968).

[12] Oliver, D. R., *Nature*, **194**, 1269 (1962).

[13] Jeffrey, R. C., and Pearson, J. R. A., *J. Fluid Mech.*, **22**, 721 (1965).

[14] Denson, C. D., Christiansen, E. B., and Salt, D. L., *J. Amer. Inst. Chem. Eng.*, **12**, 589 (1966).

[15] Cox, R. G., and Brenner, H., *Chem. Eng. Sci.*, **23**, 147 (1968).

BIOLOGICAL SCIENCES

Virus of the 1918 Influenza Pandemic

THE results of tissue culture neutralization of influenza viruses with sera obtained from ten persons from a remote Pacific island (Fais in the Western Caroline Group) have been described[1]. The persons concerned were alive in the decade before a devastating epidemic of influenza on the island in 1924, believed to have been a residual wave of 1918-type influenza. The ten sera contained no neutralizing antibody to strains of the influenza A1, A2 and equine virus, but did neutralize both human influenza AO viruses (PR8, WS and BH) and swine virus. This suggested that Fais has escaped influenza in the past twenty-five years. Because the titre of antibodies was quantitatively greater against the PR8 and BH—though not WS—than against swine virus, the authors conclude that 1918 influenza was more likely to have been caused by a virus related to the influenza AO group than to the swine virus.

In 1936, sera were examined in London for influenza antibodies in islanders from St Helena and also from Tuitula and Apia in Samoa, who were alive in 1918. Both areas were selected because historical[2] and local records confirmed that no epidemic of influenza occurred there in the 1918–19 period. The findings relating to the St Helena islanders were published[3] but not those from the Pacific. The Samoan sera were obtained by the late Sir Patrick Laidlaw from Dr P. J. Monaghan of the Western Samoan Administration, and from this information it was suggested that Pacific islanders who escaped through quarantine in the 1918 epidemic did become infected from 1920 onwards as pointed out by Brown *et al.*[1].

Sera were examined by inoculating mice intranasally with dilutions of serum and mouse lung filtrates from mice infected with either the WS or the Shope (1931) swine influenza virus. Hyperimmune horse sera prepared from each of the viruses and of similar homologous potency were included in the test. The results of the lung tests from the groups of mice are expressed in terms of the neutralization titre of each standard horse serum. Table 1 presents the distribution of antibody titres obtained with both viruses. More than half the sera possessed antibodies to swine virus, but eight of the fourteen sera did not neutralize the WS virus. In the table, S/5 represents a titre one-fifth that of the standard antiserum, and is the most potent serum that was found.

It is clear that the antibody titres against swine influenza virus were greater than those against the WS virus. The results are comparable with those of the sera obtained from St Helena islanders in 1935. Of the twenty-three islanders from St Helena aged 35 to 74 and bled in 1935, however, twenty-two were again sampled in 1936 by Dr P. B. Wilkinson of the island's health department. In the interval, a considerable epidemic of influenza occurred on the island. Table 2 shows the results of mouse lung neutralization tests with the two sets of sera using the WS and swine viruses expressed, as before, in terms of the homologous standard.

Table 1. MOUSE LUNG NEUTRALIZATION TESTS WITH FOURTEEN SAMOAN SERA COLLECTED IN 1936

	Number of sera with titres in each range					
Viruses	S/5	S/10	S/25	S/50	S/125	< S/125
WS	—	1	1	—	4	8
Shope	3	1	4	2	1	3

Table 2. ST HELENA SERA IN 1935 AND 1936 MOUSE LUNG NEUTRALIZATION TESTS

		Number of sera with titres in each range							
Virus	Collection date	> S/5	S/5	S/10	S/25	S/50	S/125	< S/125	Totals
WS	1935	—	—	1	—	4	6	12	23
	1936	2	—	10	3	3	4	—	23
Shope	1935	—	1	—	5	6	10	1	23
	1936	2	5	8	1	5	1	—	22

It is clear that the 1936 epidemic of influenza enhanced the antibodies to both viruses and the final titres against swine virus were at least as high or higher than the titres against WS virus. This suggested that the swine virus antibodies in the Samoan and the original St Helena sera resulted from some previous infection with a virus related to swine virus, and were not merely heterologous.

The results obtained in 1936 with both the sera from Samoa and St Helena showed that the distribution of anti-swine virus antibodies found in remote island groups said to have escaped the 1918 influenza were surprisingly similar to those obtained at the same time from persons living in London or in the United States and who were alive in 1918[4,5]. The fact that anti-swine virus antibodies were present in adult islanders in substantial amount and were boosted by influenza of the AO variety suggested that they were antibodies resulting from some previous infection, possibly of the 1918–20 epoch. The absence of an epidemic of influenza during the great pandemic does not rule out the possibility of subsequent infection during the 1920s with re-circulating virus of the same serological type as the 1918 influenza. Brown *et al.*[1] prefer the explanation that anti-swine virus antibodies in Fais islanders are heterologous antibodies arising from antigens of the type A viruses related to those of swine virus, but the evidence from the anti-swine virus antibodies in the European and American sera is against this interpretation.

In 1935, it was found[4] that children under ten born in London after 1925 had no demonstrable neutralizing antibodies to swine virus. Low titres were found in those aged ten to fourteen and higher titres were found in most of those older than twenty. Similar results were obtained by Shope[5] and Francis and Magill[6] with American sera and in Australia by Burnet and Lush[7]. Using pooled sera that had been collected in 1953 and 1954 from Sheffield and Ann Arbor, Michigan, and tested by the haemagglutination method, Davenport *et al.*[8] found that persons under thirty years of age had no swine virus antibodies, but peak titres were present in sera from persons aged thirty-five to forty-five. Masurel and Mulder[9] similarly tested individual sera from persons of different ages collected in 1957 and 1958. Though low titres of swine antibodies were found in sera from 10 to 40 per cent of children and young adults, high titres were found in most sera from those aged thirty and over and there was a peak in persons aged forty to fifty. In 1964, Schild and Stuart-Harris[10], using individual sera collected in 1961–2, found very low titres of H1 antibody to swine virus in sera from persons less than thirty, and peak titres in the fifty to sixty age group. This evidence strongly favours a cohort of persons aged between twenty and thirty in 1935 and forty to fifty in 1955 who continue to possess high titres of anti-swine antibodies. It does not favour a slowly rising percentage of persons in any one year with swine antibodies resulting from repeated infections with the various human influenza viruses. This does not preclude the fact that heterologous antibodies appear after infection with any one strain of influenza virus. The evidence suggests that, in the case of influenza viruses, childhood infection orients the antibody response to subsequent infection by later viruses as first suggested by Francis *et al.*[11].

The data published by Philip and Lackman[12] using sera from Alaskan villagers where influenza either did or did not occur in 1918 also strongly confirm the hypothesis that the presence of anti-swine antibodies in human sera results from exposure to an influenza virus closely related to the swine influenza virus which was circulating in the 1918 and early 1920 period. The view[1] that the 1918 influenza virus was one of the human AO type is inadequate to explain the accumulated evidence for the presence of swine virus antibodies in human sera.

C. H. STUART-HARRIS

Department of Medicine,
University of Sheffield,
The Royal Hospital,
Sheffield.

Received December 9, 1969.

[1] Brown, P., Gajdusek, D. C., and Morris, J. A., *Science*, **166**, 117 (1969).
[2] *Report on the Pandemic of Influenza 1918–1919* (Reports on Public Health and Medical Subjects No. 4) (HMSO, London, 1920).
[3] Stuart-Harris, C. H., Andrewes, C. H., and Smith, W., *MRC Spec. Rep. Series*, No. 228 (HMSO, London, 1938).
[4] Andrewes, C. H., Laidlaw, P. P., and Smith, W., *Brit. J. Exp. Path.*, **16**, 566 (1935).
[5] Shope, R. E., *J. Exp. Med.*, **63**, 669 (1936).
[6] Francis, jun., T., and Magill, T. P., *J. Exp. Med.*, **63**, 655 (1936).
[7] Burnet, F. M., and Lush, D., *Brit. J. Exp. Path.*, **19**, 17 (1938).
[8] Davenport, F. M., Hennessy, A. V., Stuart-Harris, C. H., and Francis, jun., T., *Lancet*, **2**, 469 (1955).
[9] Masurel, N., and Mulder, J., *Verh. Inst. Prev. Geneesk.*, **52** (Batteljee and Terpstra, Leiden, 1962).
[10] Schild, G., and Stuart-Harris, C. H., *J. Hyg. (Camb.)*, **63**, 479 (1965).
[11] Francis, jun., T., Davenport, F. M., and Hennessy, A. V., *Trans. Assoc. Amer. Phys.*, **66**, 231 (1953).
[12] Philip, R. N., and Lackman, D. B., *Amer. J. Hyg.*, **75**, 322 (1962).

Comparison of the Fates of Ag–B Locus Compatible Homografts of Skin and Hearts in Inbred Rats

TISSUES and organs are not all equally vulnerable to transplantation immunity. Renal homografts sometimes succeed in conditions in which homografts of skin would have failed[1] and there is suggestive evidence that liver grafts may be less exacting in their genetical requirements than those of kidney, especially in pigs[2]. A few reports during the early days of human cardiac transplantation prompted speculation that this organ might be superior to kidney with regard to its capacity to override histoincompatibility barriers. It was suggested that: (1) because the heart is essentially a muscular pump, without complex metabolic functions, it might be less vulnerable to immunological insult than kidney; and (2) being composed chiefly of muscle and devoid of epithelial components, hearts might be "less antigenic" than organs with large amounts of epithelial tissue such as kidneys.

In the light of the recent results of clinical cardiac grafting, the status of the human heart as a possible partially privileged organ has become difficult to uphold. That there is probably nothing immunologically unique about the cardiac transplant had previously been suggested by Shumway and Lower's finding that the functional survival time of cardiac homografts in non-immunosuppressed dogs is only 7 days[3], and that auxiliary rat heart grafts[4] across the major, Ag–B, histocompatibility locus in this species are rejected as promptly as homografts of skin, liver[5] and pancreas[6].

It is, however, generally agreed that the most favourable situation for revealing the existence of possible differential histocompatibility requirements is that which obtains when donor and recipient are compatible at the major locus for the species concerned. White and Hildeman[7] and Mahabier *et al.*[8] have reported that kidney homografts may enjoy prolonged (if not permanent) survival in inbred rats where Ag–B compatibility exists—for example, with the Fischer→Lewis combination—even though skin homografts are rejected promptly. By contrast, Freeman and Steinmuller[9] have recently reported that Lewis rats reject Fischer heart homografts with essentially the same vigour as skin. Freeman and Steinmuller's data are in agreement with our own previously reported findings[10] in regard to Fischer hearts in Lewis hosts. These authors interpret their study as indicative that the heart (unlike the kidney) is as susceptible to rejection as skin where minor histoincompatibilities exist. We present evidence here which challenges this generalization. We have recently found that when Fischer rats are used as the recipients, Lewis heart homografts display striking prolongation of survival as compared with those of skin.

The subjects of the work to be described were adult rats, weighing 250–450 g, of the inbred Lewis and Fischer strains, obtained from Microbiological Associates, Inc., Walkersville, Maryland. These two strains of rats are known to have similar alleles at the Ag–B locus, though they differ with respect to antigens determined by at least four other H-loci (our unpublished results).

To determine the survival times of skin homografts, animals of similar sex from these strains were paired off at random and two trimmed grafts of belly skin, 1·5–2·0 cm in diameter, were exchanged between them. Following primary inspection on the sixth day after the operation, the grafts were scored daily to determine their survival times, according to our standard procedures.

The auxiliary hearts were transplanted by the technique of Ono *et al.*[11]. The donor pulmonary artery was anastomosed to the recipient's inferior vena cava and the donor aorta to the recipient's abdominal aorta. The donor pulmonary veins and venae cavae were ligated. Thus the direction of blood flow was from recipient aorta into donor aorta and thence into the coronary arteries. After perfusing the myocardium, blood returned to the right side of the heart and was pumped into the host inferior vena cava. Ischaemia times were 60–90 min. Within 1–2 min of revascularization the hearts began to beat spontaneously and vigorously. The beating hearts could be easily palpated through the anterior abdominal wall in the unanaesthetized animals and this proved to be the most convenient means of assessing function daily. Electrocardiograms and surgical exploration were carried

Table 1.

Donor–host strain combination	Type of graft	No. of hosts studied	Skin graft survival times (days)	Functional survival times of hearts (days)	Absolute survival times of hearts (days)
Fischer→Lewis	Skin	10	7, 2 × 8, 9, 4 × 10, 2 × 11 (MST: 10·0 ± 0·8; *S.D.* 1·15)		
Fischer→Lewis	Heart	12		2 × 7, 4 × 8, 5 × 9, 11 (MST: 9·0 ± 0·5; *S.D.* 1·08)	2 × 8, 6 × 9, 10, 13, 15, 22
Fischer→Lewis (presensitized)	Heart	4		2 × 4, 7, 8	
Lewis→Fischer	Skin	10	9, 3 × 12, 13, 3 × 14, 16, 18 (MST: 13·8 ± 1·5; *S.D.* 1·18)		
Lewis→Fischer	Heart	10		7, 9, 12, 14, 16, 23, 24, 25, 26, 27 (MST: 18·6 ± 6·0; *S.D.* 1·66)	8, 11, 13*, 15, 18, 27, 50*, 61*, 88*, 100*
Lewis→Lewis	Heart	6		6 × 100	

* In the L→F heart homografts absolute survival time was shortened in these instances by removing the heart (for histology) while it was still beating weakly.

out when doubt existed as to the status of the transplanted hearts.

The results of preliminary skin graft exchanges between animals of the same inbred strain confirmed their isohistogenic status, and the survival of all of six Lewis→Lewis heart grafts for 100 days afforded evidence that the operative technique used was adequate to meet the metabolic and physiological requirements of this organ.

The experiments carried out and their results are summarized in Table 1. Fischer skin grafts on Lewis hosts were rejected more rapidly than skin transplanted in the opposite direction, the median survival times (MSTs) of the two series of skin grafts being 10·0 ± 0·8 days and 13·8 ± 1·5 days respectively.

The first indication of incipient rejection of a cardiac homograft was a decline in the forcefulness of what had previously been a palpable, strong, regular beat. Within the next 24–48 h there was usually either total cessation of heart beat or the beat became so feeble that it was no longer detectable with certainty by palpation.

In either case the animal's abdomen was explored surgically at this time. When direct observation of the heart confirmed either the complete absence of pulsatory activity or only very weak activity, this was scored as the "functional end point", for the organ's activity had fallen below the level needed to support the circulation and sustain life had it been transplanted orthotopically rather than as an accessory. In most instances the heart was removed at this time for histological study, because it clearly no longer had haemodynamic significance.

With the Fischer→Lewis combination when observation of cardiac homografts was continued beyond their "functional" end points, contractile activity of the myocardium and electrical activity on the EKG ceased completely within a few days. The "functional" and absolute survival times of the transplanted hearts were indistinguishable from the survival times of skin grafts.

The fate of the Lewis→Fischer heart transplants, however, proved to be more interesting from the immunological point of view. Four out of ten such hearts continued to beat weakly long after the functional end point was reached, and in fact displayed electrocardiographic activity and rhythmic myocardial contractility even at the time of their eventual removal for histological study, 50–100 days after grafting. Hearts removed at either the functional end point or after all signs of contractility had ceased showed typical mononuclear cell infiltration of the myocardium, though this tissue usually appeared to have a high degree of survival. With this combination, both "functional" and absolute survival times of heart transplants proved to be significantly longer than skin graft survival time.

It is interesting to note that Fischer hearts, transplanted to Lewis hosts specifically presensitized by bilateral grafting with Fischer skin, continued to beat vigorously for 4–8 days, a surprisingly long time in view of the fact that the vascular supply in these organ grafts was immediately restored surgically and did not require 2–3 days for regeneration as with skin grafts.

The findings with the Fischer→Lewis combination lend no support to the thesis that hearts are less vulnerable to transplantation immunity than skin. Lewis hearts in Fischer rats did, however, enjoy a slightly but significantly longer "functional" survival time than skin grafts, and the total survival time (with weak beats) of the Lewis hearts in Fischer hosts was in some cases 50–100 days. This finding is similar to that of White and Hildeman's with Fischer kidneys in Lewis hosts. As in the case of our heart transplants, White and Hildeman's kidney homografts, though enjoying prolonged survival, did not continue to function normally, as evidenced by the uraemia developing in their hosts. It is possible that the immunosuppressive effects of the uraemia may have helped in further prolonging graft survival.

The asymmetry in MSTs of exchanged skin grafts suggests that the histoincompatibility barrier confronting Fischer grafts in Lewis rats is a more formidable one than in the reverse direction, though both MSTs depend on antigens determined by minor loci. The heart homograft results reported here are also in keeping with this thesis. We do not know of any study in which kidneys have been transplanted from Lewis to Fischer rats, but in the opposite direction kidneys enjoy prolonged survival while hearts and skin do not. We are currently engaged in comparing the survival of skin and hearts taken from [(Fischer × Lewis) F_1 × Lewis] R_2 backcross donors transplanted to Lewis recipients, each donor giving its heart to one host and a skin graft to another. This allows the fates of these two types of graft to be compared over a wide range of histoincompatibility. In all such experiments so far (more than twenty pairs), the heart has survived longer than the skin and in many instances by more than 100 days.

We conclude from these findings that heart transplants across a histoincompatibility barrier other than Ag-B are less vulnerable to rejection than skin grafts, but it seems likely that they are more susceptible than kidney grafts.

We thank Mr George Sawchuck for technical assistance. The work was supported in part by a US Public Health Service grant. C. F. B. is a Markle scholar in academic medicine.

CLYDE F. BARKER
R. E. BILLINGHAM

Departments of Medical Genetics and Surgery,
University of Pennsylvania,
Philadelphia, Pennsylvania.

Received November 13, 1969.

[1] Murray, J. E., Sheil, A. G. R., Moseley, R., Knight, P., McGaric, J. D., and Dammin, G. J., *Ann. Surg.*, **160**, 449 (1964).
[2] Calne, R. Y., Sells, R. A., Pena, J. R., Davis, D. R., Millard, P. R., Herbertson, B. M., Binns, R. M., and Davies, D. A. L., *Nature*, **223**, 472 (1969).
[3] Lower, R. R., Dong, jun., E., and Shumway, N. E., *Surgery*, **58**, 110 (1965).
[4] Abbott, C. P., Lindsey, E. S., Creech, jun., O., and DeWitt, C. W., *Arch. Surg.*, **89**, 645 (1964).
[5] Lee, S., and Edgington, T. S., *Amer. J. Path.*, **52**, 649 (1968).
[6] Reemtsma, K., Giraldo, N., Depp, D. A., and Eichwald, E. J., *Ann. Surg.*, **168**, 436 (1968).
[7] White, E., and Hildeman, W. H., *Science*, **162**, 1293 (1968).
[8] Mahabier, R. N., Guttman, R. D., and Lindquist, R. R., *Transplantation*, **8**, 369 (1969).
[9] Freeman, J. S., and Steinmuller, D., *Transplantation*, **8**, 530 (1969).
[10] Barker, C. F., and Billingham, R. E., *J. Plastic and Reconstr. Surg.*, **44**, 20 (1969).
[11] Ono, K., Lindsey, E. S., and Creech, jun., O., *Transplantation*, **1**, No. 3, 176 (1969).

Radioimmune Assay for Histocompatibility Antigens

A MAJOR obstacle in histocompatibility testing has been the need for whole cells as the final target for antiserum action, for example, haemagglutination, complement fixation or complement-dependent cytotoxicity. Catt and Tregear[1] have reported that antibody adsorbed irreversibly onto polystyrene test tubes can specifically bind isotope-labelled antigen in nanogram amounts. We have applied a modification of their test to the assay of mouse transplantation antigens.

All operations were carried out at room temperature. One ml. of a dilution of serum in 0·05 M carbonate buffer, *p*H 9·6, was added to polystyrene (12 × 75 mm) test tubes (Falcon Plastics) and incubated for 1 h in an upright position. The antiserum was removed by suction and the tubes were rinsed twice with saline. 0·4 ml. of a solution of bovine serum albumin (25 mg/ml.) in 0·15 M saline, 0·01 M phosphate buffer at *p*H 7·2 was added to the tubes. The tubes were rotated in a tissue culture roller drum at 20 r.p.m. for 30 min. The solution was removed by suction and 0·25 ml. of a solution of the same composition, containing ^{125}I-labelled antigen, was added. Incubation was continued in the roller drum for another 3 h. After the tubes were rinsed three times with saline, the amount of ^{125}I bound to the empty tubes was determined in an automatic gamma counter. All assays were performed in triplicate. The presence of 25 mg bovine serum albumin minimized the non-specific adsorption of ^{125}I-labelled proteins onto the plastic.

The antiserum used was developed in C57Bl/10 SnJ mice inoculated with the DBA/2 lymphoblast L-5178Y microsomal lipoprotein fraction (MLP), which has been shown to be a potent source of transplantation antigens, both H2 and non-H2[2,3].

The MLP was labelled according to the method of Hunter and Greenwood[4]. A fresh, dialysed MLP preparation (20 µl. containing 50 µg protein) was iodinated with 2 mCi of carrier-free ^{125}I (Amersham Searle). The MLP being insoluble, the unused ^{125}I was removed by dialysis (4 × 100 ml.) over a 2 day period in the cold.

The extent of specific binding of labelled antigen by various dilutions of the antiserum is shown in Table 1. Even at a 1 : 25,000 dilution, a significant amount of radioactive antigen was specifically bound. This serum gave a 1 : 160 titre when assayed by conventional complement-induced lysis of DBA/2 lymphocytes, using trypan blue as an indicator.

Table 2 shows the results of absorption studies performed with this serum. Incubation of the serum with spleen MLP from the strain isogeneic to the serum donor (C57Bl/10) had no effect on the titre in either the radioimmune assay or in the conventional haemagglutination titration. The MLP of congenic resistant-strain spleens, B10.D2, reduced the bulk of the antigen-binding capacity in both tests (approximately 85 per cent in each case). Because the H2D antigen is present in this MLP[5], the antibody removed was more than likely H2 antibody. A second absorption with B10.D2 MLP did not reduce the titre any further. The antigen-combining activity was reduced by absorbing the resulting serum with DBA/2 MLP, a source of non-H2, as well as H2D antigens[6]. It therefore seems that the method will be effective for detecting non-H2 antigens, as well as antibodies directed against these antigens. The final product after these absorptions still had a residual antigen-binding capacity. The nature of this antibody is being investigated further. Further applications of the test will be published elsewhere[7].

Table 1. BINDING OF ^{125}I-LABELLED L-5178Y MLP TO ANTIBODY-COATED TUBES

Antiserum	Antiserum dilution	Antigen bound per tube (c.p.m.)	Specific binding (c.p.m.)	Specific binding (ng protein)
C57Bl/10 anti L-5178Y	1 : 1,000	6,654 ± 108	5,276	0·67
	1 : 5,000	4,485 ± 135	3,107	0·40
	1 : 6,250	3,830 ± 473	2,452	0·31
	1 : 25,000	1,966 ± 369*	588	0·08
	1 : 125,000	1,183 ± 146	− 195	—
Foetal calf	1 : 50	1,575 ± 647	197	
	1 : 250	1,468 ± 210	90	
Control		1,378 ± 302†		

The antiserum-coated tubes were exposed to 0·25 ml. of a solution described in the text containing 78,420 c.p.m. (10 ng of MLP protein) for 3 h.

* Significant at the 0·02 per cent level.

† The control tubes were treated with alkaline buffer for 1 h, then treated as described in the text.

Table 2. ABSORPTION EXPERIMENTS WITH THE ANTI-L-5178Y SERUM

Treatment	Specific binding (c.p.m.)* 1 : 250	1 : 1,000	1 : 5,000	Ha titre†
Untreated		2,943	1,752	320
(*a*) Absorbed with 1 mg C57Bl/10 MLP		2,852	1,843	320
(*b*) Absorbed (*a*) with 1 mg B10.D2 MLP		370	− 43	40
(*c*) Absorbed (*b*) with 1 mg B10.D2 MLP	1,221	702	—	—
(*d*) Absorbed (*c*) 2 × 1 mg DBA/2 MLP	767	− 207	—	—

* Absorptions were carried out sequentially with the serum used in Table 1. One ml. of the serum was incubated in step (*a*) 1 h at 37° C, then overnight at 4° C on a roller drum (1 r.p.m.). The suspension was centrifuged 30 min at 100,000*g*. The supernatant fluid was titred and subjected to step (*b*) in the same conditions as for step (*a*). Steps (*c*) and (*d*) were then carried out in succession and the values obtained entered in the table. The values in the table are the specific binding found at the dilution indicated, relative to the original serum. Non-specific absorption with the antigen preparation used was 3,465 ± 307 c.p.m. per tube.

† This titration was performed by Dr J. Palm, using DBA/2 red cells, according to a procedure previously used[3]. The values in the table are the reciprocal of the dilution that was the end-point in the titration.

We thank Dr Joy Palm for carrying out the cytotoxicity and haemagglutinating antibody titrations, and Misses R. Crocamo and B. Van Dyke for technical assistance. This work was supported by Public Health Service research grants from the US National Cancer Institute, and a training grant from the US National Institute of General Medical Sciences.

GABRIEL V. FOSCHI
LIONEL A. MANSON

Wistar Institute of Anatomy and Biology,
Philadelphia, Pennsylvania 19104.

Received August 18; revised December 17, 1969.

[1] Catt, K., and Tregear, G. W., *Science*, **158**, 1570 (1967).
[2] Manson, L. A., Foschi, G. V., and Palm, J., *J. Cell. Comp. Physiol.*, **61**, 109 (1963).
[3] Manson, L. A., Hickey, C. A., and Palm, J., in *Biological Properties of the Mammalian Surface Membrane*, **93** (edit. by Manson, L. A.) (Wistar Institute Press, Philadelphia, 1968).
[4] Hunter, W. M., and Greenwood, F. C., *Nature*, **194**, 495 (1962).
[5] Palm, J., and Manson, L. A., *J. Cell. Physiol.*, **68**, 207 (1965).
[6] Manson, L. A., Dougherty, D., Foschi, G. V., and Palm, J., *Ann. NY Acad. Sci.*, **120**, 251 (1964).
[7] Manson, L. A., *Proc. Fourth Intern. Histocompatibility Conf.* (in the press).

Simple Method of Subtyping Human G-Myeloma Proteins based on Sensitivity to Pepsin Digestion

HUMAN immunoglobulin G comprises four subclasses called IgG 1, IgG 2, IgG 3 and IgG 4. G-myeloma proteins result from the proliferation of a single clone of plasma cells and belong to only one of the four possible subclasses. At the present time such myeloma proteins represent the only readily available source of subclass specific protein and are increasingly in demand for both immunological investigations and protein sequence analysis. Subtyping of G-myeloma proteins may be achieved by immunological procedures using subclass specific antisera[1,2], by Gm typing[3] or by chemical typing[4]. Early work with G-myeloma proteins of the four subclasses revealed striking differences in sensitivity to papain[5,6] and these observations have recently been extended by other groups[7,8] to provide a basis for subtyping. In a recent investigation of the Gm markers associated with the pFc′ fragments of different IgG subclasses[9], differences were observed between the subclasses in their sensitivity to pepsin digestion and seemed to provide a further tool for subtyping myeloma proteins.

G-myeloma proteins were isolated by two-step procedures involving 'Pevikon'-block electrophoresis and DEAE-cellulose chromatography. One ml. solutions of the proteins (1·5 per cent) were prepared in 0·1 M acetate buffer *p*H 4·5 and digestion was performed at 37° C with twice recrystallized pepsin (Worthington) using an enzyme : substrate ratio of 1 : 100. Aliquots (100 µl.) of the digest mixtures were removed at 0 h, 2 h, 6 h, 10 h and 24 h. Solid Tris salt was immediately added to give a final *p*H of 8·0 and samples were stored at −20° C until required for analysis.

The digestion of the proteins was then studied as a function of time using acid urea starch gel electrophoresis at *p*H 3·0 according to the method of Edelman and Poulik[10]. A marker of pFc′ fragment (prepared from pooled human IgG) (ref. 11) was included with each analysis.

Fig. 1*a–e* shows representative photographs of the course of digestion in the following myeloma proteins: IgG 1 Gm(f), IgG 2 Gm(n–), IgG 3 Gm(g), IgG 3 Gm(b) and IgG 4 Gm(−). The starch gel patterns of the pepsin digested IgG 1 protein (Fig. 1*a*) closely resembled the patterns previously observed with pooled IgG undergoing similar digestion[12]. Thus the pFc′ fragment was present in all samples (except at 0 h) and the appearance of the fragment was paralleled by a steady increase in the F(ab′)$_2$ fragment and a decrease in the amount of intact IgG. The maximum intensity of the pFc′ band was seen after 10–24 h digestion.

The pepsin digestion of an IgG 2 protein is shown in Fig. 1*b*. In this case the pFc′ fragment was visible at 2, 6 and 10 h but not at 24 h. The maximum intensity of the pFc′ band was evident at 6 h. Another point of difference between the IgG 1 and IgG 2 classes was the absence of residual intact IgG in the 24 h aliquot from the IgG 2 protein.

The digestion of an IgG 3 Gm(g) protein is shown in Fig. 1*c* and differs strikingly from the patterns for IgG 1 and IgG 2 proteins. The pFc′ fragment was present in the 2 h aliquot as shown by a heavily stained band but was only weakly present at 6 h and absent at 10 h and 24 h. Also striking is the absence of intact IgG at 2 h. An essentially similar pattern is seen in the digestion of an IgG 3 Gm(b) protein (Fig. 1*d*). The pFc′ band at 2 h was, however, very much weaker than in the IgG 3 Gm(g) protein. The IgG band was not detectable at 2 h.

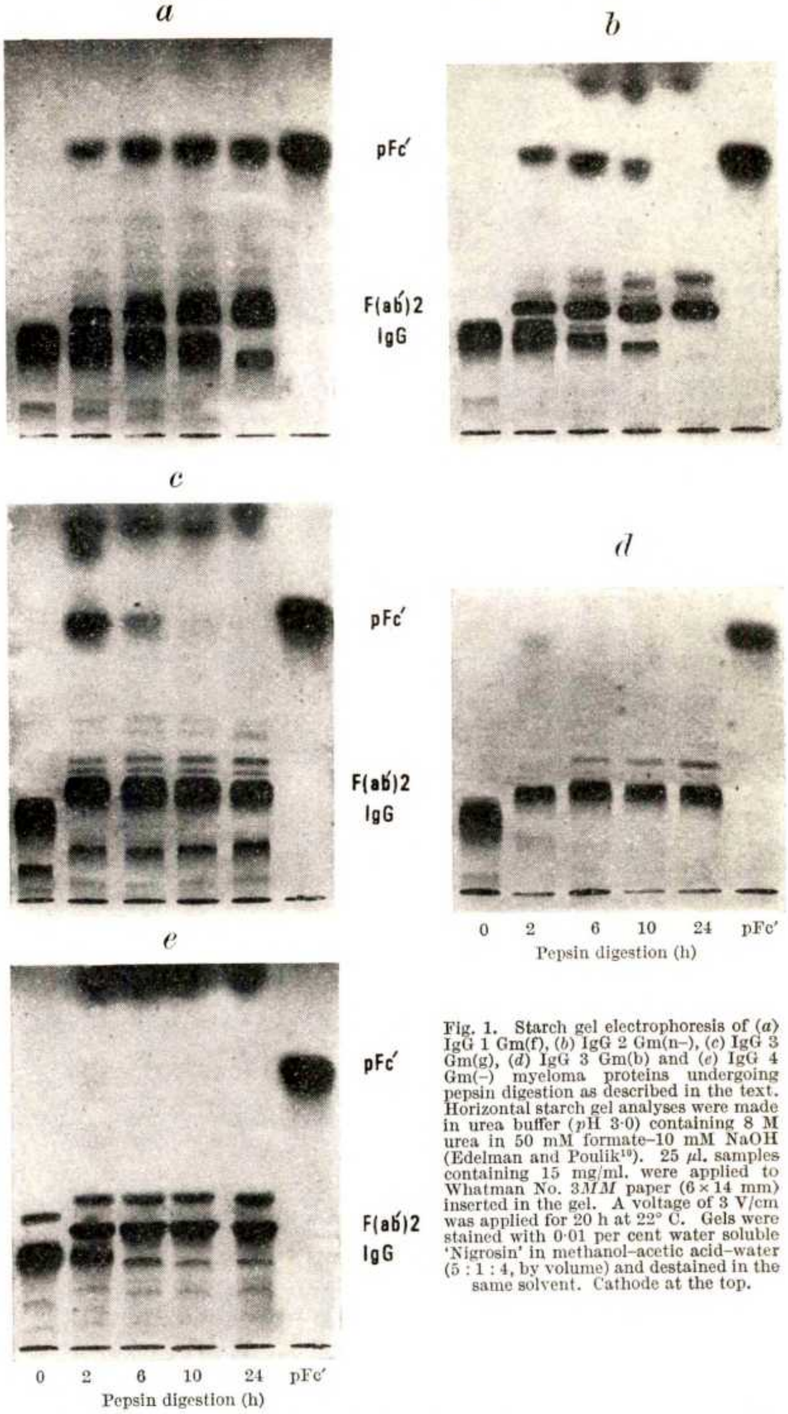


Fig. 1. Starch gel electrophoresis of (*a*) IgG 1 Gm(f), (*b*) IgG 2 Gm(n–), (*c*) IgG 3 Gm(g), (*d*) IgG 3 Gm(b) and (*e*) IgG 4 Gm(–) myeloma proteins undergoing pepsin digestion as described in the text. Horizontal starch gel analyses were made in urea buffer (*p*H 3·0) containing 8 M urea in 50 mM formate–10 mM NaOH (Edelman and Poulik[10]). 25 µl. samples containing 15 mg/ml. were applied to Whatman No. 3*MM* paper (6 × 14 mm) inserted in the gel. A voltage of 3 V/cm was applied for 20 h at 22° C. Gels were stained with 0·01 per cent water soluble 'Nigrosin' in methanol–acetic acid–water (5 : 1 : 4, by volume) and destained in the same solvent. Cathode at the top.

Finally, Fig. 1*e* shows that pepsin digestion of the IgG 4 protein produced starch gel patterns somewhat similar to those of IgG 3 Gm(b). There were, however, two differences. First, no pFc′ band was seen at any stage and second, there was residual undigested IgG at 2 h.

The results of starch gel analyses of nineteen G-myeloma proteins (Table 1) suggest that the patterns illustrated in Fig. 1 are representative of each subclass and furthermore that the human IgG subclasses fall into two groups in their sensitivities to pepsin digestion. In the conditions used the IgG 1 and IgG 2 proteins are relatively resistant to the enzyme because both the release of pFc′ fragment and the disappearance of intact IgG are slow processes; these two subclasses are distinguishable by the time of maximum production of pFc′ fragment—6 h for IgG 2 proteins and 10–24 h for IgG 1 proteins.

The IgG 3 and IgG 4 subclasses are highly sensitive to pepsin. In the case of the IgG 3 proteins, pFc′ fragment is seen at 2 h but only weakly (if at all) at 6 h. Intact monomeric IgG was not detectable after 2 h digestion in any IgG 3 protein studied. The difference between IgG 3 Gm(b) and Gm(g) proteins may indicate different conformational structures in the region where pepsin cleaves off the pFc′ fragment (near residue 332 of the γ-chain). A similar explanation may also apply to the IgG 4 proteins

Table 1. PEPSIN HYDROLYSIS OF G-MYELOMA PROTEINS

Class and designation of protein		Presence of pFc′ fragment 2 h	6 h	10 h	24 h	Presence of undigested IgG 2 h	6 h	10 h	24 h
IgG 1 Gm(f)	Hoo	+	+ +	+ + +	+ +	+ + +	+ +	+	W+
	Cus	+	+ +	+ + +	+ + +	+ + +	+ +	+ +	+
	Mar	+	+ +	+ + +	+ + +	+ + +	+ +	+ +	+
Gm(a)	Cha	+	+ +	+ + +	+ +	+ + +	+ +	+ +	+
IgG 2 Gm(n+)	New	+ +	+ +	+	–	+ + +	+	+	–
	Jas	+ +	+ +	+	Tr	+ + +	+	+	+
	Pet	+	+ + +	+ +	+	+ + +	+ +	+	–
Gm(n–)	Tsc	+	+ +	+	–	+ + +	+ +	+	–
	Wie	+ +	+ +	+	Tr	+ + +	+ +	+	Tr
IgG 3 Gm(g)	Hus	+ + +	W +	–	–	–	–	–	–
	Jon	+ +	+	W +	–	–	–	–	–
	Vil	+ + +	+	–	–	–	–	–	–
Gm(b)	Mye	W +	–	–	–	–	–	–	–
	EvL	W +	–	–	–	–	–	–	–
	Lev	+	–	–	–	(+)*	–	–	–
IgG 4 Gm(–)	Joh	–	–	–	–	+ +	+	W +	W +
	Heb	(W +)†	–	–	–	+	–	–	–
	Sta	(W +)†	–	–	–	+ +	W +	–	–
	Ger	–	–	–	–	+	–	–	–

W + = weakly stained band. Tr = trace amount, less than W +.
* Aggregated IgG. † "pFc′-like" band on anodic side of normal pFc′ mobility range.

which were characterized by the absence of a pFc′ fragment (Joh and Ger proteins) or the presence of a faint, anodically migrating, "pFc′-like" fragment. The different mobility of this IgG 4 "pFc′-like" band and the presence of residual IgG at 2 h distinguishes this subclass from the IgG 3 Gm(b) proteins.

A correlation between heavy chain subclasses and papain sensitivity of IgG myeloma proteins was suggested by earlier investigations[5,6] and has been confirmed by Jefferis *et al.*[7] and Gergely *et al.*[8]. The latter group investigated the papain susceptibilities of fifty G-myeloma proteins. IgG 2 and IgG 4 proteins were resistant to papain, whereas IgG 3 proteins were sensitive. The IgG 1 class was heterogeneous in that three out of seventeen specimens were papain resistant. This heterogeneity was not related to Gm type. In our investigation of nineteen myeloma proteins no heterogeneity was evident within the subclasses except in the case of the IgG 3 proteins where it was, apparently, related to Gm type.

Finally, because pFc′ fragment is not widely available we have investigated other possible markers for use in the starch gel electrophoretic analysis. Trypsin (see Fig. 2) was found to migrate on the anodic side of the pFc′ region but can nevertheless usefully serve as an indicator for the fragment in subtyping experiments.

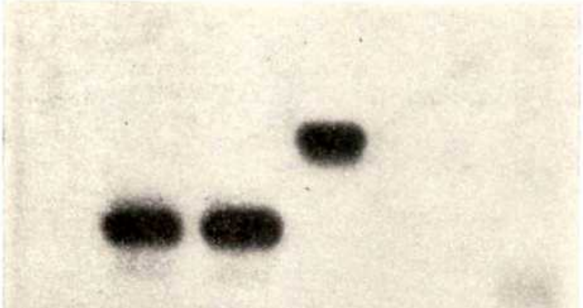


Fig. 2. Acid urea starch gel electrophoresis (*p*H 3·0) of 1 per cent solutions of bovine serum albumin (BSA); trypsin; BSA + trypsin; pFc′ fragment; IgG and reduced IgG. If trypsin is arbitrarily given an R_F value of 1·00, the pFc′ fragment has an R_F value in the range 1·19–1·26 (average 1·23). Cathode at the top.

This work was supported in part by the British Medical Research Council, the Norwegian Rheumatism Council and the Swedish Medical Research Council. One of us (MWT) is in receipt of an EMBO Travel Fellowship. We thank Drs H. Fudenberg, H. M. Grey, H. G. Kunkel, E. von Loghem and J. R. Hobbs for providing some of the myeloma sera.

M. W. TURNER

Department of Immunology,
Institute of Child Health,
London.

H. H. BENNICH

The Wallenberg Laboratory,
Uppsala University.

J. B. NATVIG

Research Institute of Immunology and Rheumatology,
Rikshospitalet University Hospital,
Oslo.

Received November 6, 1969.

[1] Grey, H. M., and Kunkel, H. G., *J. Exp. Med.*, **120**, 253 (1964).
[2] Terry, W. D., and Fahey, J. L., *Science*, **146**, 400 (1964).
[3] Natvig, J. B., and Kunkel, H. G., *Ser. Haematol.*, **1**, 1, 66 (1968).
[4] Frangione, B., Milstein, C., and Franklin, E. C., *Nature*, **221**, 149 (1969).
[5] Poulik, M. D., and Shuster, J., *Nature*, **207**, 1092 (1965).
[6] Natvig, J. B., Kunkel, H. G., and Litwin, S. D., *Cold Spring Harbor Symp. Quant. Biol.*, **32**, 173 (1967).
[7] Jefferis, R., Weston, P. D., Stanworth, D. R., and Clamp, J. R., *Nature*, **219**, 649 (1968).
[8] Gergely, J., Fudenberg, H. H., and van Loghem, E., *Immunochemistry*, **7**, 1 (1970)
[9] Turner, M. W., Natvig, J. B., and Bennich, H. H., *FEBS Letters* (in the press).
[10] Edelman, G. M., and Poulik, M. D., *J. Exp. Med.*, **113**, 861 (1961).
[11] Turner, M. W., Mårtensson, L., Natvig, J. B., and Bennich, H. H., *Nature*, **221**, 1166 (1969).
[12] Bennich, H. H., and Turner, M. W., *Biochim. Biophys. Acta*, **175**, 388 (1969).

Rheumatoid Anti Gm Factors with Specificity for the pFc′ Subfragment of Human Immunoglobulin G

THE rheumatoid factors are anti-γ-globulin antibodies directed against heavy chain determinants in the Fc region of immunoglobulin G (IgG). Although quantitative differences seem to exist, each of the four subclasses of IgG is able to interact with suitable preparations of rheumatoid factor[1,2]. In addition, Gm genetic markers are involved in certain rheumatoid factor reactions and the delineations of several new Gm markers have been performed with anti Gm antibodies from patients with rheumatoid arthritis, for example, Gm(a), (x), (b) and (g)[3].

It is now more common, however, to use anti Gm antibodies from normal individuals or heteroantisera for Gm typing[3].

The pFc′ subfragments of IgG1 molecules have recently been shown to possess Gm(a) and "non a" serological activities[4,5]. The pFc′ fragment is a dimeric subunit of molecular weight 26,000 from the C-terminal end of the heavy (γ) chains. A related but smaller fragment, termed Fc′ fragment (molecular weight 21,000), is located between amino-acid residues 14 and 105 (numbered from the C-terminal end)[6] and seems to lack both Gm(a) and "non a" serological activities[4]. We have now investigated the reactivity of the Fc′ and pFc′ fragments with rheumatoid factors of known anti Gm activity. The specificity and sensitivity of rheumatoid anti Gm sera seem to differ from those originating from normal individuals and heteroantisera[3,7,8]. The former are inhibited by concentrated solutions or aggregated material of γ-globulin, negative for a particular Gm marker. By contrast, anti Gm antibodies from normal sera and heteroantisera are not inhibited. Nevertheless, we found that the rheumatoid anti Gm reagents exhibited the same reactivity for the pFc′ and Fc′ subfragments as other anti Gm systems but were less sensitive.

The pFc′ and Fc′ fragments were prepared from pooled IgG (Kabi, Stockholm) as previously described[4]. In addition pFc′ fragments were prepared from myeloma proteins of each of the three subclasses IgG1, IgG2 and IgG3 (ref. 9). The anti Gm reagents were obtained from patients with typical rheumatoid arthritis. Two reagents were used to test each of the following specificities: anti Gm(a) (3051 and 3070), anti Gm(x) (M.S. and Wi) and anti Gm(b) (3167 and 3510). Only one anti Gm(g) reagent (3978) was available. Appropriate controls included the testing of all intact proteins and fragments with anti Gm reagents obtained from normal sera or by heteroimmunization.

Table 1. INHIBITION OF VARIOUS RHEUMATOID FACTORS WITH pFc′ AND Fc FRAGMENTS

Class and designation of protein	Protein or fragment tested	Lowest concentration (mg/ml.) of protein or fragment giving inhibition				
		Anti Gm(a)	Anti Gm(x)	Anti Gm(g)	Anti Gm(b)	Rheumatoid factor without anti Gm activity
Pooled IgG	IgG	0·06	0·06	0·12	0·12	0·03
	pFc′ fragment	0·06	0·06	> 1·00	> 1·00	> 1·00
	Fc′ fragment	> 1·00	> 1·00	> 1·00	> 1·00	> 1·00
IgG1 Gm(a)	IgG	0·06	—	> 1·00	> 1·00	0·06
	pFc′ fragment	0·06–0·12	—	> 1·00	> 1·00	> 0·5
Gm(f)	IgG	> 1·00	—	> 1·00	> 1·00	0·03
	pFc′ fragment	> 1·00	—	> 1·00	> 1·00	> 0·5
IgG2 Gm(n –)	IgG	> 1·00	—	> 1·00	> 1·00	0·06
	pFc′ fragment	> 1·00	—	> 1·00	> 1·00	> 0·5
IgG3 Gm(b)	IgG	> 1·00	—	> 1·00	0·06	> 0·5
	pFc′ fragment	> 1·00	—	> 1·00	> 1·00	> 0·5
Gm(g)	IgG	0·5	—	0·06	> 1·00	> 0·5
	pFc′ fragment	0·5	—	> 1·00	> 1·00	> 0·5

All anti Gm reagents were used in dilutions corresponding to 2 or 4 agglutinating units with red cells coated by anti Rh[3]. —, Not tested.

Table 1 shows the results of haemagglutination–inhibition tests with rheumatoid anti Gm sera. Inhibition was achieved in the rheumatoid anti Gm(a) system with pooled IgG and with pFc′ fragment prepared from it. Similar results were obtained with an isolated Gm(a) myeloma protein and its pFc′ fragment. Anti Gm(x) exhibited the same pattern of reactivity with pooled IgG and its fragments but was not tested with isolated myeloma proteins or their fragments. No inhibition of the anti Gm(a) system was obtained with IgG1 Gm(f), IgG2 or IgG3 myeloma proteins and fragments except for a faint activity with the IgG3 Gm(g) protein which probably arose from contamination. Inhibiting activity for rheumatoid anti Gm(g) and anti Gm(b) was found only in the intact IgG3 Gm(g) and IgG3 Gm(b) myeloma proteins, respectively. With these antisera no inhibition was obtained with either pFc′ fragment from pooled IgG or pFc′ fragments from isolated Gm(g) or Gm(b) myeloma proteins. Similarly, a general rheumatoid factor without specificity for Gm antigens was not inhibited by any of the pFc′ fragments. In every case the reactivities observed with the rheumatoid anti Gm antibodies corresponded to those of anti Gm reagents from normal individuals or heteroantisera. The sensitivity was, however, less in the rheumatoid systems and a four to eight-fold difference was seen between the endpoints of inhibition using the two types of reagents.

Because heat-aggregated γ-globulin shows a very strong affinity for rheumatoid factor, additional experiments were performed with pFc′ fragments (at 10 mg/ml.) from pooled IgG heated to 63° C for 15 min. No increase in inhibitory activity was observed with these pFc′ fragments or with pFc′ fragments of isolated myeloma proteins treated similarly at a concentration of 1 mg/ml. It is, however, possible that the treatment did not give an aggregation comparable with that of whole γ-globulin, and further study on this point is needed.

The observation that rheumatoid factors with anti Gm(a) and anti Gm(x) activities interact with structural regions of the pFc′ fragment is in agreement with recent studies which used inhibition of precipitation techniques to show reactivity of rheumatoid factor with both the pFc′ and Fc′ fragments[10]. Because anti Gm(a) activity is frequently detected in rheumatoid sera, the Gm(a) site is probably easily exposed and strongly antigenic in the rheumatoid process. The strong antigenicity of the Gm(a) site in heteroimmunization (see ref. 3) supports this view.

In IgG1 Gm(a–) molecules the site which corresponds to the Gm(a) structure has recently been defined and called "non a"[5]. No rheumatoid factor has yet been reported to be reactive with this "non a" site but preliminary evidence points to the existence of such antibodies in rheumatoid sera[5]. The "non a" structure is also present in IgG2 and IgG3 molecules of all genetic types. Accordingly, rheumatoid factors with anti "non a" activity would be autospecific and inhibited by IgG Gm(a–) proteins as well as by all IgG2 and IgG3 proteins and human sera.

Our study indicates that rheumatoid factors are directed against at least two distinct molecular regions in the Fc fragment of IgG molecules. One is the Gm(a) site within the pFc′ fragment. The other sites for rheumatoid factor reaction are probably located outside the pFc′ fragment. The rheumatoid anti Gm(g) and anti Gm(b) antibodies are probably directed against structures in the N-terminal half of the Fc region, but they may require the interacting presence of both the C and N terminal halves. This was also the case with a general rheumatoid factor lacking anti Gm specificity. In this system, too, which may be similar to the one described by Allen and Kunkel[11], no inhibition was obtained with any pFc′ fragment.

It is of interest that rheumatoid anti Gm(b) has a specificity similar to (b^1) (ref. 12) and that the Gm(b^1) site has now been shown to be absent from the pFc′ fragment[9]. In addition, the recent identification of "non g" which is probably antithetic to the Gm(g) structure[5] makes it likely that the Gm(b^1) and Gm(g) sites are located in different regions of the Fc fragment. Considered together, these observations indicate the presence of multiple structural regions on the IgG molecule for reactivity with rheumatoid factors.

This work was supported by the Norwegian Rheumatism Council. One of us (M. W. T.) is in receipt of an EMBO travel fellowship.

J. B. NATVIG

Research Institute of Immunology and Rheumatology,
Rikshospitalet University Hospital,
Oslo.

M. W. TURNER

Institute of Child Health,
London.

Received November 6, 1969.

[1] Schurr, P. H., and Kunkel, H. G., *Arthritis and Rheumatism*, **8**, 468 (1965).

[2] Normansell, D. E., and Stanworth, D. R., *Immunology*, **15**, 549 (1968).

[3] Natvig, J. B., and Kunkel, H. G., *Ser. Haematol.*, **1**, 1, 66 (1968).

[4] Turner, M. W., Martensson, L., Natvig, J. B., and Bennich, H. H., *Nature*, **221**, 1166 (1969).

[5] Natvig, J. B., Kunkel, H. G., and Joslin, F. G., *J. Immunol.*, **102**, 611 (1969).

[6] Turner, M. W., and Bennich, H., *Biochem. J.*, **107**, 171 (1968).

[7] Kunkel, H. G., and Tan, E. M., *Advan. Immunol.*, **4**, 351 (1964).

[8] Steinberg, A. G., *Arthritis and Rheumatism*, **5**, 331 (1962).

[9] Turner, M. W., Natvig, J. B., and Bennich, H. H., *FEBS Letters* (in the press).

[10] Turner, M. W., Stanworth, D. R., Normansell, D. E., and Bennich, H. H., *Biochim. Biophys. Acta*, **188**, 265 (1969).

[11] Allen, J. C., and Kunkel, H. G., *Arthritis and Rheumatism*, **9**, 758 (1966).

[12] Natvig J. B. Kunkel, H. G., Yount, W. J., and Nielsen, J. C., *J. Exp. Med.*, **128**, 763 (1968).

Origin of Small Lymphocytes following Blastogenesis induced by Short-term PHA Stimulation

WE have reported that short-term (24 h) exposure of human lymphocyte cultures to phytohaemagglutinin (PHA) results in the conversion of 90 per cent or more of the small lymphocytes to "blastlike" forms. Subsequently, the cultures revert to cells which resemble the original unstimulated small lymphocytes, both morphologically and metabolically, but remain fully reactive to renewed PHA stimulation[1]. In this report, we offer evidence that, in the 24 h PHA-stimulated cultures, most lymphocytes become blastoid but do not divide; instead, they return to their original form by 9 or 10 days after stimulation.

Lymphocyte cultures were prepared in replicate and subjected to 24 h stimulation with Difco 'PHA-P' as previously described[2]. Viable cell count was followed with erythrosin[3]. During the first 6 days, accurate cell counts could not be made in the stimulated cultures due to heavy clumping induced by PHA. Rough counts, however, indicated that the total cell numbers fluctuated from the initial count by less than 30 per cent. By the seventh day and thereafter, the clumps had become looser and could be dispersed by mechanical action. Daughter cell production was determined by incorporation of [^{3}H-*methyl*]-thymidine into cellular DNA, followed by isolation and measurement of this label in the acid insoluble fraction[1]. Daughter cell formation was measured daily by 24 h pulse label, that is, each day two vials were labelled and then collected after 24 h. Cumulative daughter cell production was determined by continuous label. For this purpose, additional vials were exposed to ^{3}H-thymidine and the label was maintained in the cultures throughout the experiment. Again two vials were taken daily. When continuous label was used, fresh ^{3}H-thymidine was included in the daily media changes. ^{3}H-Thymidine was not toxic to either the stimulated or the unstimulated lymphocytes at the isotope concentration used (0·01 μCi/ml.). In addition, daughter cell production was determined by autoradiography, using the same label and Eastman Kodak film emulsion 'NTB2' (ref. 4).

Data from a representative experiment showing daughter cell formation, as measured by DNA synthesis, are presented in Table 1. PHA-stimulated and control cultures were simultaneously tested. Column *A* lists daily DNA synthesis (daughter cell formation) during days 3 to 11 after PHA stimulation. This reaches a maximum at day 4, then drops to levels found in control cultures (column *D*) by day 9–10. Column *B* lists the total DNA synthesis occurring during the test period. This is determined by successive addition of the daily increments of DNA synthesis listed in column *A*. Column *C* gives the actual cumulative DNA synthesis in such cultures as determined by continuous ^{3}H-thymidine labelling. The higher values in column *B* as compared with column *C*, beginning at day 5, indicate that many of the daughter cells do not survive long. By day 11 the cumulative counts, based on continuous label, approach the level of unstimulated cells, indicating that most of the daughter cells have died.

Table 1. DAUGHTER CELL FORMATION IN HUMAN LYMPHOCYTE CULTURES

	PHA-stimulated cells			Unstimulated cells
Column	*A*	*B*	*C*	*D*
Day after stimulation	Daily DNA synthesis (c.p.m.)	Total DNA synthesis (c.p.m.)	Cumulative DNA synthesis (c.p.m.)	Cumulative DNA synthesis (c.p.m.)
3	109	—	—	8
4	450	659	670	28
5	405	1,064	720	33
6	330	1,394	560	20
7	180	1,574	390	41
8	107	1,681	260	20
9	49	1,730	100	21
10	50	1,780	70	14
11	22	1,813	40	11

Cell formation was measured by DNA synthesis in c.p.m. A tracer dose of ^{3}H-thymidine (specific activity 6·7 Ci/mmole) at a final concentration 0·01 μCi/ml. was used as a marker. Background (25 c.p.m.) has been subtracted.

Table 2 presents data on daughter cell formation as determined by autoradiographic measurements. Column *A* shows the percentage of daughter cells formed daily during days 3 to 11 after PHA stimulation, based on counts of a thousand cells. Column *B* lists the total percentage of daughter cells formed in such cultures during the test period. This is determined by successive addition of the percentages of daughter cells formed daily as listed in column *A*. Column *C* gives the actual cumulative percentage of daughter cells in such cultures as determined by continuous ^{3}H-thymidine label. Daughter cells accumulate for a few days as seen by equivalent values in columns *B* and *C* for days 4 and 5. As the rate of DNA synthesis declines, day 6, the values in column *B* begin to exceed those in column *C*, indicating the disappearance of daughter cells. By day 10, only 6 per cent of the cells were labelled, yet the number of viable cells remained at 57 per cent of the initial number. This indicates that most of the cells present at that time represent lymphocytes which have not divided. Because these cells were blasts on day 7, as shown in column *F*, they must have reverted to their original form.

The mechanism of PHA action is still unclear. There is evidence that PHA attaches to the cell, and subsequently

Table 2. AUTORADIOGRAPHIC MEASUREMENTS

	PHA-stimulated cells					Unstimulated cells
Column	*A*	*B*	*C*	*D*	*E*	*F*
Day after PHA stimulation	Daily daughter cell production (per cent)	Total daughter cell production (per cent)	Continuous daughter cell production (per cent)	Viable cell count (× 10^6/ml.)	Cell morphology	Cumulative daughter cell formation (per cent)
0	—	—	—	1·6	Small	0
3	12	—	—	—	Large	0
4	30	42	48	—	Large	0
5	22	64	63	—	Blasts	0
6	15	79	54	—	Blasts	0
7	7	87	55	2·0	> 90 per cent blasts	0
8	0	—	30	1·7	Large	0
9	0	—	12	1·3	Medium	0
10	0	—	6	0·9	Small	0
11	0	—	7	0·9	Small	0

Measurements were made of daughter cell formation in human lymphocyte cultures. ^{3}H-Thymidine (specific activity 6·7 Ci/mmole) was used as a marker. Column *E* describes type of cell: small, medium, large or blast-type lymphocyte.

appears in the cytoplasm[5-7]. If this, indeed, is the case, then reversion of blastogenesis may depend on release or inactivation of this mitogen, thus permitting reactivation of the receptor mechanism in the lymphocytes. Although the reverted cells behave metabolically and appear microscopically much like small lymphocytes, it still remains to be shown whether other aspects of the reversion are also complete, for example, enzymatic pattern.

The ability of the lymphocytes in culture to undergo repeatedly reversible transformation in metabolic activity and morphology, as well as mitosis, further increases the scope of activities demonstrated for these cells. Thus short-term lymphocyte stimulation with PHA provides a system for studying (1) the control mechanism of differentiation and dedifferentiation and (2) the mechanisms leading to blastogenesis and mitosis.

We thank Dr John Ifft for his suggestions regarding the autoradiographic studies and Michael Klein for his assistance. This work was supported by US Public Health Service grants.

PETER R. POLGAR
SIDNEY KIBRICK

Departments of Biochemistry,
Microbiology and Medicine,
Boston University School of Medicine,
Boston University Medical Center,
Boston, Massachusetts.

Received August 11; revised November 20, 1969.

[1] Polgar, P. R., Kibrick, S., and Foster, J. M., *Nature*, **218**, 596 (1968).
[2] Polgar, P. R., Foster, J. M., and Cooperband, S. R., *Exp. Cell Res.*, **49**, 231 (1968).
[3] Phillips, H. J., and Andrews, R. V., *Exp. Cell Res.*, **16**, 678 (1959).
[4] Kopriwa, B., and Lebland, C. P., *J. Histochem. Cytochem.*, **10**, 269 (1962).
[5] Polgar, P. R., Cooperband, S. R., and Kibrick, S., *Proc. Fourth Leukocyte Culture Conference* (1969).
[6] Razavi, L., *Nature*, **210**, 444 (1966).
[7] Canard, R. A., *Nature*, **214**, 709 (1967).

Cell Movement and Cell Spreading: a Passive or an Active Process?

Wolpert, Macpherson and Todd[1] have recently described some experiments on the effects of temperature on the spreading and rounding up of cultured cells. They believe their findings invalidate the "passive" theory of tissue cell movement which I have previously put forward[2]. In this theory, the advancement of the cell margin during spreading or locomotion is considered to be passive in the sense that it is analogous to the spreading of a liquid on a suitably wettable surface. It is helpful to express the theory in terms of surface (interfacial) tensions because it places adequate emphasis on the part played by the medium and the substrate.

The challenge offered by Wolpert and co-authors is based on two experiments. In the first experiment the failure of cells to spread on a (glass ?) Petri dish at 2° C, whereas they do spread at 37° C, is held to refute the idea of passive spread because passive spreading "ought not to be significantly affected by lowered temperature". It is well known that the surface tensions of pure liquids are relatively unaffected by temperature changes (the surface tension of water, for example, changes by only 7 per cent in this temperature range), but if surface-active impurities are present or if the system is intrinsically more complex, profound changes of surface tension in response to changing temperature may occur. A chief cause of such temperature dependence is the adsorption of molecules at an interface. Adsorption of very small amounts of material can cause spectacular changes in surface tension. Not only is the process of adsorption itself temperature dependent, but other temperature sensitive factors such as solubility, the formation of complexes or coacervates, or the rates of biochemical change could affect the number of molecules available for adsorption. The more complex the system and the more heterogeneous its molecular population, the more opportunities there are for such factors to operate.

The surface properties of a cell are primarily dependent on the molecular species at the cell surface. The synthesis and breakdown of these molecules (and in some cases their transport) are largely determined by metabolic processes. To claim that the wetting properties of a cell should not be significantly affected by changes of temperature is to assume that the molecular population at the cell surface will not be significantly altered whether the cell metabolism is working optimally at 37° C or virtually switched off at 2° C.

In the second experiment, cells which had already spread at 37° C were found to display no tendency to round up when the temperature was lowered to 2° C. If a cell remains permanently rounded up at 2° C, one would certainly expect—on purely thermodynamic grounds—that a spread cell would tend towards this condition if cooled to the same temperature. The factors which oppose such a tendency should not be neglected, however. One of these is hysteresis of wetting—the familiar tendency of liquids to remain spread over areas of surface which have already been wetted. Wetting hysteresis is particularly marked in complex situations involving heterogeneous molecular populations. It may also be greatly enhanced by adsorption hysteresis. Molecules already adsorbed at an interface (particularly polymeric molecules such as proteins) do not necessarily desorb in conditions which discourage adsorption.

In addition, the resistance of the cell to deformation may be increased at low temperatures. Increased stiffness could slow down the process of rounding up so that it may not be discernible in 24 h. Wolpert and co-authors believe they have eliminated this possibility by showing that cells at 2° C will round up relatively quickly if trypsinized and detached from the surface. There is no reason, however, to expect the rate of rounding up of attached cells to be the same as that for detached and trypsinized cells at the same temperature. In terms of surface thermodynamics the detached cells do not share any common factors with the spread cells; nor has the possibility that trypsin may affect the rigidity of the cell membrane been excluded.

None of the foregoing arguments is crucial to my case, however, because I am unable to confirm the reported finding that spread cells show "no sign of rounding up" at 2° C. My experiments differ from those reported only in the use of oil-sealed culture chambers[3] in order to allow adequate microscopy for quantitative experiments. After 24 h, planimetric measurements of photographs revealed a reduction in average area per cell of 39·8 per cent in the case of L cells, 38·7 per cent for HeLa cells, 23·9 per cent for monkey kidney cells, 59·7 per cent for hamster ovary cells, and 28·8 per cent for human lung cells. At least thirty randomly selected cells were measured in each case and sampling errors were eliminated by measuring the same cells before and after the temperature change. Although these measured differences are considerable, it is entirely possible that they could be overlooked when Petri dishes are examined visually without the aid of objective measurements.

My colleagues at the Dyestuffs Division of this company, P. K. Davies and H. R. Harper, have carried out an experiment with a simple aqueous surface-active agent system which imitates very neatly the behaviour reported for cells. They found that droplets of a dilute aqueous suspension of tetradecyl sodium sulphate did not spread on paraffin wax at 2° C but did so at 37° C. Moreover, no retraction of the spread droplets was observed when the system was cooled to 2° C. In this case the behaviour of the droplets is clearly related to changes in solubility of the surface-active molecules with temperature.

The experiment nevertheless illustrates the potential importance of surface-active molecules in more complex systems where their availability may be related to more subtle factors such as those already discussed.

In arguing their case, Wolpert *et al.* have used the classical principles of surface thermodynamics appropriate to pure liquids in ideal conditions. These principles cannot be applied uncritically to systems in which there are opportunities for molecular rearrangement or chemical change. Consequently they cannot be used to refute the theory of passive spread as a component of tissue cell locomotion in culture.

While rejecting the idea of passive spread, Wolpert and co-authors make it clear they would prefer to implicate pseudopod activity in cell spreading and locomotion, remarking that cell spreading is "always associated with the appearance of pseudopods about 0·1 μm thick". These structures cannot be seen in the living cell and it is begging the question to give them a title with functional implications before their significance (if any) for cell locomotion has been established.

S. B. CARTER

ICI, Pharmaceuticals Division,
Alderley Park,
Macclesfield, Cheshire, SK10 4TG.

Received October 24, 1969.

[1] Wolpert, L., Macpherson, I., and Todd, I., *Nature*, **223**, 512 (1969).
[2] Carter, S. B., *Nature*, **213**, 256 (1967).
[3] Carter, S. B., *Exp. Cell Res.*, **42**, 395 (1966).

Bonding of Small Molecules to DNA following Ionizing Radiation

IONIZATION is known to cause the scission of DNA molecules in bacterial cells[1-3]. The effects of ultraviolet radiation, by contrast, include the formation of pyrimidine dimers, covalent links between DNA molecules, crosslinks between DNA and protein molecules, and covalent bonding of amino-acids and free purine and pyrimidine bases[4-11]. We now report that ionizing radiation of DNA in defined solution or in chromatin can also bring about the formation of covalent links between the DNA molecules and a variety of small molecules.

We have used both purified commercial calf thymus DNA (Calbiochem) and DNA purified from *Tetrahymena pyriformis*[12]. To demonstrate the formation of stable bonds after irradiation, DNA solutions containing labelled small molecules were irradiated in 2–3 mm depths using the electron beam of a Varian Associates 6 meV linear accelerator (Fig. 1). After irradiation, triplicate 50 μl. samples were placed on disks of Whatman 3*MM* filter paper, washed in cold 5 per cent trichloroacetic acid, and dehydrated for liquid scintillation counting[13]. Identical non-irradiated control samples were taken at the same time intervals. To determine the chromatographic properties of the irradiation products some DNA samples were irradiated directly with one million rads in the presence of a mixture of ^{14}C-labelled amino-acids. Following enzymatic hydrolysis with deoxyribonuclease and venom phosphodiesterase the nucleotides were concentrated and chromatographed[14] as described in the caption to Fig. 2. Chromatographic migration of individual nucleotides (adenylic acid, cytidylic acid, guanylic acid and thymidylic acid) was determined for comparison. In each experiment a non-irradiated control solution was also hydrolysed. The irradiated and non-irradiated samples were processed simultaneously and then chromatographed on the same sheet to monitor transfer of non-bound radioactive label as closely as possible.

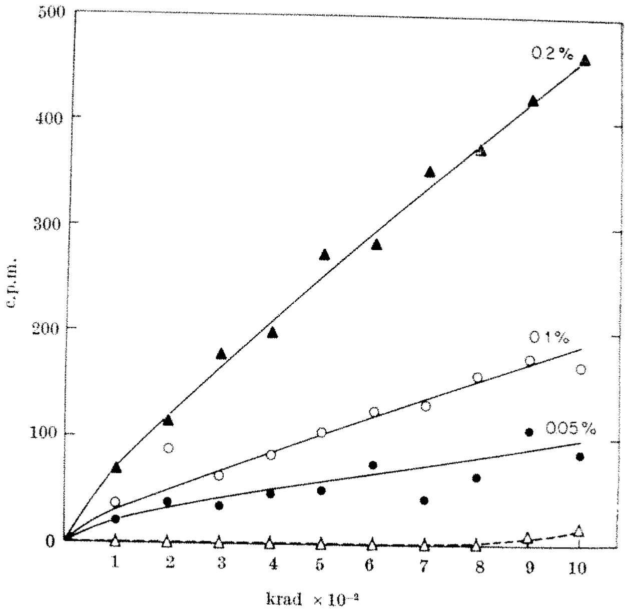


Fig. 1. Formation of acid-insoluble material following ionizing irradiation. Calf thymus DNA (0·05, 0·1, 0·2 per cent in 0·01 M, *p*H 7·4 Tris buffer) containing 20 μCi/ml. ^{3}H-leucine and 1 mg/ml. chloromycetin were irradiated with varying amounts of high-energy electrons using the electron beam of a 6 meV Varian Associates linear accelerator. Following each irradiation increment of 100 krads triplicate 50 μl. samples were removed and deposited on 2·5 cm disks of Whatman 3*MM* filter paper. Each disk was dropped wet into ice-cold 5 per cent trichloroacetic acid and processed for liquid scintillation counting[13]. Each solution was matched with a non-irradiated control which was sampled at the same time and identically processed. The formation of acid-insoluble label in each of the irradiated solutions (▲) and one of the non-irradiated (△) controls (0·1 per cent DNA) is shown. Abscissa: delivered dose in krads × 10^{-2}; ordinate: average c.p.m. for each set of triplicate samples. Specific activity of the ^{3}H-leucine: 1 mCi/0·026 mg. Counting efficiency 3·15 per cent.

One example of covalent bonding induced by ionizing radiation is seen in Fig. 1. In this case tritiated leucine and several concentrations of DNA were irradiated with progressive amounts of 6 meV electrons. The conversion of label from acid solubility to acid insolubility is proportional to the DNA concentration and to the amount of absorbed radiation. In each experiment some trichloroacetic acid insoluble label was found towards the end of the experimental period in the non-irradiated controls. Adding chloromycetin to the solution reduced this value substantially, but never to zero. We attribute this uptake to contamination of the sample with microorganisms (because of sampling time the experimental period is about 45 min). In other experiments we have found that a variety of small molecules are also bound following ionizing irradiation (including thyroxine, glyceraldehyde and other amino-acids). Similar results were obtained when DNA was replaced by RNA or albumin. No significant acid-insoluble products are formed by irradiation of a labelled amino-acid solution in the absence of a high molecular weight carrier molecule.

If the irradiated DNA is hydrolysed and chromatographed the label is found to be distributed over the entire chromatogram with a considerable amount remaining near the origin (Fig. 2). Examination of the chromatograph under ultraviolet light reveals only the expected four nucleotides. The radiation products therefore probably exist in minute amounts and may include a wide variety of compounds. Label is not localized on the chromatograph even if single amino-acids are used as the labelled source.

In another series of experiments we found that label can be bound to DNA existing as native chromatin. To show this we isolated nuclei from logarithmic stage *T. pyriformis* and purified them so as to be free of cytoplasmic contamination[12]. Because these nuclei incorporate many labelled materials spontaneously we used ^{14}C-labelled D-phenylalanine. This optical isomer is not normally incorporated by the cell or isolated nuclei.

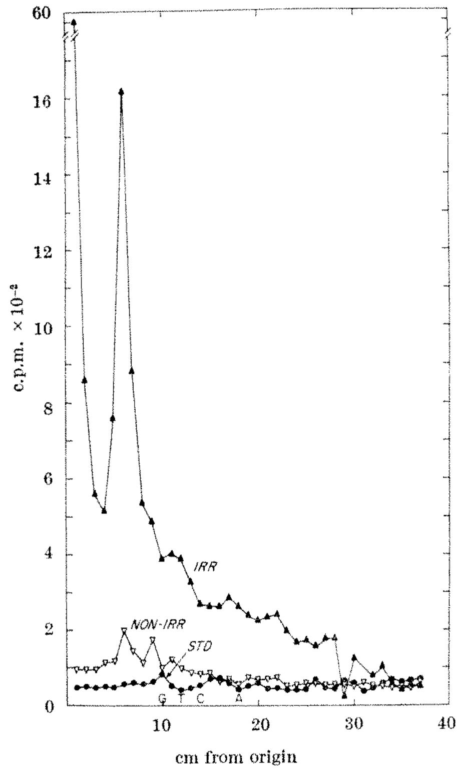


Fig. 2. Chromatography of labelled DNA products. A 0·1 per cent solution of calf thymus DNA (in 0·01 M Tris buffer, *p*H 7·6, 0·005 M $MgCl_2$) containing 10 μCi of a uniformly labelled ^{14}C-amino-acid mixture (New England Nuclear specific activity 0·1 mCi/0·067 mg) was irradiated (1×10^6 rads, electron beam, 6 meV linear accelerator) in a shallow (2–3 mm) solution. Following irradiation the DNA solution was precipitated and washed three times using ice-cold 5 per cent trichloroacetic acid. The pellet was then dissolved in 0·2 ml. of 0·01 M Tris buffer, *p*H 7·6 (containing 0·005 M $MgCl_2$), and digested by adding 0·2 ml. of a solution containing 1 mg/ml. each deoxyribonuclease and bovine serum albumin (in 0·001 M NaCl). The mixture was incubated for 150 min at 37° C. The *p*H was then raised to 8·6 using 0·1 M NaOH. Snake venom phosphodiesterase (0·02 ml. of a 1,000 unit/ml. solution in 1·0 M NaCl) was added, and the solution was again digested for 150 min at 37°. The hydrolysate was evaporated to dryness and resuspended in 50 μl. of distilled water. The entire solution was spotted on chromatographic filter paper and chromatographed for 17 h using an isobutyric acid–ammonium hydroxide solvent system[14]. After being dried, the chromatogram was cut into 1·0 cm transverse strips and each strip was counted in a Beckman *CPM*-200 liquid scintillation counter at an efficiency of 60·7 per cent. Shown are the radiochromatograms obtained from an irradiated DNA solution (▲), a non-irradiated DNA solution (▽), and a control strip containing the four DNA nucleotides (●). No differences in the ultraviolet patterns could be found when the three strips were examined before cutting.

Again we found label associated with material which was acid insoluble following irradiation and which could be concentrated using standard DNA purification procedures.

These experiments strongly suggest that small amounts of amino-acids and carbohydrates can become covalently linked to nucleic acids following ionizing radiation. The reactions may be similar to those linking DNA and protein following ultraviolet irradiation. Our data suggest that they are chemically quite non-specific. So far no material tested has failed to become linked although the doses required for binding vary. Because highly purified DNA and DNA in intact nucleoprotein will both accept such small molecules it seems reasonable to assume that similar reactions occur following ionizing irradiation *in vivo*. Amino-acids and peptides linked to DNA are known to occur in the absence of irradiation[15–17]; the relationship between such naturally occurring materials and the irradiation products described here is unknown. If reactions such as we have found do occur *in vivo* it is obvious that the multiplicity of products must be large. Accordingly, the chance of observing individual products in acid hydrolysates of DNA irradiated *in vivo* is probably small because the concentrations within the cell would not be expected to reach those used in these experiments. It is interesting to speculate on whether the relatively large production of pyrimidine dimers found following ultraviolet irradiation is the result of a high probability of such reactions occurring or of the ease with which the products can be identified. Because of the possible genetic implications it seems important to determine whether lesions such as we have shown here can be repaired in the same fashion as pyrimidine dimer excision. For example, products resistant to repair excision might effectively act as mutagens.

This work was supported by research and fellowship funds from the California Institute for Cancer Research, the Atomic Energy Commission and the US Public Health Service. We thank Miss M. Carlsson for assistance.

JOHN E. BYFIELD
YOUNG C. LEE
LESLIE R. BENNETT

Radiology Department,
UCLA Medical Center,
Los Angeles, California 90024.

Received August 11; revised December 11, 1969.

[1] Freifelder, D., *Proc. US Nat. Acad. Sci.*, **54**, 128 (1965).
[2] McGrath, R. A., and Williams, R. W., *Nature*, **212**, 534 (1966).
[3] Kaplan, H. S., in *Radiation Research* (edit. by Silini, G.), 397 (Wiley, New York, 1967).
[4] Setlow, R. B., and Setlow, J. K., *Proc. US Nat. Acad. Sci.*, **48**, 1250 (1962).
[5] Setlow, J. K., and Setlow, R. B., *Nature*, **197**, 560 (1963).
[6] Setlow, R. B., in *Radiation Research* (edit. by Silini, G.), 525 (Wiley, New York 1967).
[7] Smith, K. C., *Photochem. Photobiol.*, **3**, 415 (1964).
[8] Smith, K. C., *Biochem. Biophys. Res. Commun.*, **8**, 157 (1962).
[9] Smith, K. C., and Aplin, R. T., *Biochemistry*, **5**, 2125 (1966).
[10] Smith, K. C., and Meun, D. H., *Biochemistry*, **7**, 1033 (1968).
[11] Smith, K. C., *Biochem. Biophys. Res. Commun.*, **34**, 354 (1969).
[12] Lee, Y. C., and Scherbaum, O. H., *Nature*, **208**, 1350 (1965).
[13] Bollum, F. J., *J. Biol. Chem.*, **234**, 2733 (1959).
[14] Wyatt, G. R., in *The Nucleic Acids* (edit. by Chargaff, E., and Davidson, J. N.), **1**, 243 (Academic Press, New York, 1955).
[15] Balis, M. E., Salser, J. S., and Elder, A., *Nature*, **203**, 1170 (1964).
[16] Webb, S. J., *Canad. J. Microbiol.*, **13**, 57 (1967).
[17] Salser, J. S., and Balis, M. E., *Cancer Res.*, **28**, 595 (1968).

In vitro Synthesis of Hb Hammersmith (CD1 Phe→Ser)

RED cells of patients heterozygous for Hb A and the unstable haemoglobin, Hb Köln (FG5 Val→Met)[1] contain only 10–15 per cent of the abnormal haemoglobin. This was found to arise from defective synthesis of the abnormal β Köln chain[2–4], and not from its removal from cells by Heinz body formation. The techniques used in these studies might, however, have caused denaturation of Hb Köln and produced falsely low values for the proportion of Hb Köln synthesized. The relative rates of synthesis of Hb A and Hb Hammersmith (CD1 Phe→Ser)[5], another unstable haemoglobin, have now been studied, using identical techniques, to test the validity of the Hb Köln experiments and to compare the synthesis of Hb Hammersmith with that of Hb Köln.

The subject studied was a female, 40 per cent of whose haemoglobin was Hb Hammersmith. Whole blood was washed three times with isotonic saline and the red cells were incubated at 37° C with 500 μCi of ^3H-leucine according to the method of Lingrel and Borsook[6]. Samples of cells were removed at known times and after washing and lysis, a stroma-free lysate was prepared. Free ^3H-leucine was removed from the lysate by passage down a column of 'Sephadex *G* 25'. The two haemoglobins were then separated on a column of 'Sephadex *CM* 50' as previously described[4]. The whole of each fraction was collected and its protein concentration measured at $A_{280\,nm}$ (phenylalanine does not absorb at this wavelength). The lysates were concentrated by ultrafiltration for 10 h at 4° C and globin was prepared by acid-acetone precipitation at −20° C. The specific activity of the whole globin from each lysate was measured by liquid scintillation counting and the total counts incorporated into globin A and globin Hammersmith were determined. The α and β chains of each globin were then separated according to the method of Clegg, Naughton and Weatherall[7], the specific activity of the chains were measured and the total radioactivity incorporated into each chain was calculated.

Table 1. INCORPORATION OF ^{3}H-LEUCINE INTO HB A AND HB HAMMERSMITH

Time (min)	Total ^{3}H-leucine incorporated HbA	Total ^{3}H-leucine incorporated Hb Hammersmith	Proportion of total counts in Hb Hammersmith (per cent)
30	36,719	73,540	66·6
60	45,600	84,771	65·0
120	47,624	88,100	64·8

Table 2. INCORPORATION OF ^{3}H-LEUCINE INTO THE α AND β CHAINS OF HB A AND HB HAMMERSMITH

Time (min)	Hb A (c.p.m.)		Hb Hammersmith (c.p.m.)	
	α	β	α	β
30	1,638	4,505	7,583	4,730
60	2,275	6,783	12,835	6,701
120	2,726	7,791	13,939	7,826

The "apparent" synthesis of the two haemoglobins was determined by the proportion of total counts incorporated into whole globin A and whole globin Hammersmith. Thirty-five per cent of the counts were in globin A and 65 per cent in globin Hammersmith (Table 1). When the "true" proportion of each haemoglobin synthesized was calculated from the total counts incorporated into the β A chains and β Hammersmith chains, however, it was found that they were synthesized in equal amounts (Table 2). The discrepancy between "apparent" synthesis and "true" synthesis is likely to arise from the unequal α chain labelling of the two haemoglobins. This unequal α chain labelling is probably the result of unequal exchange of labelled α chains between the Hb A and Hb Hammersmith (as was found in the Hb Köln study[4]) but requires further investigation. The equal synthesis of Hb A and Hb Hammersmith suggests that our earlier finding of unequal synthesis of Hb A and Hb Köln is probably valid because Hb Hammersmith is at least as unstable as Hb Köln, and loss *in vitro*—if it occurred—would be expected to a similar degree. The finding of equal synthesis of β A chains and β Hammersmith chains implies that the substitution of phenylalanine by the smaller, polar amino-acid serine at the CD1 position of the chain does not impede the assembly of the chain. This is in contrast to the substitution of valine by the large amino-acid methionine at the FG5 position in the β Köln chain.

Hb Köln and Hb Hammersmith are both very unstable haemoglobins, yet the degree of haemolysis caused by them differs considerably. The first produces a mild and the second a severe haemolytic disease.

It seems unlikely that the differences in haemolysis can be explained by differences in the stability of the two haemoglobins, for when heated both haemoglobins appear to precipitate with equal rapidity. The fact that the red cells of patients with Hb Hammersmith contain about three times as much unstable protein (35–40 per cent) as do the cells of patients with Hb Köln (10–15 per cent) may be relevant, for it has been shown that haemolysis is related to the interaction of the precipitated haemoglobin with membrane thiol groups[8]. The concentration of unstable haemoglobin within cells must therefore be an important factor in determining the degree to which cells are damaged. Patients heterozygous for Hb Köln may therefore be protected from severe haemolysis because the amino-acid substitution which imparts instability to Köln β chains may also result in a deficiency in the number of these chains synthesized.

J. M. WHITE
J. V. DACIE

MRC Group for Haemolytic Mechanisms,
Royal Postgraduate Medical School,
Hammersmith Hospital,
London W12.

Received September 25, 1969.

[1] Carrell, R. W., Lehmann, H., and Hutchinson, H. E., *Nature*, **210**, 915 (1966).

[2] White, J. M., and Brain, M. C., *Abst. Thirteen Cong. Int. Soc. Haematol.*, 65 (Abstr.) (1968).

[3] Brain, M. C., and White, J. M., *J. Physiol.*, **202**, 26P (1969).

[4] White, J. M., and Brain, M. C., *Brit. J. Haemat.*, **18**, 195 (1970).

[5] Dacie, J. V., Shinton, N. K., Gaffney, P. J., Carrell, R. W., and Lehmann, H., *Nature*, **216**, 663 (1967).

[6] Lingrel, J. B., and Borsook, H., *Biochemistry*, **2**, 309 (1963).

[7] Clegg, J. B., Naughton, M. A., and Weatherall, D. J., *J. Molec. Biol.*, **19**, 91 (1966).

[8] Jacob, H. S., Brain, M. C., Dacie, J. V., Carrell, R. W., and Lehmann, H., *Nature*, **218**, 1214 (1968).

Alteration of Normal Development of Drug Metabolism by Injection of Growth Hormone

GROWTH hormone (STH) may affect the normal development of the rat hepatic microsomal drug-metabolizing enzyme system, for there is a normal postnatal, age-related increase in activity of this system[1,2]. A relation between pituitary hormones and liver drug metabolism was suggested by the observation that the normal postnatal development of hexobarbital, aminopyrine and *p*-nitrobenzoic acid metabolism was prevented in young male rats which had high levels of STH (ref. 2) from a pituitary mammotropic tumour (MtT–F4) (ref. 3). In adult rats injected with STH a decrease in hepatic microsomal drug metabolism was observed[4]. This report demonstrates a reduction in the level and rate of increase with age of aminopyrine and ethylmorphine metabolism by liver from STH injected young rats and thus suggests STH as an endogenous agent responsible for the observed postnatal development pattern of this metabolism.

Fischer rats were purchased from a local distributor and placed in plastic nesting boxes or stainless steel cages over a hardwood bedding. They were given purina chow and water *ad libitum* and exposed to alternate 12 h periods of light and darkness. Soon after birth, male rat pups were selected and randomized with different dams for suckling. Rats from several litters were used for each hepatic drug metabolism determination. Injection solutions were prepared by dissolving albumin (fraction V from plasma) or STH in alkaline 0·9 per cent aqueous saline, and powdered gelatine was added to a final gelatine concentration of 14 per cent. The gelatine was dissolved by gentle shaking in a water bath at 37° C. One gram of rat liver was homogenized with 2 ml. of 1·15 per cent KCl in 0·5 M Tris buffer (*p*H 7·35) by using a glass homogenizer with 'Teflon' pestle. This homogenate was centrifuged at 9,000*g* for 20 min at 4° C to obtain the 9,000*g* supernatant fraction. An aliquot (0·25 ml.) of this fraction was added to a reaction mixture which contained cofactors, phosphate buffer and substrate (hexobarbital, aminopyrine or ethylmorphine) as previously described[4]. This mixture was incubated at 37° C in an atmosphere of oxygen for 20 min in a Dubnoff type shaking water bath incubator. Measurements of the disappearance of hexobarbital[5] and the formation of formaldehyde from aminopyrine or ethylmorphine[6,7] were used to estimate hepatic microsomal drug-metabolizing enzyme activity. Liver 9,000*g* supernatant fraction protein was measured by the method described by Lowry *et al.*[8]. Evaluation of the statistical significance between paired means was performed with Student's *t* test[9].

An alteration in the normal postnatal development of the hepatic microsomal drug metabolizing enzyme system in young rats was observed after one injection of STH. The hepatic demethylation of ethylmorphine or aminopyrine was decreased at 30, 42 and 58 days of age in rats injected once with STH compared with albumin injected controls (Fig. 1). Because activity of this enzyme system in

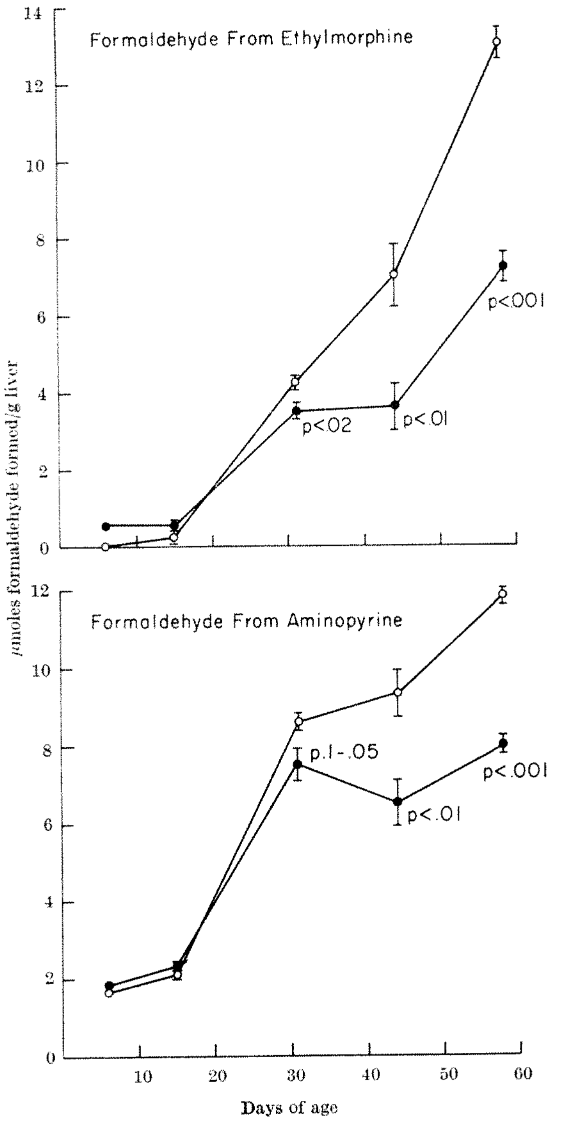


Fig. 1. Hepatic metabolism of ethylmorphine or aminopyrine in young rats after one injection of STH. Approximately eight male Fischer rats less than 28 days old were placed with a female rat for suckling. Each young suckling or older non-suckling animal was injected once subcutaneously with albumin or STH prepared as 1 mg/ml. in 14 per cent gelatine and given as 1 mg/100 g body weight. A single injection was given 48 h before each measurement, that is, 48 h before the determination at 15, 30, 42 and 58 days of age. Results are depicted as the mean ± *S.E.*, and values not shown with $P < 0{\cdot}05$ were not statistically significant as compared with albumin-injected controls. Livers from three animals were combined to produce one sample for the determination at 6 days of age; livers from six rats were combined to produce three samples at 15 days of age. Four to six rats per group were used for the other determinations. ○, Control; ●, STH injected.

control rats 5 and 15 days old is quite low, it is not surprising that further depression with STH treatment could not be detected. The normal age-related increase in the hepatic metabolism of two drugs was, however, prevented by a single injection of STH in young rats. Similar results were noted when young rats were injected every other day with a smaller dose (0·25 mg/100 g body weight) of STH (unpublished observation).

Two possible explanations for this effect on liver drug metabolism in STH treated young rats are: (i) the appearance of inhibitors in rat liver, and (ii) constant total activity with reduced specific activity secondary to hepatomegaly without an increase in enzyme. The metabolism of hexobarbital and aminopyrine was measured in 52 day old rats which had been treated with albumin or STH, and various amounts of each liver fraction were added to a reaction mixture to detect the presence of inhibitors. Mixtures which contained 1, 2 or 3 parts of control 9,000*g* liver supernatant fraction and 3, 2 or 1 parts respectively of liver fraction from STH injected rats showed the expected activity. Thus no *in vitro* inhibitors were demonstrated in liver from STH treated rats. Direct inhibition of hepatic drug metabolism by the presence of STH in liver of STH treated young rats is also unlikely, because no *in vitro* inhibition was detected when an STH containing hormone mixture was added to a reaction vessel with control adult rat liver 9,000*g* supernatant fraction[10]. No enlargement of the liver was noted after injection of STH, and in young rats injected once with STH (Fig. 1), no significant change in 9,000*g* liver supernatant fraction protein (mg/g liver) was found as compared with control rats of the same age.

The mechanism by which STH treatment decreases liver drug metabolism in the young rat remains unknown. Some insight into this problem, however, was gained by observing the effect of phenobarbital in STH treated rats (Table 1). The injection of young rats with STH did not prevent a post-phenobarbital increase in the liver metabolism of hexobarbital or aminopyrine. Similar results were found when adult rats implanted with the STH secreting pituitary tumour MtT–F4 were treated with phenobarbital[11]. These observations suggest that different independent mechanisms may be responsible for the effect of STH or phenobarbital on this system.

Table 1. THE EFFECT OF PHENOBARBITAL ON LIVER DRUG METABOLISM IN YOUNG RATS INJECTED WITH STH

Treatment	Hexobarbital metabolized (μmole/g liver)	Formaldehyde from aminopyrine (μmole/g liver)
Albumin	6·5 ± 0·3	10·8 ± 0·3
Albumin and phenobarbital	10·9 ± 0·4	15·0 ± 0·6
STH	3·2 ± 0·6	7·6 ± 0·5
STH and phenobarbital	9·2 ± 0·4	13·2 ± 0·9

Male Fischer rats, 45 days old, were injected with bovine albumin or STH (1 mg/ml./100 g body weight) subcutaneously. One intraperitoneal injection of phenobarbital sodium (75 mg/kg) was given 24 h after the hormone injection. Determinations of rat liver drug metabolism were made 48 h after the rats received albumin or STH. The μmoles of hexobarbital metabolized or formaldehyde formed from aminopyrine per g liver is expressed as the mean ± *S.E.* The metabolism found with liver from phenobarbital treated animals was statistically significant ($P < 0{\cdot}05$) as compared with that from rats which did not receive phenobarbital. There were six rats per group.

As the animal grows older, certain physiological events occur which allow for an increase in liver drug metabolic enzyme activity to adult levels. Fouts *et al.*[12,13] have proposed that an increase in activity may occur as a result of enzyme protein synthesis in the developing animal. Identification of physiological influences which allow for increased enzyme activity in the young rat may increase our understanding of the normal sequence of biochemical events in drug metabolism. Such influences may not be recognized in the adult as a result of slight changes in activity, masked by high levels of metabolism. If, however, conditions which regulate hepatic drug-metabolic activity in the young animal were produced in the adult, a level of this activity in the adult similar to that found in the young rat would be expected. High levels of STH in blood may be one of these conditions.

Maternal steroids characteristic of pregnancy and post-partum may also affect liver drug metabolism. Low levels of drug metabolism in pregnant dams and nursing rat pups may be related to high levels of gonadal hormones in the dam as suggested by Feuer and Liscio[14]. *In vitro* inhibitors (which would have produced an increase in hexobarbital sleeping time or a decrease in liver drug metabolism) were, however, not identified. A relationship between maternal sex steroids and liver drug metabolism probably exists, but does it offer a primary or secondary explanation for low levels of drug metabolism in the newborn? Studies on the mechanism of action with regard to an *in vitro* versus *in vivo* effect of specific maternal steroids and competitive inhibition by alternative substrates would be helpful.

I propose that STH is a factor which alters the normal postnatal developmental pattern of liver drug metabolism by an action *in vivo*. Alteration of this pattern by injection of STH, failure to find *in vitro* inhibitors in liver microsomes of STH treated rats, and a post-phenobarbital increase in metabolism in STH treated rats are consistent with this hypothesis. In the human newborn, elevated levels in blood of STH (ref. 15) and low levels of drug metabolism were found[16]. *In vitro* inhibitors of drug metabolism have not been demonstrated in liver microsomes from young rabbits[13], and phenobarbital increased liver drug metabolic enzyme activity in young animals[17]. Thus liver drug metabolism in young rats has certain features in common with that of STH treated rats. A high blood level of STH in the young animal may affect liver metabolism to produce a low level of drug biotransformation.

This work was supported in part by a grant from the US National Institutes of Health and a fellowship grant from the National Association for Retarded Children. J. T. W. is recipient of a research career development award from the NIH. I thank the Endocrine Study Section of the National Institutes of Health for the bovine STH.

JOHN T. WILSON

Laboratory of Developmental Pharmacology,
Perinatal Health Center,
Children's Hospital of San Francisco,
San Francisco, California 94119.

Received July 8; revised October 27, 1969.

[1] Kato, R., Vassanelli, P., Frontino, G., and Chiesara, E., *Biochem. Pharmacol.*, **13**, 1037 (1964).

[2] Wilson, J. T., *Ped. Res.*, **2**, 514 (1968).

[3] Bates, R. W., Milkovic, S., and Garrison, M. D., *Endocrinology*, **71**, 943 (1962).

[4] Wilson, J. T., *Biochem. Pharmacol.*, **18**, 2029 (1969).

[5] Cooper, J. R., and Brodie, B. V., *J. Pharmacol. Exp. Ther.*, **114**, 409 (1955).

[6] Nash, T., *Biochem. J.*, **55**, 416 (1953).

[7] Cochin, J., and Axelrod, J., *J. Pharmacol. Exp. Ther.*, **125**, 105 (1959).

[8] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

[9] Snedecor, G. W., *Statistical Methods* (Iowa State Univ., Ames, 1956).

[10] Wilson, J. T., *Biochem. Biophys. Res. Commun.*, **32**, 903 (1968).

[11] Wilson, J. T., *Proc. Soc. Exp. Biol. and Med.*, **128**, 445 (1968).

[12] Fouts, J. R., *Embryopathic Activity of Drugs* (edit. by Robson, J. M., and Smith, R. L.) (Churchill, London, 1965).

[13] Fouts, J. R., and Adamson, R. H., *Science*, **129**, 897 (1959).

[14] Feuer, G., and Liscio, A., *Nature*, **223**, 68 (1969).

[15] Cornblath, M., Parker, M. L., Reisner, S. H., Forbes, A. E., and Doughaday, W. H., *J. Clin. Endocrinol.*, **25**, 209 (1963).

[16] Nitowsky, H. M., Matz, L., and Berzofsky, J. A., *J. Ped.*, **69**, 1139 (1966).

[17] Conney, A. H., Davison, C., Gastel, R., and Burns, J. J., *J. Pharmacol. Exp. Therap.*, **130**, 1 (1960).

Potent Peripheral and Splanchnic Vasodilator Peptide from Normal Gut

BIOLOGICALLY active polypeptides, such as bradykinin and angiotensin, have considerable importance in a variety of physiological, pharmacological and pathological reactions. We have recently reported on the extraction from normal lung tissue of a peptide fraction capable of causing gradual and prolonged peripheral vasodilatation[1,2]. This finding led us to pursue the search for similar vasoactive principles in extracts of other metabolically active organs. We report here on the extraction from small intestine of a highly active, hitherto unidentified vasodepressor peptide substance. The vasodilator effect of this compound, several times as prolonged as that of bradykinin, may be important as a regulator of blood flow to the digestive organs, and as a mediator in various hypotensive states.

Hog small intestine was treated as for the preparation of secretin[3,4]. Like secretin, the vasoactive peptide was concentrated in the methanol extract. This extract was chromatographed in 0·125 M sodium phosphate, *p*H 8, on a column of carboxymethylcellulose. Using the same buffer, the bulk of the secretin was eluted in one fraction, which also exhibited moderate vasoactivity. An intensely vasoactive fraction was, however, retained on the column and recovered by eluting with 0·2 M HCl. Further purification of this eluate was obtained by chromatography in 0·2 M acetic acid on a column of 'Sephadex *G*-25'.

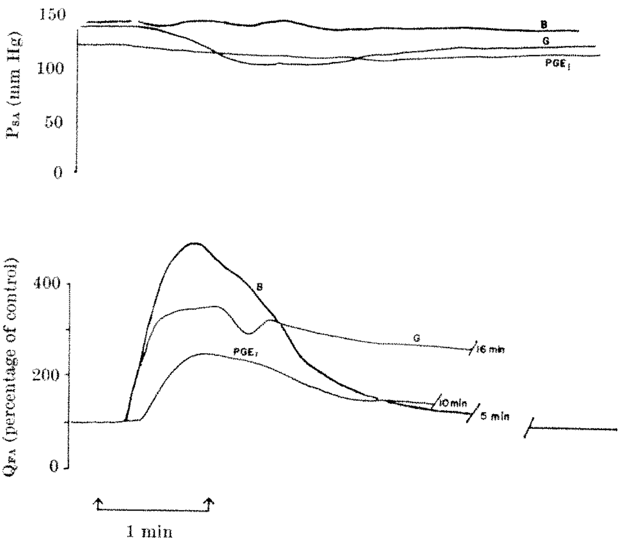


Fig. 1. Increase in femoral arterial blood flow ($\dot{Q}_{FA}$) and fall in systemic arterial blood pressure (P_{SA}) following infusion into the femoral artery of: methanol-soluble gut peptide fraction (G, 100 µg/ml.), bradykinin (B, 1 µg/ml.), and prostaglandin E_1 (PGE_1, 0·5 µg/ml.). All infusions were given at 1 ml./min for 1 min, between the two arrows. Curves, traced from the records, were superimposed to start at the same time. Interruptions in tracings show length of time until return to control value.

We assayed the vasoactivity of different preparations by measuring the increase in femoral arterial blood flow on their infusion, at a constant speed, into a cannulated branch of a femoral artery of dogs, anaesthetized with pentobarbital. Mean blood flow was measured by a square-wave electromagnetic probe (Nycotron) placed on the main artery, and recorded continuously by an Elema-Schönander amplifier and a Siemens oscillographic recorder. Simultaneously, systemic arterial blood pressure was recorded by a catheter in the aorta and a strain-gauge transducer.

Marked and prolonged increase in femoral arterial blood flow followed the intra-arterial injection of the methanol-soluble extract (Fig. 1). The onset of the flow increase was almost as rapid as from a dose of bradykinin causing a similar increase in flow, but lasted several times as long. The effect of the extract was qualitatively similar to that of prostaglandins E_1 and E_2. The increased flow was accompanied by a fall in systemic blood pressure of as much as 50 mm Hg, lasting about the same length of time.

On intravenous infusion of the purified vasoactive peptide fraction, arterial blood pressure fell and peripheral blood flow increased, both changes being dose-dependent. After a 3 min infusion of 20 µg per kg per min, the dogs remained hypotensive for 45 min. The increase in peripheral blood flow, together with systemic hypotension, clearly signifies peripheral vasodilatation.

In a group of six experiments, in collaboration with P. Olsson and L. Thulin, we recorded the blood flow in the hepatic and superior mesenteric arteries, and the portal vein, by probes placed around these vessels. Infusions of the peptide into the inferior vena cava increased the blood flow in the splanchnic vessels along with lowering the arterial blood pressure. Infusions of the same doses into the portal vein, however, caused no change in the blood pressure, suggesting that the peptide is inactivated or removed by the liver.

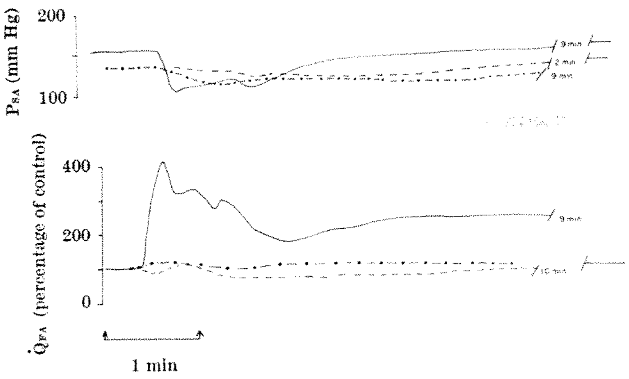


Fig. 2. Comparison of the effects on peripheral blood flow and systemic arterial pressure of equal doses (100 μg) of the vasoactive gut peptide material (——), secretin (· – · – ·) and cholecystokinin–pancreozymin (– – – –).

The vasoactivity in the peptide fraction was totally abolished by incubation with α-chymotrypsin (CDS, Worthington, 60 μg per mg), and almost completely lost by incubation with trypsin ('TRTPCK', Worthington). On high-voltage paper electrophoresis at *p*H 6·5, the peptide material moved toward the cathode more slowly than and distinctly from bradykinin.

A vasodepressor effect of intestinal extracts was observed many years ago by Bayliss and Starling[5]. Since then the gut has been shown to contain several vasoactive substances, including histamine[6], prostaglandins[7] and 'substance P' (ref. 8). In addition, the digestive hormones secretin and cholecystokinin–pancreozymin (CCK-PZ) also have certain vascular effects[9]. The gut fraction we are describing was free of histamine (eliminated by salt precipitation and 'Sephadex' chromatography), and free of prostaglandins (removed by ether precipitation at *p*H 2). It contained virtually no CCK-PZ, as this hormone is not extracted by methanol. The peptide fraction was slightly contaminated with secretin, though in concentrations of approximately 1 per cent (bioassay). To establish the possible role of any contamination with secretin and CCK-PZ, however, we measured the separate effects of equal doses of these hormones on intra-arterial injection in the femoral artery (Fig. 2). CCK-PZ (refs. 3 and 4) caused a decrease, rather than an increase, in femoral flow, along with a fall in blood pressure of about 15 mm Hg. Secretin (synthetic, Squibb) gave a 25 per cent increment in flow with a 20 mm Hg drop in pressure, compared with a three-fold increase in flow and a 50 mm Hg lowering of pressure, resulting from the vasoactive peptide fraction. Thus the vasoactivity of the newly identified gut peptide is distinct from, and many times greater than, that of secretin and CCK-PZ.

It is difficult to assess the relationship, if any, between this peptide and 'substance P', for the latter is probably a mixture of several peptides the characteristics of which may differ in different preparations[10-12]. Several lines of evidence, however, suggest that the active principle described here is unrelated to 'substance P'. The prolonged vasodepressor effect we observed contrasts with the transient effect of 'substance P' which, on intravenous infusion, decreases or vanishes even while the substance is being infused[10-12].

It will be of interest to examine what effects this systemic vasodilator peptide may have on other vascular beds, for example, the pulmonary circulation, as well as on other smooth-muscle tissues, such as the bronchial tree and the digestive tract itself. It also remains to be determined whether this peptide is in any way related to the vasoactive lung peptide[1] (for example, formed in the gut and modified by the lung). In any case, however, its strong and unusually prolonged vasodilator action on the peripheral and splanchnic circulations raises the possibility that it might have a physiological role in the regulation of blood flow to the digestive organs. This process remains poorly understood[13]. Release of this peptide could be an important mechanism in the production of systemic hypotension and splanchnic vasodilatation in such conditions as endotoxin shock[14]. It is also reasonable to speculate that, if the liver is normally the main site for inactivating this vasoactive intestinal peptide, its failure of destruction when liver function is seriously impaired, for example, in hepatic cirrhosis, could account for the peripheral vasodilatation complicating that disease[15].

We thank Professor S. Bergström, Emeritus Professor E. Jorpes and Dr E. Berglund for their support and encouragement, Dr M. A. Ondetti for a gift of synthetic secretin and bradykinin, and Mrs K. Librén, Mrs M. Christofferson, Miss A-C. Öhrlund, Mrs K. Thermaenius, Mrs L. Melin and Mr P-O. Bäckman for skilled assistance. This work was supported by research grants from the US Public Health Service, the American Heart Association, the National Tuberculosis Association and the Swedish Medical Research Council. S. I. S. is a recipient of a career development award from the National Heart Institute.

SAMI I. SAID*
VIKTOR MUTT

Department of Chemistry,
Karolinska Institute, and
Thoracic Surgery Research Laboratory,
Karolinska Hospital,
Stockholm, Sweden.

Received September 22, 1969.

---

* On leave from the Department of Medicine, Medical College of Virginia, Richmond 23219.

[1] Said, S. I., and Mutt, V., *Scand. J. Clin. Lab. Invest.* (in the press).
[2] Said, S. I., and Mutt, V., *Nature*, **224**, 699 (1969).
[3] Jorpes, J. E., and Mutt, V., in *Ciba Foundation Symposium on the Exocrine Pancreas*, 150 (Churchill, London, 1962).
[4] Mutt, V., and Jorpes, J. E., in *Recent Progress in Hormone Research*, **23**, 483 (Academic Press, New York, 1967).
[5] Bayliss, W. M., and Starling, E. H., *J. Physiol.*, **28**, 325 (1902).
[6] Feldberg, W., in *Ciba Foundation Symposium on Histamine*, 4 (Churchill, London, 1956).
[7] Bergström, S., Carlson, L. A., and Weeks, J. R., *Pharmacol. Rev.*, **20**, 1 (1968).
[8] von Euler, U. S., and Gaddum, J. H., *J. Physiol.*, **72**, 74 (1931).
[9] Dorigotti, L., and Glässer, A. H., *Experientia*, **24**, 806 (1968).
[10] Haefely, W., and Hürlimann, A., *Experientia*, **8**, 297 (1962).
[11] Meinardi, H., and Craig, L. C., in *Hypotensive Peptides* (edit. by Erdös, E. G., Back, N., and Sicuteri, F.), 594 (Springer, New York, 1966).
[12] Zuber, H., in *Hypotensive Peptides* (edit. by Erdös, E. G., Back, N., and Sicuteri, F.), 584 (Springer, New York, 1966).
[13] Lundgren, O., *Proc. Internat. Union Physiol. Sciences, vol. 6 (Twenty-fourth Internat. Cong.)*, 175 (1968).
[14] Wyler, F., Forsyth, R. P., Nies, A. S., Neutze, J. M., and Melmon, K. L., *Circ. Research*, **24**, 777 (1969).
[15] Fomon, J. J., and Warren, W. D., *Ann. Rev. Med.*, **20**, 277 (1969).

Active Water-insoluble Derivative of Renin

ALTHOUGH the action of renin on the blood pressure has been explored by many investigators, some of its basic enzymatic properties are yet to be established[1]. While studying plasma kinins, we found it to be of advantage to couple enzymes that release or inactivate kinins covalently to water-insoluble complexes[2-4]. The active insoluble derivatives of enzymes are convenient to use, because, among many other attractive properties, they are stable, re-usable and readily removed from incubation mixture by sedimentation. We have extended these investigations to renin. Our previous results with killikrein, carboxypeptidases, kallikrein and trypsin inhibitors and renin were briefly reported[3,4]. This report deals with the binding of renin to 'Sepharose 4*B*'.

Partially purified hog kidney renin was obtained from commercial sources. It contained a 4 ng of renin pressor

▶**Amino Acids—^{14}C Labeled**
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700

(SEE ADVERTISEMENT THIS PAGE)

▶**Amino Acids—^{3}H Labeled**
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700

(SEE ADVERTISEMENT THIS PAGE)

▶**Amino Acids—^{15}N and ^{35}S Labeled**
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700

(WRITE FOR INFORMATION)

▶**Amino Acids—Reconstituted Protein Hydrolysates (^{14}C, ^{3}H)**
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700

(WRITE FOR INFORMATION)

▶**Amino Acids—Special Contract Orders**
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700

(WRITE FOR INFORMATION)

▶**Amino Acids—t-Boc**
Schwarz/Mann
We supply virtually every starting material needed for peptide synthesis: 33 Boc-amino acids, 22 ^{14}C and ^{3}H labeled Boc-amino acids, 12 Boc-amino acid resin esters, etc. Write or call for brochure.
Orangeburg, New York
10962 (914) 359-2700

▶**Amino Acids—Unlabeled**
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700

(WRITE FOR INFORMATION)

▶**Amino Acids-**
Schwa

^{14}C Amino Acids from Schwarz/Mann

DIVISION OF BECTON, DICKINSON AND COMPANY

Examples of the unusually high specific activities available from Schwarz/Mann:

L-[^{14}C] Alanine	159mc/mM	L-[^{14}C] Lysine	320mc/mM
L-[^{14}C] Arginine	320mc/mM	L-[^{14}C] Methionine	255mc/mM
L-[^{14}C] Aspartic Acid	212mc/mM	L-[^{14}C] Phenylalanine	455mc/mM
[^{14}C] Glycine	106mc/mM	L-[^{14}C] Threonine	212mc/mM
L-[^{14}C] Leucine	320mc/mM	L-[^{14}C] Tyrosine	455mc/mM

Further re our high specific activities: ALL of our ^{14}C amino acids are higher than 50mc/mA. About purity. Every lot of phenylalanine and tyrosine is repurified every month. Every lot of everything else: reassayed every ten weeks and repurified if necessary. And *all* assay and reassay data goes onto the Product Analysis Report that you receive. About availability: these compounds are available *from stock*. For the complete story on our complete labeled amino acid line call (914) 359-2700 or write: Schwarz/Mann, Orangeburg, New York 10962. Division of Becton, Dickinson and Company.

(914) 359-2700

(SEE OUR OTHER ADVERTISEMENT AND LISTINGS ON THIS PAGE.)

^{3}H AMINO ACIDS FROM SCHWARZ/MANN

Examples of the unusually high specific activities available from Schwarz/Mann:

Catalytic

L-[^{3}H] Alanine	6c/mM
L-[^{3}H] Arginine	10c/mM
L-[^{3}H] Histidine	8c/mM
L-[^{3}H] Lysine	6c/mM
L-[^{3}H] Phenylalanine	7c/mM
L-[^{3}H] Valine	8c/mM

Specifically Labeled

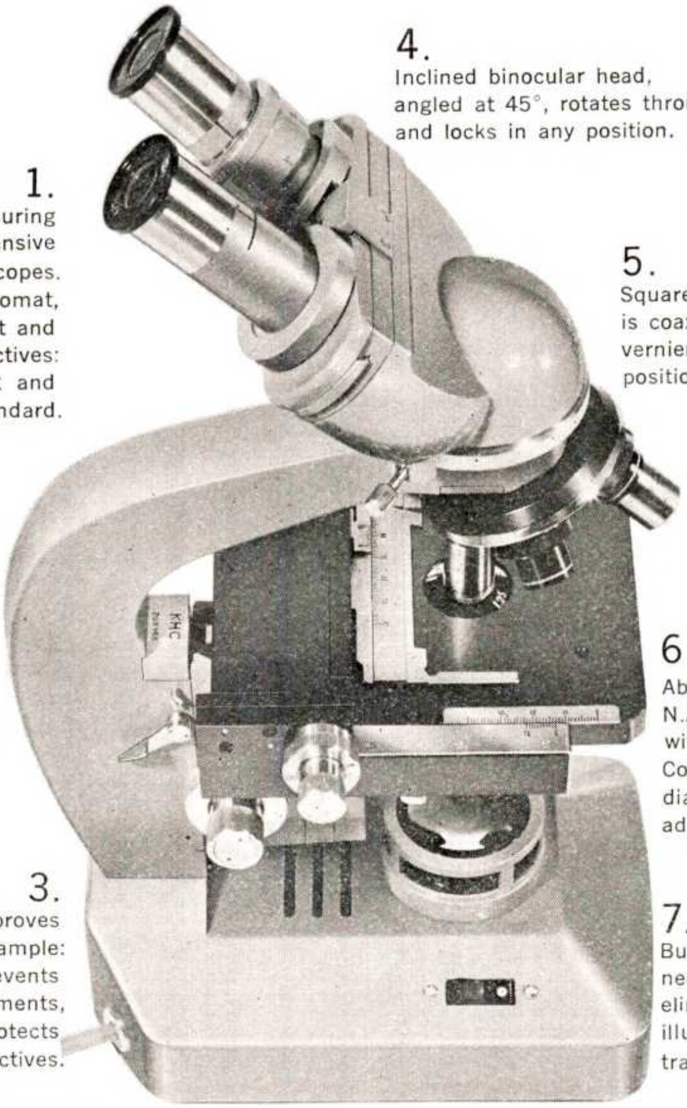
L-[4^{3}H] Arginine	20c/mM
L-[2,3^{3}H] Aspartic Acid	15c/mM
L-[3,4^{3}H] Glutamic Acid	15c/mM
L-[4,5^{3}H] Leucine	50c/mM
L-[4,5^{3}H] Lysine	20c/mM
L-[5^{3}H] Proline	15c/mM
L-[2,3^{3}H] Valine	15c/mM

These high specific activity ^{3}H-labeled amino acids are reassayed every ten weeks—as is *every* radiochemical we make—and repurified when necessary. All relevant analytical data gets forwarded to you. For detailed information call (914) 359-2700 or write: Schwarz/Mann, Orangeburg, New York 10962. Division of Becton, Dickinson and Company.

(SEE OUR OTHER ADVERTISEMENT AND LISTINGS ON THIS PAGE.)

**For the rest of the Schwarz/Mann amino acid story write or call Schwarz/Mann
Orangeburg, N.Y. 10962
(914) 359-2700**

Olympus KHC is top choice for latest features and low budget



1.
Wide field or measuring eyepieces as in expensive research microscopes. Highest quality Achromat, Plan Achromat and Fluorite objectives: Achromat 4×, 10×, 40× and 100× (oil) standard.

2.
Fine and coarse focusing knob are coaxial and positioned low for ease of operation.

3.
Modern design improves handling ease. For example: vertical stage focusing prevents distracting head movements, and pre-set focus lock protects slides and objectives.

4.
Inclined binocular head, angled at 45°, rotates through 360° and locks in any position.

5.
Square mechanical stage is coaxially-controlled and vernier calibrated to give exact positioning of slides.

6.
Abbe type condenser, N.A. 125, is focusable with rack and pinion. Continuously variable iris diaphragm allows adjustment of illumination.

7.
Built-in 20-watt illuminator needs no centering, eliminates accessory illuminators and transformers.

Olympus KHC is the latest improvement for every-day laboratory work, a microscope for the lowest budget and still featuring eye-fatigue-free clearness and high precision. Its seven outstanding features have made KHC the most used and valued instrument for medical students and laboratory workers all over the world.

Total magnification: 40x～1000x

50

OLYMPUS

OLYMPUS OPTICAL CO., LTD. 7, 3-chome, Kanda-Ogawamachi, Chiyoda-ku Tokyo, Japan
OLYMPUS OPTICAL CO., (EUROPA) GmbH. 2, Hamburg 1, Steindamm 105, Germany
OLYMPUS CORPORATION OF AMERICA 2 Nevada Drive, New Hyde Park, N.Y. 11040, U.S.A.

activity per mg protein[5] according to the manufacturer's specifications (Pentex Biochemicals). Renin substrate angiotensinogen was purified from hog plasma as described previously[6,7]. The activity of renin was measured by assaying the amount of angiotensin released by the enzyme on the systemic arterial blood pressure of the pithed rat against synthetic Ile^5–angiotensin I.

Renin was bound covalently to 'Sepharose *4B*' according to Axen *et al.*[8] and Cuetrecasas *et al.*[9], after 'Sepharose' was activated with cyanogen bromide. Twenty ml. of settled volume of 'Sepharose *4B*' were coupled with 100 mg renin. Renin was allowed to react with 'Sepharose' for 18 h at 4° C. Afterwards the complex was washed in a column with 2 l. of 0·1 M $NaHCO_3$ containing 1 M NaCl followed by 2 l. of distilled water. The release of angiotensin by the water-insoluble complex was done under constant shaking at 37° C. Five mg of angiotensinogen was dissolved in 1 ml. 0·05 M phosphate buffer of *p*H 6·5 containing 0·9 per cent NaCl. The solution was incubated with 0·5 ml. settled volume of the covalently bound renin–'Sepharose' (RS) complex and 0·5 ml. water. Samples were withdrawn at regular intervals. In one group of control studies equal amounts of soluble renin were used. In other control experiments, human albumin was coupled to 'Sepharose', and the albumin–'Sepharose' complex was used instead of the RS complex. Albumin–'Sepharose' did not release angiotensin from angiotensinogen.

The water-insoluble RS complex liberated a peptide from angiotensinogen that on intravenous injection raised the blood pressure of the rat. The activity of the vasopressor released expressed in terms of synthetic Ile^5–angiotensin I is summarized in Table 1. About half of the soluble renin used was bound to 'Sepharose' in our conditions; the renin so complexed had about half the activity of the equivalent amount of soluble enzyme (Table 1). The vasopressor material released from angiotensinogen by RS was inactivated by trypsin[7]. The trypsin preparation used was a water-insoluble complex of trypsin with 'Sepharose *4B*' (5 mg per ml. 'Sepharose'). The disappearance of the biological activity of angiotensin incubated with trypsin was followed on the isolated rat colon[10]. This inactivation by trypsin also indicated that the liberated vasopressor substance was a peptide. The RS preparation was stable when kept in the refrigerator for several months. After the completion of the reaction, RS can be sedimented in the centrifuge, washed and used again.

Table 1. RELEASE OF ANGIOTENSIN BY WATER-INSOLUBLE RENIN–SEPHAROSE DERIVATIVE

Amount of enzyme coupled with 'Sepharose' (mg per ml.)	Per cent $A_{280\,nm}$ absorbing material bound	Amount of peptide released from 5 mg substrate per h*	Per cent activity of bound renin
5	58	2·8	61

* In μg of synthetic Ile^5–angiotensin I equivalents.

The experiment showed that renin stayed active after being complexed with the agarose polymer 'Sepharose' and cleaved off a vasopressor, very likely angiotensin I, from the high molecular weight plasma protein substrate, angiotensinogen. Angiotensin I was subsequently converted to angiotensin II in the circulation of the rat.

The use of stable, water-insoluble enzyme preparations may have particular importance in systems which depend on the interaction of a number of factors that result in the release and inactivation of vasoactive peptides. Here the role of insoluble components can be studied by adding them or removing them at will. In future investigations stable RS preparations may serve as standards for renin activity or RS could be used in columns for concentrating and purifying renin inhibitors by means of affinity chromatography.

We thank Miss Deborah R. Downs for assistance. These studies were supported in part by a grant from the National Institutes of Health, US Public Health Service, by the US Office of Naval Research and by a fellowship (T. S.) of the Oklahoma Heart Association.

T. SEKI*
T. A. JENSSEN
Y. LEVIN†
E. G. ERDÖS

Department of Pharmacology,
University of Oklahoma School of Medicine,
Oklahoma City,
Oklahoma.

Received November 27, 1969.

* Permanent address: Tohoku University, Department of Medicine, Sendai, Japan.

† Permanent address: Weizmann Institute of Science, Rehovoth, Israel.

[1] *Renal Hypertension* (edit. by Page, I. H., and McCubbin, J. W.) (Year Book Medical Publishers, Inc., Chicago, 1968).
[2] Erdös, E. G., Seki, T., Yang, H. Y. T., and Tague, L. L., *Pharmacol. Res. Commun.*, **1**, 152 (1969).
[3] Seki, T., Yang, H. Y. T., Levin, Y., Jenssen, T. A., and Erdös, E. G., in *International Symposium on Cardiovascular and Neuro-actions of Bradykinin and Related Kinins* (Fiesole-Florence, July 21–25, 1969).
[4] Seki, T., Yang, H. Y. T., Levin, Y., Jenssen, T. A., and Erdös, E. G., *Advances in Experimental Medicine and Biology* (in the press).
[5] Sen, S., Smeby, R. R., and Bumpus, F. M., *Biochemistry*, **6**, 1572 (1967).
[6] Green, A. A., and Bumpus, F. M., *J. Biol. Chem.*, **210**, 281 (1954).
[7] Chiang, T. S., Erdös, E. G., Miwa, I., Tague, L. L., and Coalson, J. J., *Circ. Res.*, **23**, 507 (1968).
[8] Axen, R., Porath, J., and Ernback, S., *Nature*, **214**, 1302 (1967).
[9] Cuatrecasas, P., Wilchek, M., and Anfinsen, C. B., *Proc. US Nat. Acad. Sci.*, **61**, 636 (1968).
[10] Regoli, D., and Vane, J. R., *Brit. J. Pharmacol. Chemother.*, **23**, 351 (1964).

Net Synthesis of ATP by Reversal of the Sodium Pump

A FEW years ago, Garrahan and Glynn[1,2] described experiments in which very steep concentration gradients of Na^+ and K^+ across the membranes of human red cell ghosts led to a ouabain-sensitive incorporation of ^{32}P-labelled inorganic phosphate into ATP, apparently by a reversal of the cation transport system. These findings have been confirmed and extended in experiments on ghosts[3,4] and in experiments on intact red cells[5–7] in which the incorporation of phosphate was shown to parallel the ouabain-sensitive downhill movements of the cations. Although the interpretation of these experiments seems clear, attempts in a number of laboratories to demonstrate a net ouabain-sensitive synthesis of ATP at the expense of downhill ion movements have not been successful. The failure of these attempts can be attributed to the relatively low rate of ouabain-sensitive phosphate incorporation in human cells, ATP breakdown by processes unconnected with the pump being rapid compared with the expected rate of formation of ATP by reversal of the pump. Nevertheless, in the absence of a demonstrable increase in the amount of ATP, it is difficult to be certain that the observed incorporation of radioactivity is brought about by the formation of new ATP rather than by some kind of exchange reaction. The finding that guinea-pig red cells showed a surprisingly large ouabain-sensitive efflux of K^+ into K^+-free high-Na^+ media[8] suggested that it would be worth looking for a net ouabain-sensitive synthesis of ATP in these cells, and we now report that such a synthesis does in fact occur.

The results of three experiments are summarized in Table 1. In each experiment, washed, pooled, guinea-pig red cells were pre-incubated in a nominally glucose-free high-K^+ medium for 2 h or more. During at least part of this period iodoacetamide (5 mM) was present. In the first two experiments, after the pre-incubation, the cells were loaded with inorganic phosphate by incubating them for 15 min in a phosphate–citrate medium[7]; this step was omitted in the third experiment because phosphate loading did not seem to have much effect on the magnitude of the ouabain-sensitive K^+ efflux from guinea-pig red cells. The

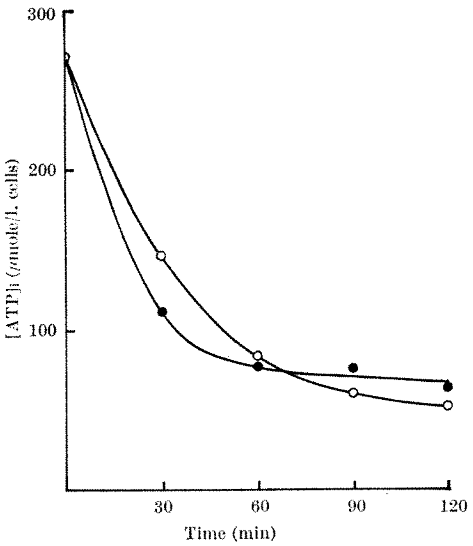


Fig. 1. The change in ATP concentration with time when guinea-pig red cells that had been stored for 24 h at 5° C in the presence of glucose were incubated at 37° C in a medium containing 150 mM NaCl, 10 mM Tris–HCl (pH 7·4 at 37° C) and 5 mM iodoacetamide. ○—○, Ouabain absent; ●—●, ouabain 5×10^{-5} g/ml. Loss of K^+ from the cells raised the K^+ concentration in the medium from 0·032 mM at the beginning of the incubation to 1·04 mM at the end. The cells used in this experiment were from the same batch as those used in the third experiment of Table 1. Note that at first the ATP level fell less steeply in the absence of ouabain, presumably because hydrolysis of ATP was partly offset by synthesis through reversal of the pump. The crossing of the curves can be explained by the accumulation of K^+ in the initially K^+-free medium. During the latter part of the incubation the K^+ concentration in the medium was sufficient to allow the pump to run forwards, so that ouabain at this stage must have been inhibiting hydrolysis more than synthesis.

cells were then washed thoroughly in a high-Na^+, K^+-free medium and resuspended at a haematocrit of about 5 per cent in more of the same medium to which iodoacetamide had been added to give a final concentration of 5 mM. At the end of the incubation period the concentration of ATP in the cells that had been kept at 0° C, and in the incubated cells, was measured[2].

In all three experiments, the concentration of ATP in the cells that were not exposed to ouabain increased during the incubation. In the presence of ouabain the ATP concentration did not change during the incubation in experiments 1 and 3; in experiment 2 there was an increase in concentration, though a much smaller increase than was found in the cells not exposed to ouabain. The increase in ATP concentration in the presence of ouabain in experiment 2 is, at first sight, surprising, for at the concentration at which it was used ouabain should have inhibited the pump completely. When guinea-pig red cells are incubated with iodoacetamide, however, the ATP concentration falls steeply at first (Fig. 1) but then levels off, presumably when the rate of hydrolysis of ATP equals the rate of formation from 2,3-diphosphoglycerate plus—if conditions are suitable—the rate of formation by reversal of the pump. If the steady state is attained in a high-K^+ medium, that is, when the pump is running forwards, the addition of ouabain will decrease ATP hydrolysis and may therefore cause an increase in the ATP level.

Table 1. CHANGES IN ATP CONCENTRATION DURING INCUBATION IN A HIGH-Na^+ K^+-FREE MEDIUM, WITH AND WITHOUT OUABAIN

	ATP content		
Experiment	Before incubation (μmole/l. cells)	After incubation without ouabain (μmole/l. cells)	After incubation with ouabain (μmole/l. cells)
1	179 ± 5	243 ± 16	170 ± 3
2	292 ± 7	391 ± 7	333 ± 5
3	44·5 ± 0·2	62·6 ± 1·0	46·6 ± 0·7

Guinea-pig red cells, pretreated as described in the text, were incubated for 30 min in a medium containing 150 mM NaCl, 10 mM Tris–HCl (pH 7·4 at 37° C) and 5 mM iodoacetamide. The ouabain concentration was 5×10^{-5} g/ml. Each figure is the mean of three estimates ± *S.E.* The details of the preliminary incubation were different in the three experiments. In the first, the cells were incubated for 5 h at 30° C in a medium containing 150 mM KCl and 10 mM Tris–HCl (pH 7·4 at 37° C), and then for another hour at 37° C in a similar medium containing 5 mM iodoacetamide. In the second experiment, the cells were incubated for 2 h at 37° C without iodoacetamide and for 40 min with iodoacetamide present. In the third experiment, the preliminary incubation was for 2 h at 37° C and iodoacetamide was present during the whole of this period. The cells used in the third experiment had been stored overnight in a medium containing glucose, and some of this glucose was probably transferred with the cells to the nominally glucose-free solution used for the preliminary incubation. The use of ATP in the conversion of glucose to triose phosphate may account for the very low ATP levels found later in the experiment[9].

The finding, in all three experiments of Table 1, that incubation in a high-Na^+, K^+-free medium led to an increase in the concentration of ATP which could be wholly or partly prevented with ouabain provides strong evidence that reversal of the pump is associated with a net synthesis of ATP.

We thank the Medical Research Council for financial support.

V. L. LEW
I. M. GLYNN

Physiological Laboratory,
University of Cambridge.

J. C. ELLORY

ARC Institute of Animal Physiology,
Babraham, Cambridge.

Received December 15, 1969.

[1] Garrahan, P. J., and Glynn, I. M., *Nature*, **211**, 1414 (1966).
[2] Garrahan, P. J., and Glynn, I. M., *J. Physiol.*, **192**, 237 (1967).
[3] Lant, A. F., and Whittam, R., *J. Physiol.*, **199**, 457 (1968).
[4] Lant, A. F., Priestland, R. N., and Whittam, R., *J. Physiol.*, **207**, 291 (1970).
[5] Glynn, I. M., and Lew, V. L., *J. Physiol.*, **202**, 89P (1969).
[6] Glynn, I. M., and Lew, V. L., *J. Gen. Physiol.*, **54**, 289s (1969).
[7] Glynn, I. M., and Lew, V. L., *J. Physiol.*, **207**, 393 (1970).
[8] Ellory, J. C., and Lew, V. L., *J. Physiol.*, **206**, 36*P* (1970).
[9] Dunham, E. T., *Physiologist*, **1**, 23 (1957).

Phosphorylase Activity in Acutely Exercised Muscle

MUSCLE glycogen serves as an important source of energy for muscular contraction. The initial glycogen degradation reaction is catalysed by the enzyme, phosphorylase.

A controlled histochemical approach to the study of phosphorylase in resting and exercised skeletal muscles has enabled us to localize phosphorylase responses to treadmill running in specific muscle fibres. Phosphorylase activity has previously been studied in homogenates and frozen sections of skeletal muscle immediately following electrical stimulation but not after exercise[1-3].

Twelve adult guinea-pigs (mixed strain) were run on a treadmill at a rate of 1·6 km/h for 5 min, 10 min or to exhaustion. Each exercised animal was paired with a non-exercised control animal of the same age. Immediately after the run, each experimental animal was removed from the treadmill and killed with a sharp blow to the head. The left plantaris and soleus muscles were removed; the belly of each muscle was ablated and frozen with isopentane which was previously cooled to a viscous fluid with liquid nitrogen. Muscle samples from the control and exercised animals were mounted in a frozen state on the same chuck; 6 μm sections were cut, thawed onto coverslips at room temperature, briefly air-dried and simultaneously incubated on the same day the animal was killed. A slightly modified technique of Eränkö and Palkama was used to demonstrate phosphorylase[4]. To determine which fibre types showed the phosphorylase reaction, serial sections were also tested for succinate dehydrogenase (SDH), malate dehydrogenase (MDH), reduced nicotine-adenine dinucleotide diaphorase (NADH–D), menadione linked α-glycerophosphate dehydrogenase, and "myosin" adenosine triphosphatase activity[5-8]. Activity of each of the enzymes in each fibre type has been described earlier[9,10]. The terms white, intermediate and red muscle fibre types are used in this paper and correspond to the classification of A, B and C, respectively, first suggested by Stein and Padykula[9]. A modified trichrome was used for demonstration of morphological details[11]. Analyses were completed from black-and-white photographic prints. The relatively white (high proportion of white fibres) and red (high proportion of red fibres) regions of the plantaris[10] and the central portion of the soleus, which consists of only intermediate type fibres, were studied. Approximately 150 fibres from each of the three regions were analysed.

Table 1. HISTOCHEMICAL PHOSPHORYLASE ACTIVITY IN EXERCISED MUSCLE

Exercise duration	No. of experiments	Percentage of fibres lacking phosphorylase activity Region of muscle "Red"	"White"
None	12	0	0
5 min	1	7	4
10 min	4	10	21
Exhaustion	7	61	36

Table 1 summarizes the results obtained for the histochemical demonstration of phosphorylase. In control animals, neither the red nor the white regions of the plantaris contained any phosphorylase-negative fibres. Fibres with moderate SDH, MDH and NADH–D activity contain moderate amounts of phosphorylase in control animals (Fig. 1*A* and *B*). With increasing durations of exercise, higher proportions of phosphorylase-negative fibres were found (Table 1). Because red fibres were found to be the most susceptible to phosphorylase "inactivation", the relatively high percentage of negative fibres in the red region relative to the white region of the acutely exercised plantaris muscles is to be expected. On the other hand, white fibres were the most resistant to becoming phosphorylase-negative after exhaustive exercise (Fig. 2*A* and *B*). No consistent changes were observed in the soleus. This may be because the soleus is composed of intermediate fibres, which normally show negligible phosphorylase activity even in control guinea-pigs.

It could be suggested that the relatively greater resistance of white fibres to loss of phosphorylase activity was due to higher glycogen levels in white than red fibres which may, in turn, affect the histochemically measured level of phosphorylase[12]. It is more likely that red fibres were used selectively during the treadmill exercise. The latter suggestion has some merit, for it has been proposed that white fibres are innervated by large motor horn cells having higher thresholds for activation than do smaller motor horn cells which innervate the red fibres[13]. This suggests that the motor horn cells which innervate red muscle fibres are more susceptible to activation than those that innervate white fibres. This would result in greater red than white muscle fibre activity and, consequently, a preferential phosphorylase "inactivation" of red fibres might be expected. This explanation fits well with the idea that the biochemistry of white fibres is geared mainly to short bursts of high intensity work, and hence may not be activated to contract during prolonged, lower intensity work such as treadmill exercise.

Localization of an either strongly negative or strongly positive histochemical reaction for phosphorylase in specific fibre types clearly demonstrates significant differences between individual fibre types during exercise. Kugelberg and Edström have reported similar results with electrical stimulation in that fibre responses were localized in specific fibre types and apparently motor units[3]. They observed, however, that white fibres were selectively depleted of phosphorylase activity, whereas some red fibres were never depleted. This is exactly the reverse of our observations in animals that were run on a treadmill. These findings leave some question as to the appropriateness of making biochemical inferences about normally exercised muscles from electrically stimulated muscles.

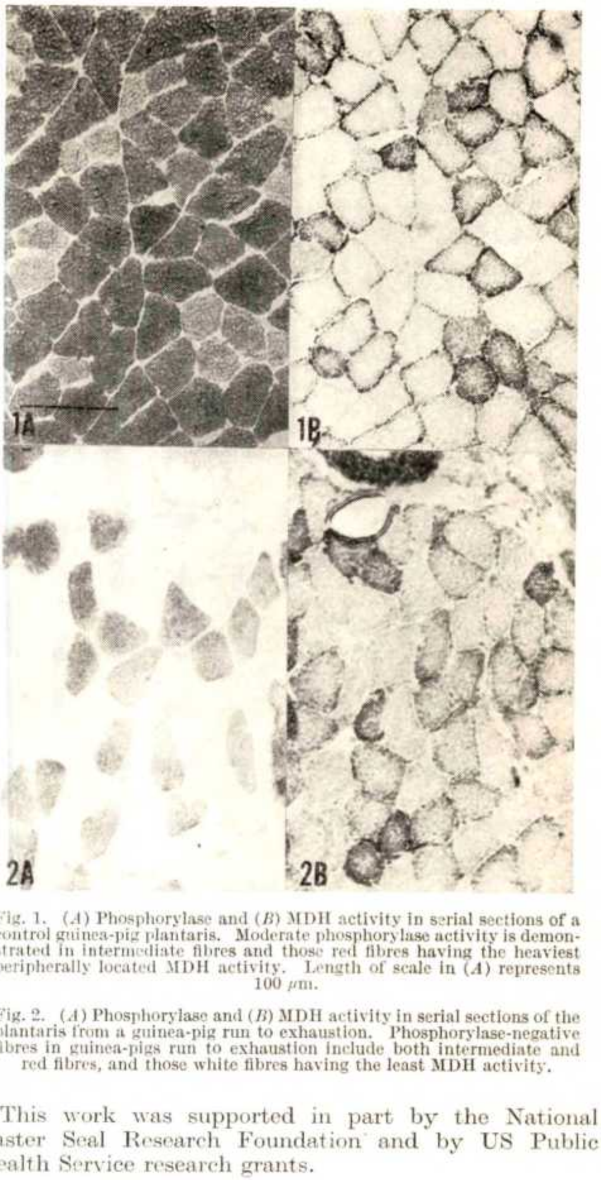


Fig. 1. (*A*) Phosphorylase and (*B*) MDH activity in serial sections of a control guinea-pig plantaris. Moderate phosphorylase activity is demonstrated in intermediate fibres and those red fibres having the heaviest peripherally located MDH activity. Length of scale in (*A*) represents 100 μm.

Fig. 2. (*A*) Phosphorylase and (*B*) MDH activity in serial sections of the plantaris from a guinea-pig run to exhaustion. Phosphorylase-negative fibres in guinea-pigs run to exhaustion include both intermediate and red fibres, and those white fibres having the least MDH activity.

This work was supported in part by the National Easter Seal Research Foundation and by US Public Health Service research grants.

V. R. EDGERTON
DONALD SIMPSON
R. JAMES BARNARD
J. B. PETER

Departments of Physical
Education and Medicine,
University of California,
Los Angeles,
Los Angeles, California 90024.

Received August 26; revised November 18, 1969.

[1] Danforth, W. H., and Helmreich, E., *J. Biol. Chem.*, **239**, 3133 (1964).
[2] Harwood, J. P., and Drummond, G. I., *Fed. Proc.*, **28**, 541 (1969).
[3] Kugelberg, E., and Edström, L., *J. Neurol. Neurosurg. Psychiat.*, **31**, 415 (1968).
[4] Eränkö, O., and Palkama, A., *J. Histochem. Cytochem.*, **9**, 585 (1961).
[5] Barka, T., *Histochemistry Theory, Practice and Bibliography*, 313 (Harper and Row, New York, 1963).
[6] Novikoff, A. B., Shin, W., and Drucker, J., *J. Biophys. Biochem. Cytol.*, **9**, 47 (1961).
[7] Wattenberg, L. W., and Leong, J. L., *J. Histochem. Cytochem.*, **8**, 296 (1960).
[8] Padykula, H. A., and Herman, E., *J. Histochem. Cytochem.*, **3**, 170 (1955).
[9] Stein, J. M., and Padykula, H. A., *Amer. J. Anat.*, **110**, 103 (1962).
[10] Edgerton, V. R., Gerchman, L., and Carrow, R., *Exp. Neurol.*, **24**, 110 (1969).
[11] Engel, W. K., and Cunningham, G., *Neurology*, **13**, 919 (1963).
[12] Meijer, A. E. F. H., *Histochemie*, **16**, 134 (1968).
[13] Henneman, E., and Olson, C. B., *J. Neurophysiol.*, **28**, 581 (1965).

Dopamine Excretion in Affective States and following Li_2CO_3 Therapy

THE interrelated biochemical changes which occur in severe recurrent depression and in mania include a disordered metabolism of cerebral monoamines[1]. It has been suggested[2,3] that in severe depression, especially of the endogenous type, there is a deficiency of amines, and in particular of norepinephrine, at certain sites in the brain. On this hypothesis, mania is marked by an excess of amines at the same sites. Determination of the urinary excretion of dopamine, the precursor of norepinephrine, and its chief metabolite vanillylmandelic acid (VMA), may therefore help to test the validity of this hypothesis. In this connexion it is relevant to note that the manic state may be associated with increased muscular activity; it has, moreover, been reported that epinephrine and norepinephrine excretion increase following muscular activity[4,5] while dopamine excretion does not increase under the same circumstances[5].

We have investigated the urinary excretion of dopamine and VMA in manic and depressive states and after stabilization with lithium carbonate. Psychiatric judgments, using an analysis of the Overall and Gorham brief psychiatric rating scale, were made on patients receiving lithium carbonate on the days of urine collection. Twenty-four hour urine collections were obtained during a period in which seven patients were in a manic phase and six other patients were in a depressive state. Urinary excretion rates of dopamine and VMA were determined during these states and following lithium therapy when the patients were essentially normal.

Creatinine was determined by the Folin procedure[6] and VMA by the method of Pisano *et al.*[7]. Dopamine was isolated from urine by adsorption on Amberlite 'CG-50' that had been cycled by a modification[8] of the procedure of Hirs *et al.*[9]. The column effluent served for the determination of conjugated dopamine after acid hydrolysis. The hydrolysate, after adjustment to *p*H 6·6, was applied to a second column. The fluorometric assay for the quantitative determination of dopamine[10] was similar to that reported by Laverty and Taylor[11]. The fluorophor formed in the 5,6-dihydroxyindole reaction was developed at *p*H 5·2 for 90 min at 100° C and its fluorescence measured at 390 nm with excitation at 332 nm.

The results are summarized in Table 1. Dopamine excretion in twenty normal volunteers, aged 22–58, did not vary with sex or age. During the manic phase, both conjugated and free dopamine excretion increased for six of seven patients. The patient P.B. was moderately hypomanic at the time of urine collection. During the depressive state, the dopamine excretion rate remained within normal limits, except for one (C.S.) of two patients (C.S. and Y.B.) with affective disorders which were highly cyclic. Following treatment with lithium carbonate, the excretion of dopamine by all patients in the manic group returned to normal levels. The excretion of dopamine by three patients in the depressed group was not significantly changed throughout administration of lithium. For one (C.S.) of the two cyclic cases, however, there was no change; in the other (Y.B.) there was an unexplained increase. Excretion of VMA was unaltered in both groups before and after therapy.

Excreted dopamine has been reported to be largely of peripheral origin[12]. Circulating and excreted norepinephrine arises from the adrenals or other chromaffin tissue or from adrenergic components of the autonomic nervous system, with little contribution from the brain[13]. It is of interest, however, that decreased dopamine excretion observed in extrapyramidal syndromes[14] seems to be correlated with a decreased concentration of this amine in the basal ganglia[15]. Moreover, a gradual decrease in urinary excretion of dopamine with increasing severity of the illness has been reported[16].

The data suggest that an increased excretion of dopamine is one of the biochemical changes associated with the manic phase of an affective disorder. This is compatible with previous single case reports of cyclothymic patients[12,17]. In another study[18], excretion of dopamine was significantly increased in only one of three cases studied. Normal excretion rates of VMA in the manic phase found in this study do not agree with a previous report[12]. The fact that lithium ion in low concentration inhibits the electrical stimulation-induced release of norepinephrine and serotonin[19] may be related to the observation that, after treatment with lithium carbonate, excretion of dopamine by patients in the manic group decreased to amounts found for the control group.

Table 1. DOPAMINE AND VANILLYLMANDELIC ACID EXCRETION DURING MANIA AND DEPRESSION BEFORE AND AFTER LITHIUM CARBONATE THERAPY

	Dopamine excretion					
Patients and clinical state	Free		Total		VMA excretion	
	Before Li_2CO_3	After Li_2CO_3	Before Li_2CO_3	After Li_2CO_3	Before Li_2CO_3	After Li_2CO_3
No. normal controls	20	—	20	—	8	—
	(μg/24 h)		(μg/24 h)		(mg/24 h)	
Average ± *S.D.*	370 ± 80·7	—	530 ± 97·0	—	2·5 ± 0·9	—
Range	(180–490)	—	(380–660)	—	(0·6–3·5)	—
Mania						
S.C.	1,340	430	1,790	570	2·3	1·7
C.L.	690	250	910	450	1·2	1·5
H.S.	400	170	880	380	0·8	2·1
R.R.*	900	180	1,520	270	3·1	3·3
R.P.†	610	310	1,380	630	2·8	2·8
I.B.†	760	300	980	650	0·9	0·8
P.B.	240	120	410	270	1·7	0·9
Mean	710	250	1,120	460	1·8	1·9
t	5·96		6·68			
P	< 0·01		< 0·01			
Depression						
M.S.	550	490	660	650	1·8	3·0
H.B.†	320	440	650	540	2·0	1·7
K.S.	340	480	630	530	1·1	1·2
C.S.†	910	590	1,060	1,030	0·9	1·3
A.L.	510	350	650	510	2·1	1·8
Y.B.†	490	530	570	1,740	1·3	1·3
Mean	520	480	700	830	1·5	1·7
t	0·39		0·51		—	—
P	Not significant		Not significant			

* Thioridazine, † chlorpromazine, administered during this period and before lithium carbonate.

t Corresponds to correlated means on square root transformation data.

The significance of these biochemical changes remains to be determined. It will be of particular interest to learn whether the brain is a significant source of the increase in dopamine excretion. Alternatively, a disturbance in catecholamine metabolism in the peripheral nervous system may reflect a similar disturbance in the central nervous system.

We thank Dr R. W. Von Korff for encouragement and discussions and Dr T. E. Hanlon for the statistical analysis.

F. S. MESSIHA

Maryland Psychiatric Research Center.

D. AGALLIANOS
C. CLOWER

Spring Grove State Hospital,
Department of Mental Hygiene,
State of Maryland,
Baltimore, Maryland 21228.

Received August 13; revised December 19, 1969.

[1] Coppen, A., *Brit. J. Psychiat.*, **113**, 1237 (1967).
[2] Schildkraut, J. J., *Amer. J. Psychiat.*, **122**, 509 (1965).
[3] Schildkraut, J. J., and Kety, S. S., *Science*, **156**, 21 (1967).
[4] Von Euler, U. S., *Clin. Pharmacol. Therap.*, **5**, 398 (1964).
[5] Häggendal, J., and Werdinius, B., *Acta Physiol. Scand.*, **66**, 223 (1966).

[6] Folin, O., *Z. Physiol. Chem.*, **228**, 268 (1934).
[7] Pisano, J. J., Crout, R., and Abraham, D., *Clin. Chem. Acta*, **7**, 258 (1962).
[8] Gey, K. F., and Messiha, F., *Experientia*, **20**, 298 (1964).
[9] Hirs, C. H. W., Moore, S., and Stein, W. H., *J. Biol. Chem.*, **200**, 493 (1953).
[10] Messiha, F. S., and Von Korff, R. W., *Fed. Proc.*, **28**, 543 (1969).
[11] Laverty, R., and Taylor, K. M., *Anal. Biochem.*, **22**, 269 (1968).
[12] Takahashi, R., Nagao, Y., Tsuchiya, K., Takamizawa, M., Kobayashi, T., Toru, M., Kobayashi, K., and Kariya, T., *J. Psychiat. Res.*, **6**, 185 (1968).
[13] Kety, S., *Pharmacol. Rev.*, **18**, 787 (1966).
[14] Barbeau, A., Murphy, G. F., and Sourkes, T. L., *Science*, **133**, 1096 (1961).
[15] Hornykiewicz, O., *Deutsch. Med. Wschr.*, **87**, 1807 (1962).
[16] Barbeau, A., *Agressology*, **9**, 2 (1968).
[17] Shinfuku, N., Omura, M., and Kayano, M., *Yonago Acta Med.*, **5**, 109 (1961).
[18] Stroem-Olsen, R., and Weil-Malherbe, H., *J. Ment. Sci.*, **104**, 696 (1958).
[19] Katz, R., Chase, T., and Kopin, I., *Science*, **162**, 466 (1968).

Nervous Activity and RNA Metabolism in the Visual Cortex of Rat Brain

THERE is increasing evidence that nervous activity and brain RNA metabolism are closely related[1–5]. The present investigations were instigated to obtain further evidence for a connexion between RNA turnover and nervous activity in the visual cortex. We wished to accomplish this without recourse to electrical, chemical or any other form of non-physiological stimulation, and were fortunate in having available two strains of rats which seemed to be ideal preparations for this purpose.

One strain, of inbred, tan-hooded albinos (originally described by Tansley *et al.*[6]), was totally blind[7], with a condition resembling human retinitis pigmentosa, in which there is a failure in the final stages of development of the rod layer of the retina. It can therefore be assumed that after the rats have attained the age of 3 weeks, their visual cortices receive negligible nervous stimulation. The second strain was a normal sighted strain (PVG rats). Rats belonging to this strain very rarely, if ever, show spontaneous retinal degeneration. Direct ophthalmoscopic and electroretinographic observations confirmed that these rats were sighted (personal communication from A. Sorsby), so that it was possible to assume that the visual cortex would be stimulated throughout life by light falling on the retina. We considered that by comparing RNA turnover in the visual cortices of rats of both strains, any differences in RNA metabolism associated with nervous activity in a particular area of brain would be maximal. All rats used were 3 months old, of either sex, and weighed between 200 and 250 g.

Rats of both strains were injected intraventricularly with 4 μCi of 6-^{14}C-orotic acid (specific activity 60·8 mCi/mmole, Radiochemical Centre, Amersham) under mild halothane anaesthesia. In some experiments, the rats were merely kept in a constantly illuminated room, and in others the rats were exposed to a flashing light source (60 W bulb. 30 flashes per min). At given time intervals after the injection of the nucleic acid precursor, the rats were killed by decapitation, and the brains removed.

The visual cortex from each brain was removed by dissection and simultaneously a sample of equal size was removed from an area of the motor cortex equidistant with the visual cortex from the site of the orotic acid injection. All operations were performed as rapidly as possible after chilling the brain. The area of cerebral tissue taken as the visual cortex corresponded to that described by Adams and Forrester[8].

RNA was extracted from the sample of brain tissue by the method of Scherrer and Darnell[9], modified by deproteinizing with a mixture in the following proportions: 500 g phenol, 70 ml. *m*-cresol, 55 ml. water and 0·5 g 8-hydroxyquinoline. RNA precipitates obtained were dissolved in 1 ml. of 0·01 M acetate buffer, *p*H 5·1, containing 10^{-4} M EDTA, 0·05 M NaCl and 0·005 per cent polyvinyl sulphate. Samples (0·1 ml.) of these solutions were layered onto a 5–20 per cent continuous sucrose gradient (volume 2·5 ml.) and centrifuged at 45,000*g* for 4 h at 5° C on an MSE Superspeed 50*TC* centrifuge using a 3 × 3 ml. swing out rotor. Fractions were then collected and the RNA in each estimated spectrophotometrically at 260 nm. The absolute radioactivity in each fraction was determined by liquid scintillation counting, using a dioxane based scintillant. The specific activities of the RNA in each fraction could thus be calculated.

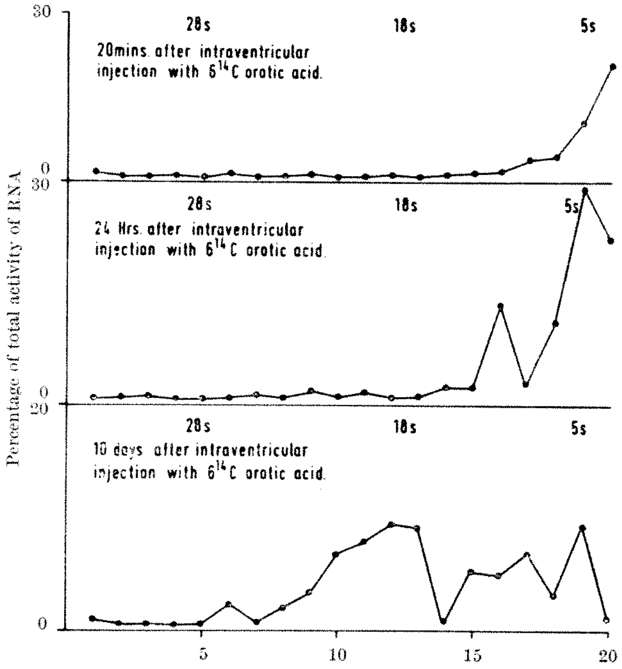


Fig. 1. The pattern of labelling of cerebral RNA fractions from PVG rats following the intraventricular injection of 4 μCi of 6-^{14}C-orotic acid.

Fig. 1 shows how the pattern of labelling in cerebral RNA fractions after the intraventricular injection of 6-^{14}C-orotic acid varies with time. These results were obtained using the whole forebrain of PVG rats, and those obtained with the whole forebrain of blind rats were identical. There is an initial labelling of the "rapidly labelled" 5S RNA followed by labelling of 5–8S fraction, and subsequently labelling of ribosomal RNA. Fig. 2 shows the pattern of labelling in the RNA fractions obtained from the visual cortex and motor cortical sample of the blind strain. The RNA from the motor cortical sample shows a pattern of labelling similar to that in Fig. 1, whereas the visual cortex RNA is labelled to a considerably lesser extent. The specific activity of the visual cortex RNA is lower, and the labelling of the 5–8S RNA and the ribosomal RNA is retarded. In Fig. 3, however, which shows the labelling patterns obtained from the visual cortex and motor cortical sample of the PVG rats, the labelling pattern from the motor cortical sample is identical to that seen in Fig. 1, but the labelling of the RNA from the visual cortex is considerably increased, both in magnitude and extent. There is a far greater labelling of the heterogeneous 5–8S RNA and ribosomal RNA. This effect is enhanced by using a flashing light source. Thus the increased nervous activity in the rat visual cortex is apparently accompanied by an increase both in the speed and the extent of the labelling of the

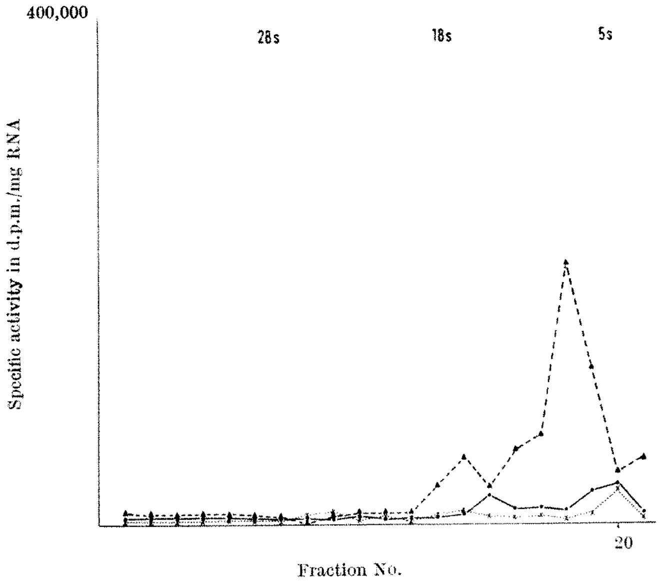


Fig. 2. The pattern of labelling of cerebral RNA fractions from the blind rats 24 h after the intraventricular injection of 4 μCi of 6-^{14}C-orotic acid. ▲- - -▲, RNA obtained from sample of motor cortex from rats subjected to a flashing light source of 30 flashes/min. Identical results were obtained using constant illumination. ●—●, RNA obtained from visual cortex of rats subjected to constant illumination. × . . . ×, RNA obtained from visual cortex of rats subjected to a flashing light source of 30 flashes/min.

RNA fraction, whereas a reduction in nervous activity seems to result in a retardation of the RNA labelling.

A possible explanation of the decrease in RNA synthesis in the visual cortex of the blind rats is that cellular degeneration had occurred. When sections from "sighted" and "blind" visual cortex were compared after thiamine blue and cresyl-fast violet staining, however, the distribution of Nissl granules was very similar, and there was no sign of gross cellular degeneration. It has, however, been established that deprivation of light causes a number of morphological changes in the visual cortex of rats[10,11]; we merely wished to confirm that the "blind" visual cortex had the potential for RNA synthesis. We intend to investigate this further by electrical stimulation of the visual pathway in the blind rats and observation of any change in the rate of RNA synthesis.

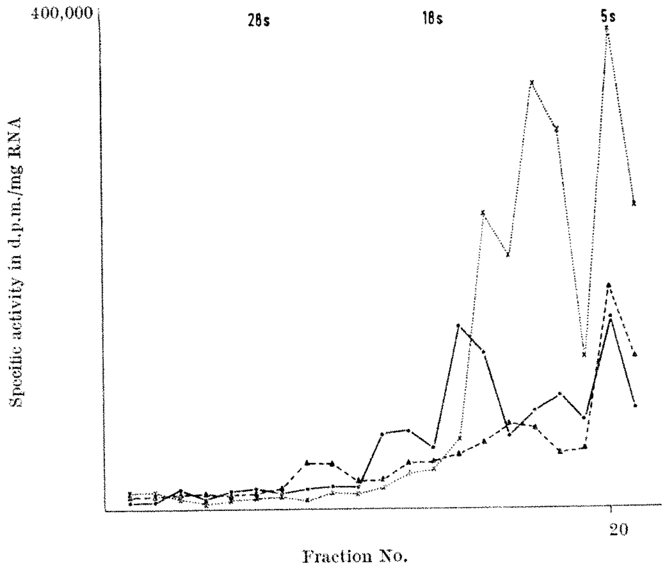


Fig. 3. The pattern of labelling of cerebral RNA fractions from the PVG rats 24 h after the intraventricular injection of 4 μCi of 6-^{14}C-orotic acid. ▲- - -▲, RNA obtained from sample of motor cortex from rats subjected to a flashing light source of 30 flashes/min. Identical results were obtained using constant illumination. ●—●, RNA obtained from visual cortex of rats subjected to constant illumination. × . . . ×, RNA obtained from visual cortex of rats subjected to a flashing light source of 30 flashes/min.

These results lend additional support to those of Kogan[3] and Baranov and Pevzner[4], but contradict those of Talwar *et al.*[12], who found that, in monkey occipital cortex, synthesis of RNA measured by incorporation of ^{32}P increased in the dark. The consequences of an increased RNA synthesis after nervous excitation are obscure; the most obvious consequence—an increase in protein synthesis—has been observed by Talwar *et al.*[13], but this seems to be contradicted by the work of Metzger *et al.*[14], who, by using a monkey split brain preparation, found that visual stimulation had no effect on the rate of protein synthesis in the occipital cortex. The observation of Rose[15], that the first exposure of rats to light produces an initial stimulation of protein synthesis in the visual cortex followed by a depression in protein synthesis, may explain some of the apparent contradictions in the literature.

We thank Miss K. F. Mackay for technical assistance.

A. J. DEWAR
H. W. READING

MRC Unit for Research in Brain Metabolism,
Department of Pharmacology,
University of Edinburgh Medical School.

Received October 17; revised November 28, 1969.

[1] Satake, M., Matsukawa, S., and Miyazawa, N., *Nature*, **218**, 768 (1968).
[2] Smirnov, V. A., *Dokl. Akad. Nauk. USSR*, **101**, 913 (1955).
[3] Kogan, A. B., *UCLA Forum in Medical Sciences No. 2, Brain Function*, **2** (edit. by Brazier, H. A. B.) (Univ. of California Press, Berkeley and Los Angeles, 1964).
[4] Baranov, M. N., and Pevzner, L. Z., *J. Neurochem.*, **10**, 279 (1963).
[5] *Macromolecules and Behavior* (edit. by Gaito, J.) (Appleton-Century-Crofts, New York, 1966).
[6] Bourne, M. C., Campbell, D. A., and Tansley, K., *Brit. J. Ophthal.*, **22**, 613 (1938).
[7] Dowling, J. E., and Sidman, R. L., *J. Cell Biol.*, **14**, 73 (1962).
[8] Adams, A. V., and Forrester, J. M., *Quart. J. Exp. Physiol.*, **53**, 327 (1968).
[9] Scherrer, K., and Darnell, J. E., *Biochim. Biophys. Res. Commun.*, **7**, 486 (1962).
[10] Cragg, B. G., *Nature*, **215**, 251 (1967).
[11] Fifkova, E., *Nature*, **220**, 379 (1968).
[12] Talwar, G. P., Goel, B. K., Chopra, S. P., and D'Monte, B., in *Macromolecules and Behavior* (edit. by Gaito, J.) (Appleton-Century-Crofts, New York, 1966).
[13] Talwar, G. P., Chopra, S. P., Goel, B. K., and D'Monte, B., *J. Neurochem.*, **13**, 109 (1966).
[14] Metzger, P., Cuenod, M., Grynbaum, A., and Waelsch, H., *Life Sciences*, **5**, 1115 (1966).
[15] Rose, S. P. R., *Nature*, **215**, 253 (1967).

Rhodopsin Content of Frog Photoreceptor Outer Segments

VISUAL receptor rod outer segments transduce the energy of absorbed photons into nerve signals. These membranous organelles can be isolated in quantities sufficient for biochemical studies, and are a favourable preparation for studying the chemistry of sensory nerve membrane excitation. Unlike other nerve membrane systems they contain an excitable protein component which binds an easily identified prosthetic group. This prosthetic group, 11-*cis*-retinaldehyde, combines with the protein opsin to form the light sensitive visual pigment rhodopsin. Retinaldehyde has been used as a label in the isolation and analysis of the opsin protein[1-3]. Opsins prepared from frog, cattle and rat outer segment membranes by detergent solubilization and column chromatography are now reported to have molecular weights in the range of 27,000 to 40,000 g per mole of retinaldehyde bound[3-6]. These opsins are thought to constitute a significant fraction of the total protein present in outer segment membranes[6-8].

The purpose of this communication is to present the data shown in Table 1 which demonstrate that intact and unfractionated frog rod outer segments contain not more than 28,500 g of protein per mole of retinaldehyde bound in visual pigment. This suggests that a single protein species, opsin itself, may account for virtually all of the protein present in frog photoreceptor membranes.

Table 1

a. COMPOSITION OF *Rana catesbeiana* OUTER SEGMENT PREPARATIONS

Preparation number	Amino-acid residues per prosthetic group	Protein molecular weight per prosthetic group
41	247	27,900
42	256	29,000
44	273	31,100
48	254	29,000
50	228	26,100
51	237	27,300
53	239	27,400
54	265	29,800
56	254	28,500
Average	250	28,500 ± 500

b. PUBLISHED COMPOSITION VALUES FOR PURIFIED RHODOPSINS

Frog rhodopsin[3]	237	26,600
Rat rhodopsin[3]	236	26,500
Cattle rhodopsin[3]	235	26,400
Cattle rhodopsin[4]	255	27,700
Cattle rhodopsin[5]	267	28,600

The ratio of protein/retinaldehyde found for frog rod outer segments in these studies is lower than previous estimates[6,8]. This is most likely because of preparation procedures which minimize contamination from other retinal membranes and protect outer segments from severe mechanical disruption and osmotic shock. In preparation for each of the analyses shown in Table 1, retinas were removed from five large dark adapted frogs (*Rana catesbeiana*), and 6 ml. of Ringer's solution containing 'Ficoll' (Pharmacia) was added to each ml. of retinal material. This suspending solution was made by dissolving 33 g of 'Ficoll' to a final volume of 100 ml. in Ringer's solution: 115 mM NaCl, 2 mM KCl, 2 mM $CaCl_2$, 10 mM N-2-hydroxyethyl - piperazine - N′ - 2 - ethanesulphonic acid ('HEPES', Calbiochem), *p*H 7·5. The retinas were homogenized by slow passage through a No. 17 syringe needle whose tip had been blunted on a grinding wheel. All manipulations were carried out under red light at 4° C. The homogenate was divided into three portions, each of which was layered under 1–2 ml. of 20 per cent (w/v) 'Ficoll' in Ringer's solution in Beckman–Spinco 302232 centrifuge tubes. These tubes were centrifuged using a Spinco *SW* 25.1 rotor for 30 min at 18,500 r.p.m. The outer segments floated up to form a thick "carpet" at the interface between 20 per cent and 33 per cent 'Ficoll' and were withdrawn in a minimum volume using a syringe. These outer segments were suspended in 10–12 ml. of 20 per cent 'Ficoll', layered over 1–2 ml. of 33 per cent 'Ficoll' in three similar centrifuge tubes, and centrifuged again as here. Outer segments which sedimented to the interface to form a "carpet" were again withdrawn. Microscopic examination revealed both intact and broken outer segments which retained their characteristic *in vivo* shape and birefringence. To remove most of the 'Ficoll' present, these outer segments were diluted with 10 ml. of Ringer's solution, sedimented in a Spinco 40 rotor for 10 min at 10,000 r.p.m., and the supernatant discarded. The outer segments were then used without further manipulations in the analyses shown in Table 1.

While these procedures yielded outer segment suspensions with fairly consistent protein/retinaldehyde ratios, there clearly was some variation between different preparations, reflected by small differences in total amino-acid composition (Table 1*a*) and rhodopsin yields. In some experiments outer segments which collected at the 'Ficoll' interface during centrifugation did not form a "carpet" of material which could be easily withdrawn, but rather formed a diffuse layer. Such preparations contained relatively more protein than those shown in Table 1.

Amino-acid analyses were performed on the Beckman model 120 *C* analyser[9]. Samples were hydrolysed for 24 h in glass tubes which had been evacuated and sealed; longer times of hydrolysis did not produce significantly different results. Cysteine and methionine were analysed after performic acid oxidation according to Moore[10]. Tryptophan was measured in preparations solubilized with 0·04 M hexadecylammonium chloride (Eastman) in 10 mM HEPES, *p*H 7·5 (ref. 11). Retinaldehyde bound in rhodopsin was determined from difference spectra caused by bleaching, using the established molar extinction coefficient at 500 nm of 42,000 (refs. 5 and 12). Rhodopsin yields varied from 0·8 to $1 \cdot 6 \times 10^{-8}$ moles/retina. No analyses of the six sugar residues which Heller[3] reports covalently bound to rhodopsin were performed. Phosphorus analyses[13] of ten different preparations indicated an average of 80 (± 8) moles of phosphorus per mole of retinaldehyde present. Ninhydrin analyses[14] of these same preparations after alkaline hydrolysis gave values varying from 270 to 377 leucine equivalents/mole retinaldehyde. The data of Table 1*a* are expressed as moles of amino-acid present (determined by amino-acid analysis) per mole of retinaldehyde bound in visual pigment (determined by difference spectroscopy). Total protein weight per mole of retinaldehyde bound in rhodopsin was calculated separately for each preparation from the amino-acid analyses.

The averaged data from nine different preparations indicate that frog outer segment suspensions contain not more than 28,500 ± 500 g of protein per mole of retinaldehyde bound in visual pigment (Table 1*a*). Because these outer segments consist almost entirely of extensively folded photosensitive membranes, one is chiefly considering the protein of these membranes. The low levels of soluble enzymes thought, however, to be present in outer segments[15] should also contribute to the total analysis, for it seems unlikely that they would be lost during the gentle preparation procedures used. The value of 28,500 is very similar to molecular weights recently reported for the protein (opsin) portions of purified frog, rat and cattle rhodopsin (Table 1*b*). If Heller's value[3] for the protein weight of a single preparation of *Rana pipiens* (26,600) is taken for reference, this opsin represents approximately 92–95 per cent of the total protein of outer segments. With opsin present in these concentrations, the enzyme activities which have been associated with outer segments[15] must be accounted for by less than 10 per cent of the total protein present. Other outer segment components such as elements of the ciliary stalk or plasma membrane must also contribute relatively little to the total analysis.

The composition data listed in Table 1*a* and 1*b* suggest then that frog photoreceptor membranes contain only one major protein component, in striking contrast to most biological membranes. The recent work of Hall *et al.*[7] is consistent with this interpretation. The high concentration of visual pigment protein (opsin) in these membranes optimizes their quantum gathering capacity and has probably been favoured by selection processes. Opsin is thought to be a single polypeptide chain[3–5], and preliminary efforts in this laboratory to dissociate it further have been unsuccessful. This polypeptide chain then may be the major structural and functional protein of outer segment membranes. The X-ray diffraction data of Blaurock and Wilkins[8] would indicate further that this polypeptide is constrained in these membranes to two layers approximately 10·5 Å thick which enclose a lipid bilayer.

If rhodopsin is the main component of vertebrate photoreceptor membranes individual rhodopsin molecules must be surrounded chiefly by other rhodopsin molecules. The decrease in apparent current flow across these membranes which can be caused by the photoexcitation of one rhodopsin molecule[16,17] might then involve cooperative interactions between this excited molecule and adjacent rhodopsin molecules, or between rhodopsin and other membrane proteins present in much smaller amounts. Such cooperative interactions could be involved in either of the two mechanisms currently suggested to account for visual excitation: that light causes an increase in the resistance of the outer segment membrane system, or that light activates an electrogenic pump which utilizes metabolic energy to move ions across the membrane[16]. If the 10^7–10^9 rhodopsin molecules present in an outer segment's membrane system were equally involved in maintaining its resistance to an observed dark current of approximately one to two cations/rhodopsin/second[17], then the alteration of just one of these molecules by photoexcitation could not cause the net movement of 10^5 cations within milliseconds, which Penn and Hagins have observed in rat outer segments[17]. Any change in resistance would have to be a cooperative phenomenon, with the excited rhodopsin molecule influencing adjacent areas of the photoreceptor membrane. Activation of an electrogenic pump, unless the pump were rhodopsin itself, would also require cooperative interaction between rhodopsin and the presumed pump molecules. Sodium–potassium activated adenosine triphosphatase activity in outer segments, which might be involved in active ion movements, is not greater than 10^{-1} moles ATP hydrolysed/mole rhodopsin/minute (D. B., in preparation), and no effect of illumination has been observed on this activity.

We thank Dr A. Gordon-Walker, Dr G. Craven and C. Sakowski for their advice and assistance. This work was supported by a grant from the US National Institutes of Health.

D. BOWNDS
A. C. GAIDE-HUGUENIN

Laboratory of Molecular Biology,
Department of Zoology,
University of Wisconsin, Madison.

Received December 27, 1969.

[1] Bownds, D., *Nature*, **216**, 1178 (1967).
[2] Hubbard, R., Bownds, D., and Yoshizawa, T., *Cold Spring Harbor Symp. Quart. Biol.*, **30**, 301 (1965).
[3] Heller, J., *Biochemistry*, **8**, 675 (1969).
[4] Shields, J. E., Dinovo, E. C., Henricksen, R. A., Kimbel, R. L., and Millar, P. G., *Biochim. Biophys. Acta*, **147**, 238 (1967).
[5] Shichi, H., Lewis, M. S., Irreverre, F., and Stone, A. L., *J. Biol. Chem.*, **244**, 529 (1969).
[6] Hubbard, R., *J. Gen. Physiol.*, **37**, 381 (1954).
[7] Hall, M. D., Bok, D., and Bacharach, A. D. E., *J. Mol. Biol.*, **45**, 397 (1969).
[8] Blaurock, A. E., and Wilkins, M. H. F., *Nature*, **223**, 906 (1969).
[9] Spackman, D. B., Stein, W. H., and Moore, S., *Anal. Chem.*, **30**, 1190 (1958).
[10] Moore, S., *J. Biol. Chem.*, **238**, 235 (1963).
[11] Patchornik, A., Lawson, W. B., and Eitkor, B., *J. Amer. Chem. Soc.*, **80**, 4747 (1958).
[12] Matthews, R. C., Hubbard, R., Brown, P. K., and Wald, G., *J. Gen. Physiol.*, **47**, 215 (1963).
[13] Bartlett, G. R., *J. Biol. Chem.*, **234**, 466 (1959).
[14] Moore, S., *J. Biol. Chem.*, **243**, 6281 (1968).
[15] Lolley, R. N., and Hess, H., *J. Cell Physiol.*, **73**, 9 (1969).
[16] Toyoda, J., Nosake, H., and Tomita, T., *Vision Res.*, **9**, 453 (1969).
[17] Penn, R. D., and Hagins, W. A., *Nature*, **223**, 201 (1969).

Interocular Transfer of Suppressive Effects of Retinal Image Displacement

WHEN the retinal image of the visual field is suddenly displaced by a saccadic eye movement, perceptual sensitivity to a weak test flash at the time of displacement is reduced. Flashes presented from 40 ms before until at least 100 ms after the displacement may be affected. I have discovered[1] that visibility is similarly reduced when the visual field is displaced as a whole before a stationary (fixating) eye. This throws doubt on the idea that either oculomotor activity[2] or mechanical acceleration of the eyeball[3] is required to account for such effects. It suggests rather that the surge of neural activity caused by the rapid displacement of the retinal image has side-effects that interfere with processing of the signals generated by the test flash, and so raise the perceptual threshold[1].

If such interference between the two signals took place only at the retina or in the early stages of the visual pathway before signals from the two eyes were combined, then with dichoptic presentation, exposing only one eye to the moving visual field and presenting the test flash to the other, one might expect to find no corresponding suppressive effects. A reduction of visibility in these circumstances would imply that the interference had a more central origin.

I have now found that interference is not abolished when the two stimuli are presented dichoptically. On the contrary, the frequency of seeing drops quite as convincingly as when both the flash and the moving field were presented to the same eye; and once again the time range over which flash visibility is reduced begins about 40 ms before onset of displacement of the field presented to the other eye.

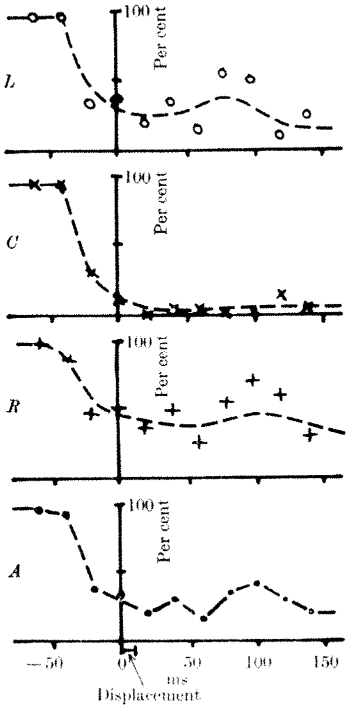


Fig. 1. Typical frequency-of-seeing curves for fixation to left (*L*), right (*R*) and on centre (*C*) of test spot. Abscissa shows time from onset of image displacement in right eye to onset of test flash to left eye. Average for *L*, *R*, *C* combined is shown at *A*.

The same sizes and dispositions of field and test spot were used as in the previous experiment[1]; but the 2° test flash, now controllable in duration, was presented via a mirror to the subject's left eye only. The right eye viewed the 10° circular field, which was displaced by 4° in 10 ms from right to left about the time of each flash, at intervals of 1 s, returning 0·5 s later. Both eyes viewed a fixation field of three luminous red dots, optically superposed via a semitransparent mirror. The dots formed a horizontal row, their separation being equal to the diameter of the test spot. The subject was instructed to fixate each dot in turn in a pseudo-random order, so as to reduce the effects of retinal adaptation in the eye exposed to the faint test flash.

The time interval between flash and field displacement was also varied at random, and the frequency of seeing was calculated separately for each interval and fixation position. Typical results on a coarse time-scale are shown in Fig. 1, for the same subject as in the previous report[1].

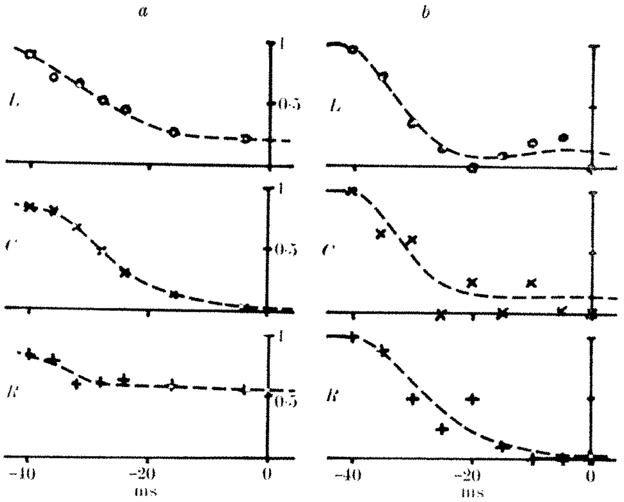


Fig. 2. *a*, Cumulative frequency-of-seeing curves for five experiments, expanded time-scale, dichoptic condition. Notation as in Fig. 1. *b*, Corresponding curves obtained when both stimuli were presented to both eyes (see ref. 1).

The drop from 100 per cent in the neighbourhood of −40 ms (that is, when the flash occurred 40 ms before displacement) is marked in every case. The detailed form of the curves in the region from −40 to 0 ms was explored in a separate series of experiments, spread over 5 successive days. The general features (though not the mean sensitivity level) were remarkably consistent from day to day, and the cumulative results shown in Fig. 2*a* are typical.

The sensitivity curves with dichoptic presentation are very similar in form and timing to those obtained when the same eye viewed both stimuli[1]. For ease of comparison, the curves of Fig. 3 in ref. 1 are re-drawn as Fig. 2*b*, on the same scale. The maximum elevation of threshold measured was of the order of 3 dB; but to avoid unduly prolonging experiments this measurement was made only in a few typical cases. It should be noted that any tendency to interocular rivalry would have had the effect of raising the "threshold" as statistically defined, quite apart from the interference between signals from the two stimuli, which is our present concern.

Significant differences were found between results for different fixation points. Visibility was lowest overall with foveal presentation of the test spot (curve *C*). This is to be expected, for the left eye is in scotopic condition (see below). More interesting is the marked and consistent contrast between response frequencies with fixation left (*L*) and right (*R*) of the test flash. Subjects reported the flash to be "much easier to see" when presented to the left of the fixation point.

In control tests with the moving field occluded, no comparable asymmetry was found between the thresholds with *L* and *R* fixation. In a total of 123 trials, using a flash duration close to scotopic threshold, the frequencies of seeing were 0·7(8), 0·7(7) and 0·1 with fixation on left, right and centre respectively. This confirms that the difference in scotopic sensitivity between extra-foveal and foveal areas is more than enough to account for the difference between curves *L* and *C* in Fig. 2; indeed, it suggests that the interference effects may be stronger with fixation on *L* than with central fixation. It also, however, shows clearly that the difference between curves *R* and *L* cannot be put down to a bias in scotopic sensitivity.

It remains to be seen whether this difference reflects a weakening of the interference process, or whether a flash in the left half-field of the left eye is "helped over the threshold" at some central stage by the breakthrough of signals from the corresponding half-field of the right eye. Because the area of the right eye-field actually corresponding to that of the test flash in the left eye does not change in luminance (the 10° field is displaced by only 4°), it seems more likely that it is the asymmetry of the disturbance caused by the displacement which accounts for the bias in favour of signals from the left half-field.

In any case, the results suggest that the effects of a moving field on the visibility of a test flash depend mainly on central rather than purely peripheral physiological interactions.

I thank Miss M. A. Steele and other subjects for their patient cooperation, and the MRC and SRC for financial support.

D. M. MACKAY

Department of Communication,
University of Keele, ST5 5BG.

Received December 24, 1969.

[1] MacKay, D. M., *Nature*, **225**, 90 (1970).
[2] Holt, E. B., *Harvard Psychol. Studies*, **1**, 3 (1903).
[3] Richards, W., *J. Opt. Soc. Amer.*, **58**, 1559A (1968).

Effect of Presenting Novel Verbal Material during Slow-wave Sleep

THE topic of sleep learning has come increasingly to the fore during the past decade, partly because of the considerable scientific interest that has been shown in the Soviet Union, where a number of unusual claims have been made[1-3], and also because of the growing commercial interest in marketing devices designed to enable individuals to make use of the supposedly "waste periods" of sleep by storing useful, if perhaps dull and repetitive, material while in a state of unconsciousness. In principle the idea is attractive, but it depends on at least two assumptions: (1) that during sleep no other important process is taking place which would be disrupted by input of data; (2) that it is indeed possible for the brain to store (and subsequently extract) information fed in during those periods of sleep known as levels *C* to *E*[4], during which consciousness is generally believed to be absent. The function of sleep is still largely not understood, but present evidence suggests that important information processing may be taking place at frequent periods throughout the night. Experimental evidence for the second assumption is contradictory[5-7] and often obscure. Fox and Robbin[5], for example, seem to have obtained clear-cut evidence for "sleep learning" (of Chinese–English equivalents) but their experiment is open to criticism because the subjects were not actually observed while the material was being fed in and it is possible that the learning took place when the subjects were at least partially awake.

It is now generally agreed that there is no reliable way of monitoring the state of sleep other than by the use of the electroencephalogram (EEG). This precaution was taken by Emmons and Simon[6,7] in their series of experiments, and the result was a clear correlation between fall in cerebral vigilance and fall in the amount of retention or learning as indicated by subsequent recall and recognition tests. For example, with the exception of those cases where the subject had been drowsy and immediately aroused by the signals, subjects who had had words repeatedly played to them during sleep showed no evidence of recall and were no better than control subjects in selecting the test words from a list of fifty. Although the experimental techniques were more satisfactory in the Emmons and Simon study, it is still possible to raise objections on the grounds of inadequate control. For example, in each experiment the tests of recall or recognition were carried out the next day. It is therefore possible to argue that learning might have taken place during the sleep presentations but have been vitiated by the retroactive interference effect of experiences intervening between presentation and test.

In an attempt to improve the adequacy of control, we combined some of the features of the Fox and Robbin and Emmons and Simon studies with certain additional precautions. Three reasonably well matched groups of subjects, most of whom were students (none reading psychology), were made up on the basis of pre-test learning performance, the material being fifteen pairs of nonsense syllables of low association value[8] the response members of which had to be anticipated. This material was chosen for use in the experiment to reduce the possible effect of differential experience.

Subjects were divided into three groups—"facilitation", "interference" and control. During sleep, subjects allocated to the "facilitation" group were presented with fifteen further pairs of low association value nonsense syllables, the pairing being as for the post-test which all subjects would subsequently undergo; members of the "interference" group were given the same material, but with the syllable pair items scrambled. The input for each subject was twenty repetitions of the nonsense syllable list, the order of pairs being randomized from trial to trial. Subjects in the control group were presented with computer-generated random musical noise for an equivalent period of time (half an hour). Our hypothesis was that if information was being stored during the sleep period, this would (*a*) assist the "facilitation" group which should show some saving on post-test learning, (*b*) have no effect on the post-test learning of the control group (which had heard only music) and (*c*) should have a deleterious effect on the post-testing of the "interference" group which would have been exposed to conflicting input data. The taped presentation was made to each subject via a pillow speaker, and EEG monitoring was continuous. Input was started when subjects had reached stage *E* sleep and maintained regardless of level unless subjects showed signs of approaching stage *B*. If this degree of arousal occurred, the input was cut and not resumed until the subject had returned to a deeper level of sleep. None of our subjects entered REM sleep during the experimental session. Subjects were allowed a few minutes continuation of sleep after the completion of the stimulus presentations; they were then woken, given a cup of coffee, and when thoroughly alert were asked to undertake the post-test learning. By reducing the interval between sleep presentation and subsequent test of retention to a minimum, we hoped that any retroactive interference effect would be precluded.

Table 1

	"Facilitation"		"Interference"		Control	
Subject	Pre	Post	Pre	Post	Pre	Post
1	16	20	16	11	17	30
2	18	15	18	50	20	10
3	19	13	24	33	21	14
4	23	26	25	16	24	24
5	25	33	29	15	27	27
6	32	32	30	15	31	27
7	36	22	35	27	38	40
Mean χ	24·1	23·0	25·3	23·9	25·4	24·6
SD	7·1	7·2	6·2	12·8	6·8	9·2

The results of the experiment are shown in Table 1, where the number of trials to criterion (one errorless set of anticipations) for the three groups on pre and post-test is compared. In each case the group means show a slight downward shift from pre to post-test, perhaps attributable to practice, but these differences are not statistically significant. There is no indication of the clear differences between groups on post-test such as those reported by Fox and Robbin[5]. Isolated individuals show more pronounced differences, in line with expectations based on the assumption of sleep learning—for example, subject 7 in the "facilitation" group and subject 2 in the "interference" group—but there is no detectable overall pattern of improvement and deterioration in performance. The differences in the number of trials to reach criterion for matched subjects show no clear trend (Table 2).

The experiment thus shows no evidence to support the suggestion that learning can occur during sleep, although information may of course be absorbed and retained when the individual is in a drowsy state. Previous work, for example that quoted by Emmons and Simon, has already indicated that such learning takes place—though efficiency is drastically reduced compared with the waking state.

Table 2

	I–F		I–C		C–F	
Subject	Pre	Post	Pre	Post	Pre	Post
1	0	−9	−1	−19	+1	+10
2	0	+35	−2	+40	+2	−5
3	+5	+20	+3	+19	+2	+1
4	+2	−10	+1	−8	+1	−2
5	+4	−18	+2	−12	+2	−6
6	−2	−17	−1	−12	−1	−5
7	−1	+5	−3	−13	+2	+18

We thank the Maudsley Hospital for facilities and Thomas Nelson and Sons (Publishers) Ltd and Imperial Chemical Industries for financial support.

D. J. BRUCE

Department of Education,
University of Cambridge.

C. R. EVANS

Division of Computer Science,
National Physical Laboratory,
Teddington, Middlesex.

P. B. C. FENWICK

The Maudsley Hospital,
London.

VALERIE SPENCER

Watford College of Technology.

Received January 7, 1970.

[1] Pushkina, I., and Zukhar, V., *Nauka i Zhizn*, **4**, 60 (1964).
[2] Rybchinskiy, T., *Soviet Union*, **171**, 16 (1964).
[3] Svyadoshch, A. M., *Voprosy psikhologii*, **1** (1962).
[4] Loomis, A. L., Harvey, E. N., and Hobart, G. A., *J. Exp. Psychol.*, **19**, 249 (1936).
[5] Fox, B. H., and Robbin, J. S., *J. Exp. Psychol.*, **43**, 75 (1952).
[6] Emmons, W. H., and Simon, C. W., *J. Exp. Psychol.*, **51**, 89 (1956).
[7] Emmons, W. H., and Simon, C. W., *Amer. J. Psychol.*, **69**, 76 (1956).
[8] Archer, E. J., *Psychol. Monogr.*, **74**, No. 10 (1960).

High Frequency Haploid Production in Barley (*Hordeum vulgare* L.)

HAPLOIDS have been sought and investigated in many plant species since their first identification in *Datura*[1]. The chief limitation in using them to produce homozygous lines for plant breeding programmes or genetic research has been the lack of techniques by which large numbers of haploids can be obtained from any given stock[2]. Reports[3,4] of haploid plants obtained from anther cultures offer a potential technique and here we report our first observations on interspecific crosses, followed by embryo culture, illustrating an equally promising method for obtaining haploids in barley and perhaps other species.

Interspecific crosses between 28-chromosome autotetraploid cultivated barley (*H. vulgare*) and tetraploid *H. bulbosum* were made reciprocally and the progeny were nearly all 14-chromosome "diploid" plants resembling cultivated barley[5]. The progeny contained gamete chromosome numbers, so investigations on crosses between the diploid forms of these species were carried out and haploid plants were obtained.

The percentage seed set obtained from the crosses between the diploids was high, as illustrated in Table 1. The average seed set was 51·5 per cent on nineteen different lines or plants, including one "diploid" plant that set no seed on the few florets that were pollinated. The "diploid" parental plants (Table 1) were fertile *H. vulgare*-like progeny obtained from crosses between lines of induced autotetraploid *H. vulgare* and naturally occurring tetraploid plants of *H. bulbosum*. The female parent was chosen mainly on the basis of plants available in the growth room at the time when the diploid *H. bulbosum* pollen parent (consisting of two plants from PI No. 318,649 of the U.S.D.A. *Hordeum* species collection) was flowering.

The induced seeds from the crosses listed in Table 1 develop vigorously for about 10 days and then begin to

Table 1. SEED SET INDUCED FROM INTERSPECIFIC CROSSES BETWEEN *H. vulgare* ($2n=14$) AND *H. bulbosum* ($2n=14$)

H. vulgare, female parent		No. of florets	No. of seeds	Percentage of
Type	Nos. used	pollinated	induced	seed set
Varieties	3	516	223	43·2
F_1 hybrids	5	196	124	63·3
"Diploid" plants*	11	526	291	55·3
Total	19	1,238	638	51·5

* Fertile *vulgare*-like plants with 14 chromosomes, derived from crosses between *H. vulgare* ($2n=28$) and *H. bulbosum* ($2n=28$).

Table 2. PLANTS OBTAINED FROM INTERSPECIFIC CROSSES BETWEEN DIPLOID *H. vulgare* AND DIPLOID *H. bulbosum* USING EMBRYO CULTURE TECHNIQUES

Method of embryo culture	Number of embryos cultured	Number of plants obtained	Percentage of embryos giving plants
1. Fruit with stigmatic end cut off	38	7	18·4
2. Fruit coat removed	131	11	8·4
3. Embryo dissected from seed	40	5	12·5
Total	209	23	11·0

show signs of abortion, making it necessary to rely on embryo culture methods to obtain the haploid plants. (The embryo culture medium we used in this study was the B5 medium described by Gamborg *et al.*[6], modified by omitting the 2,4-dichlorophenoxyacetic acid, and adding 0·7 g agar per 100 ml. of solution.) Although experiments are still in progress on embryo culture techniques, a small number of studies have been summarized in Table 2. The culture of the undissected fruit on the medium has been unsuccessful, whereas the three different degrees of dissection listed in Table 2 have made haploid plant development possible. The dissections were all performed 8–10 days after pollination. It would seem that the removal of the top half (stigmatic end) of the fruit may be as successful as attempting to dissect out the very small embryo that is present 8–10 days after pollination. After development on medium from 6–10 days using method 1, the embryo is much easier to remove from the fruit coat and endosperm.

The chromosome numbers of all twenty-three plants so far obtained from inter-specific crosses between the diploid forms have been checked by root-tip squash techniques and found to be haploids with seven chromosomes. These plants are vigorous, *vulgare*-like and produce numerous tillers with well formed spikes. They are, however, only about three-quarters of the size of diploid plants. The spikes on ten plants that have now headed are completely sterile. The pollen grains are empty and shrivelled, resulting in anthers that remain small and non-dehiscent.

Further evidence that the induced seeds contain haploid embryos was obtained from cytological observations on embryos collected and fixed 4–7 days after pollination. In each of the twenty-three embryos thus examined, most of the dividing cells contained seven chromosomes. In six embryos, however, there was one dividing cell that contained more than seven but fewer than fourteen chromosomes, the range being from eight chromosomes and a fragment to eleven chromosomes.

The elucidation of the mechanism for the development of this high frequency of haploids needs further detailed cytological and genetical studies. The present evidence, however, leads us to suggest that, rather than parthenogenesis of the gametes from *H. vulgare*, fertilization of the egg cell has occurred in at least as many cases and that the chromosomes of *H. bulbosum* have been subsequently lost during the first few mitotic divisions of the embryo. This evidence comes from four sources. (*a*) The observation of an occasional cell in the young embryo that contained more than seven chromosomes—frequently fragments of chromosomes. (*b*) The percentage seed set (equivalent to haploid frequency) is much higher than in previously reported instances of haploidy attributed to parthenogenesis, even when the techniques of pollen irradiation or delayed pollination have been used. On some spikes, pollinated two days after emasculation, we have observed 100 per cent seed development. (*c*) When diploid *H. vulgare* plants were pollinated with tetraploid *H. bulbosum* the percentage seed set obtained was similar to that from diploid crosses. Fertilization had occurred in the former because all the progeny obtained were triploid hybrids[5]. This phenomenon leads us to suggest that a certain chromosome balance is necessary in order to obtain chromosomally stable hybrids between these species. (*d*) Haploids of the *H. vulgare* type are also consistently obtained when *H. vulgare* is used as the pollen parent in crosses with *H. bulbosum*.

The unusual and important aspects of our findings are that (1) a very high frequency of haploid embryos can be obtained; (2) only haploids have been obtained from the cross between the diploid forms; (3) these haploids can apparently be obtained from any given line of cultivated barley; and (4) the haploids always apparently have the cultivated barley genome.

These observations lead us to suggest that haploidy could be used in barley breeding programmes. By selecting haploids in the first generation (F_1) from a cross and subsequently doubling their chromosome number, homozygous lines could be obtained within two generations. Present conventional barley breeding methods require six to seven generations of selfing in order to obtain an adequate level of homozygosity. The success of the application of haploidy to barley breeding programmes depends on efficient embryo culture techniques and the achievement of doubling of the haploids into homozygous diploid lines.

The discovery of a similar mechanism for producing haploids in other species might be directed on the basis of certain characteristics. First, it must be possible to induce seed set with the interspecific cross. Second, actual hybrids may only be obtained between specific ploidy levels of the species, indicating that hybrid stability requires a certain chromosomal or genomal balance. Third, embryo culture techniques may be essential for the development of haploid plants. Although embryo culture techniques have not been attempted, the interspecific cross in alfalfa between *Medicago sativa* and *M. dzhawakhetica* would appear to meet the first two requirements[7,8]. Because cultivated alfalfa tends to behave genetically like an autotetraploid, the desirable application of haploidy for breeding programmes would be to derive diploids from the tetraploid forms. This approach is being used in the potato, where dihaploids (diploids) are obtained from the tetraploid potato (*Solanum tuberosum*) by crossing with pollen from the diploid *S. phureja*[9,10]. Again, embryo culture techniques have not been used and only a portion of the very few seeds obtained per cross give rise to dihaploids. It is possible that the mechanisms of haploid production from interspecific crosses in *Hordeum* and in *Solanum* are similar.

We thank Dr E. Reinbergs for his suggestions and encouragement. Financial support from the National Council of Canada is gratefully acknowledged.

Note added in proof. Since the submission of our manuscript, a publication on haploid barley from the interspecific cross of *H. bulbosum* by *H. vulgare* has appeared (S. Symko, *Canad. J. Genet. Cytol.*, **11**, 602; 1969). This cross was made reciprocally to the one we are reporting and the haploids obtained exhibit some characteristics of both *H. vulgare* and *H. bulbosum*, probably due to the presence of *H. vulgare* chromosomes in the cytoplasm of *H. bulbosum*. Although of scientific interest, such haploids are less suitable in a barley breeding programme.

K. J. KASHA

Crop Science Department, University of Guelph,
Guelph, Ontario, Canada.

K. N. KAO

Prairie Regional Laboratory,
National Research Council,
Saskatoon, Saskatchewan, Canada.

Received November 7, 1969.

[1] Blakeslee, A. F., Belling, J., Farnham, M. E., and Berger, A. D., *Science*, **55**, 646 (1922).

[2] Kimber, G., and Riley, R., *Bot. Rev.*, **90**, 480 (1963).

[3] Niizeki, H., and Oono, K., *Proc. Jap. Acad.*, **44**, 554 (1968).
[4] Nitsch, J. P., and Nitsch, C., *Science*, **163**, 85 (1969).
[5] Kao, K. N., and Kasha, K. J., in *Proc. Second Intern. Barley Genetics Symp.*, July 6–11, 1969 (edit. by R. A. Nilan) (Wash. State Univ. Press, in the press).
[6] Gamborg, O. L., Miller, R. A., and Ojima, K., *Exp. Cell Res.*, **50**, 151 (1968).
[7] Lesins, K., *Canad. J. Genet. Cytol.*, **3**, 135 (1961).
[8] Clement, W. M., *Canad. J. Genet. Cytol.*, **5**, 427 (1963).
[9] Hougas, R. S., Peloquin, S. J., and Ross, R. W., *J. Hered.*, **49**, 103 (1958).
[10] Wangenheim, K. H. V., Peloquin, S. J., and Hougas, R. S., *Z. Vererbung.*, **91**, 391 (1960).

Carbon Monoxide as a Basis for Primitive Life on Other Planets

In considering the possibility of life on other planets, we are naturally prejudiced towards some variant of the terrestrial photosynthetic mechanism for CO_2 fixation. Such a prejudice is reasonable: photosynthesis is efficient and we know that it works. But there are reasons why such a cycle may not be likely on a planet such as Mars. The chemical machinery required for photosynthesis is exceedingly complex and is unlikely to be available to the most elementary organisms. Furthermore, on many planets, such as Mars, there is a considerable flux of high energy ultraviolet radiation which reaches the planetary surface[1] and which will tend to rupture bonds in organic molecules. Because photosynthetic organisms must be exposed to light, it seems unlikely that they could survive for long in the face of this radiation. It is, of course, conceivable that an organism could develop protective or self-repairing capabilities, but this would be particularly difficult for primitive life: in these conditions, life would be easiest in the shade. This would disfavour, but not exclude, an energy cycle based on photosynthesis.

In looking for an alternative metabolic cycle and energy source, we should consider the fact that the atmosphere of primitive planets may well be predominantly CO_2. This is the case for Mars and Venus[2]. A small but significant fraction of the solar flux striking the atmosphere will be absorbed to dissociate CO_2 to carbon monoxide[1,3]:

$$CO_2 \xrightarrow{h\nu} CO + O$$

Reoxidation of the CO (or a CO derivative) within an organism would provide a ready source of energy to drive metabolic reactions in which carbon is fixed and transformed into organic compounds. The complex chemical machinery required for transforming light energy into high energy chemical compounds is unnecessary, for this function is now carried on outside the organism. Furthermore, the living entity need not expose itself to any destructive ultraviolet flux.

A metabolic energy cycle based on oxidation of CO will certainly be far less efficient in terms of the utilization of the total solar energy striking the planetary surface. Only a fraction of a percentage (2×10^{-3} per cent) of the total light energy flux is in the form of photons sufficiently energetic directly to dissociate CO_2 (refs. 1 and 3), whereas of the order of 10 per cent may be useful for photosynthesis[4]. Thus very primitive life forms subsisting on CO would eventually tend to be supplanted by more sophisticated photosynthetic organisms. Perhaps this has happened on Earth. On a planet like Mars, however, this subsequent phase might well not have occurred in view of the continuing presence of the destructive ultraviolet flux, and because of the shortage of water.

There are two facts which further support this hypothesis of a metabolic energy cycle based on CO. The first is that CO metabolizing organisms are known to exist on Earth. An example is *Bacillus oligocarbophilus*[5], which seems to be widely distributed, and is particularly abundant in mines which contain appreciable amounts of CO.

The nature of the Martian atmosphere itself provides an interesting clue. If the CO_2 is slowly photodissociated to CO and oxygen, why do we not see more of these latter species? Plausible mechanisms for efficient atmospheric recombination of CO and oxygen are hard to find, not only on Mars but on Earth[3,6]. It was on this basis that we suggested some years ago[6] that terrestrial ^{14}CO produced by ^{14}C arising from cosmic radiation, as well as ^{12}CO, was biologically oxidized by *B. oligocarbophilus* and similar organisms. Certainly, biological activity on Mars based on the oxidation of CO would provide a natural answer to the problem of why the Martian atmosphere is predominantly CO_2 and contains so little of the expected CO and oxygen photodissociation products.

There are some obvious objections to this hypothesis. Perhaps the most serious is the inefficiency of the postulated process. But then, as has already been said, efficiency is not a requirement of the most primitive forms of life. It is only necessary that there is no more efficient process in direct competition. Another objection is the low concentration of CO and of oxygen, its probable oxidant. The molar fraction of CO in the Martian atmosphere is only 0·1 per cent[2,7]. Measurements of O_2 have so far only given a rather high upper limit, 0·3 per cent (ref. 7 and personal communication from C. Barth). Because O_2 is presumably produced by the same process which yields CO, it is, however, plausible that these two species are present in similar concentrations. Obviously simultaneous collisions of CO and O_2 molecules with an active site on an organism are unlikely. But relatively simple chemical forms are known to be capable of complexing and holding both CO and O_2. It seems plausible that an organism can thus accumulate both types of molecule and then cause the oxidation to proceed by an indirect route (Lederberg[8] mentions the possibility of CO conversion on Mars, and suggests that the exergonic reaction $2CO + H_2O \rightarrow CH_2O + CO_2$ might be involved). This mechanism provides a good example of the indirect path probably involved in such metabolism. Oxygen will be required for the eventual oxidation of organic material so produced, and this oxidation would then close the cycle. Finally, the fact that terrestrial organisms make do with a 0·03 per cent concentration of CO_2 implies that low partial pressures are not necessarily a limitation.

This hypothesis seems to be in accordance with known facts, but there may well be unknown factors which make it unlikely. It is not suggested that on a planet like Mars a life cycle based on CO, or for that matter any life, is probable. But, if there is biological activity, it seems necessary to consider the hypothesis that it is based on the metabolism of CO.

It should be possible to search directly for CO-fuelled life cycles by incubating planetary soil with ^{14}CO and looking for labelled metabolic products.

These views have been stimulated by laboratory experiments supported by the US National Aeronautics and Space Administration and the US Atomic Energy Commission and discussions with W. F. Libby and others.

RICHARD WOLFGANG*

Department of Chemistry,
University of Colorado,
Boulder.

Received January 16, 1970.

* Present address: Department of Chemistry, Yale University, New Haven, Connecticut.

[1] Johnson, F. S., *Satellite Environment Handbook*, 98 (Stanford Univ. Press, 1965).
[2] Goody, R. M., *Atmosphere of Venus and Mars* (edit. by Brandt, J. C., and McElroy, M. B.) (Gordon and Breach, 1968).
[3] Bates, D. R., *The Earth as a Planet* (edit. by Kuyper, G. P.) (Chicago, 1964).
[4] NAS–NRC, *Biology and the Exploration of Mars*, 230 (Publication 1296, 1966).
[5] Stephenson, M., *Bacterial Metabolism*, third ed. (Longmans, Green, London 1949).
[6] Pandow, M., MacKay, C., and Wolfgang, R., *J. Inorg. Nucl. Chem.*, **14**, 15 (1960).
[7] Belton, M. J. S., and Hunten, D. M., *Astrophys. J.*, **153**, 963 (1968).
[8] Lederberg, J., *Applied Optics*, **8**, 1269 (1969).

Book Reviews

MULTIFARIOUS ORGANELLES

Lysosomes in Biology and Pathology

Edited by J. T. Dingle and Honor B. Fell. Vol. 1: Pp. xxiii + 543. Vol. 2: Pp. xxiv + 668. (North-Holland Research Monographs: Frontiers of Biology, Vols. 14A and 14B.) (North-Holland: Amsterdam and London, 1969.) 490*s* for the 2 vols.

According to de Duve, "The lysosome introduced itself to us on December 16, 1949", brought in by what he describes as a subtle gremlin. Only a great scientist could welcome such a gremlin and realize, from a series of "dismal failures", the fact that he had, in his hands, a "structure-linked latency". His essay in this book should be compulsory reading for all scientists—an essay on the true experimentalist's philosophy of science. As he says, "As scientists we do not simply read the book of nature. We write it. . . . But we must accept our concepts for what they are, provisional approximations that are as much fictions of our minds as they are faithful depictions of facts". During the first surge of excitement over the newly established concept it was considered fashionable by the scientific intelligentsia, as distinct from the scientific experimentalists, to dismiss lysosomes as being predominantly mental fiction. The wealth of detailed information on the structure and function of these organelles now presented in these two volumes shows how justified were those who welcomed the first sketch of the lysosome concept and recognized its heuristic value.

The editors have been faced with the almost impossible task of ordering the plethora of information on the lysosomal and vacuolar systems, in both the plant and animal kingdoms. Moreover, because the lysosomal system is a real depiction of a biological fact, it is constantly changing in any one type of organism. This is typified by the enormous variety of its morphological forms, as discussed by Daems and his colleagues; by the different types of lysosomes, with their own special characteristics, found in plants (Matile); and even by the complexities of unravelling the biochemical features of lysosomal systems in the heterogeneous lymphoidal tissues which makes such fascinating and instructive reading in the chapter by Bowers.

The first volume consists of five separate parts; the second volume has four. "Lysosomes in Pathology" is dealt with in both volumes and consists of twelve chapters including reviews on the role of these organelles in teratogenesis, muscular dystrophy and arthritis. Two chapters deserve special mention. Slater's description of the behaviour of lysosomes in experimentally induced tissue injury should give reasoned order to a subject which has been a vortex of controversy for many years. The admirably clear and concise account by Coombs and Fell of the place of lysosomes in tissue injury mediated by allergic reactions is both timely and most welcome especially in the light of the recent rapid increase in interest in this field both from the medical and the scientific viewpoints. Other chapters of both fundamental and applied interest are those by Hers and van Hoof on genetic abnormalities of lysosomes and by Allison on lysosomes and cancer.

The other main parts of these volumes deal with lysosomal form and function; lysosomal physiology in vertebrate tissues; lysosomes in reproductive physiology in mammals; lysosomal physiology in non-vertebrate tissues, including insects, sponges and plants; structure and enzymology; lysosomes in secretion and digestion; and a section on methods. The valuable contributions of Weissmann on the effects of steroids and drugs and of Roels on the influence of vitamins A and E on lysosomes are included under "Physiology of Vertebrate Tissues"; the closely related topic of the extracellular secretion of lysosomal enzymes is reviewed by Dingle in a stimulating chapter in the section dealing with secretion and digestion.

In dealing with such a complex and all-embracing subject, the reader must realize that the subject of his interest may be treated in different chapters, often disguised under a more generalized title. For example, if his concern is with arthritis he will find much value in the chapter on connective tissue catabolism (Reynolds) as well as in the two chapters formally devoted to this subject by Lack and by Page Thomas. The student of lysosomes in microbiology and infection is well served by two chapters, one on bacterial infection (Allen) and the other on cell–virus interactions (Dale), but will be well rewarded by the chapter by Cohn and Fedorko on the fate and formation of lysosomes which deals with these organelles in polymorphs and macrophages. As might be expected in a vast and developing subject, some facets have been intensively investigated whereas others, possibly newer or dealing with more refractory tissue such as bone, are less well documented and sometimes even involve special pleading to relate observable effects to the general concept.

The second volume is completed by several chapters dealing with methods used in lysosome research. These contain much valuable and detailed information and several superb colour reproductions illustrate dramatically the chapters on vital staining and special methods. Many chapters have fine examples of cytochemical techniques with convincing photographs which match those, published some years back, of such pioneers in this subject as Novikoff and Holt. It is regrettable that the chapter in this book devoted explicitly to histochemistry and electron microscopy does not approach this standard.

These volumes fill an essential need in the scientific literature and should be treasured in all bio-scientific departments. Lucille Bitensky

LIPOPROTEIN SYSTEMS

Structural and Functional Aspects of Lipoproteins in Living Systems

Edited by E. Tria and A. M. Scanu. Pp. xxiii + 661. (Academic Press: New York and London, November 1969.) 170*s*; $26.

This book represents an attempt to cover lipoproteins in the widest sense. About one half of it is devoted to "soluble" lipoproteins and less than one third to "structural" or membrane lipoproteins. Most of the chapters seem to have been written during 1967 but some references for 1968 have been added. They provide very thorough reviews of progress in the studies of the major classes of lipoproteins and, on the whole, they constitute a very informative and readable book. There are extensive author and subject indexes which were perhaps compiled by a "literary assistant"—I felt compelled to look up the first entry under "S" which was "Salts, 210", and discovered that the extractability of protein from tissue by organic solvents was affected by (among other things) salts.

The chapters by Chapman and by Pethica are concerned with the contributions to knowledge of lipoprotein interactions made by a variety of physico-chemical methods. Pethica's contribution provides a particularly realistic and welcome new assessment of the significance of measurements made on phospholipid monolayers. Sjostrand provides the very high resolution electron micrographs of membranes, especially of mitochondrial membranes, and also speculations as to how they might be interpreted at the molecular level. Tria and Barnabei contribute a brief independent assessment of progress towards a detailed knowledge of membrane structure but are equally concerned with the possible ways in which phospholipids and lipopeptides are involved in some membrane functions. Criddle makes a remarkably good case for a biological significance for "structural protein", and Lebaron covers the very varied facets of proteolipid chemistry; Lebaron concludes that there are several lipid–protein complexes which are constituents of myelin. In a very brief chapter, entitled "A Physico-chemical Model of Nerve Membrane", Liquori derives a qualitatively satisfactory explanation of resting potential and action potential in terms of a three layer model for the axonal membrane in which a central layer of protein is preferred. The longest and certainly one of the most useful contributions comes from Utrecht. Op den Kamp, Van Deenen and Tomasi have collected together extensive information on bacterial lipids, their metabolism and their role in membrane structure. Blood lipoproteins are very extensively covered. Chylomicrons (Zilversmit), low density (Margolis) and high density (Scanu) lipoproteins, their biosynthesis (Marsh), the hormonal control of their levels in blood (Galli, Sirtori and Paoletti) and their involvement in blood coagulation (Barton) are all reviewed very thoroughly. To complete the lipoprotein scene there are two authoritative contributions on milk lipoproteins (Brunner) and egg lipoproteins (Cook and Martin).

This is perhaps the most extensive coverage of lipoproteins attempted in one book since the first international conference on lipoproteins (*Discussions of the Faraday Society*, No. 6, 1949). The topics covered are remarkably similar in both books, but only one person, Oncley, who contributes the foreword to the present volume, has contributed to both. A comparison of the two volumes provides an interesting assessment of twenty years of progress in this field. The most striking advance has been in the quantity and quality of the analytical data. Lipid components are now very precisely characterized and there is a great deal of chemical, physical and immunological data relating to the protein components. A greater emphasis on the significance of non-polar interactions between lipid and protein components is also evident, but there is continued uncertainty as to the precise molecular basis of most of the specific activities of these lipoprotein systems.

J. B. FINEAN

MANUAL OF METHODOLOGY

Fluorescent Antibody Techniques and their Applications

Edited by Akiyoski Kawamura, jun. Pp. xiii + 203 + 8 colour plates. (University of Tokyo Press: Tokyo; University Park Press: Baltimore and Manchester, England, June 1969.) $16.50.

THIS is a compilation on immunofluorescence from the experience of forty Japanese exponents of the technique, and it deals with the subject in two sections: the first on theoretical and general aspects, the second on practical applications, chiefly in virology.

In a manual of methodology, such as this claims to be, the first section should include a straightforward account of the immunochemistry relevant to labelling and to the reactivity of labelled antisera, of the common causes of non-specific staining, and of how the conditions of excitation of fluorochromes conjugated to globulins govern the choice of lamps and filters for microscopy. Here, alas, there is scant attention to the requirements essential for the different components in immunofluorescence—substrates, antisera, microscopy. Instead, the reader is plunged into a flurry of detail on serum fractionation, immuno-analysis, formulae, absorption spectra, and molar ratios that cannot but confuse the beginner. Indeed, some of the splendidly coloured plates—on the direct and indirect methods, and on fluorescence microscopy, for example—are less illustrations than obfuscations.

The second part of the book is a series of notes on applications of immunofluorescence, arbitrarily selected by the many workers who have contributed them. These give us glimpses into laboratory notebooks recording mostly identification procedures for a range of human and animal viruses and their antibodies in clinical and experimental material, and are as unrewarding as might be expected of this method of communication. The bacteriological section is even more severely limited; a section on treponemal antibody, for instance, is concerned only with methods of determining its immunoglobulin classes and types. The reader looking for help in studying tissue antigens and autoantibodies will find only a very incomplete account of antinuclear antibodies, a note on nephrotoxic serum, and some remarks on staining of arteries by sera from cases of pulseless disease. The illustrations of the two latter have transposed captions.

E. J. HOLBOROW

SCHOLARLY BOTANY

Précis de Biologie Végétale

Vol. 1: Cytologie. By A. Nougarède. Pp. xii + 598. 130 francs. Vol. 2: Nutrition et Métabolisme. By R. Heller. Pp. viii + 578. 115 francs. Vol. 3: Croissance Morphogenèse Reproduction. By P. Champagnat, P. Ozenda and L. Baillaud. Pp. viii + 510. 115 francs. (Précis de Sciences Biologiques.) (Masson: Paris, 1969.)

THESE three volumes are part of a library, "Précis de Sciences Biologiques", under the editorship of P. P. Grassé. Grassé's aim is that each volume should be scholarly but not esoteric, and up to date but not neglecting the "classical" observations on which our knowledge of biology is founded. Let me say at once that these books fulfil these aims to a very large degree. Such an aim, however, inevitably leads all the authors to assume a pedagogic (though never pedantic) style of writing. As for the content of the volumes, emphasis is laid on the presentation of established facts; consequently, controversial topics tend to be dismissed briefly with "malgré les progrès realisés le déterminisme reste encore mal connu", and there is no place for speculation in these pages. All the major areas of plant biology are covered with a clarity that never allows the reader to become bogged down by details. Yet the exposition of fundamental truths often makes for informative rather than interesting reading.

Nougarède's contribution is "cytologie" in its widest sense. The first section of her book is devoted to the techniques used in cell biology (chromatography, the centrifuge, isotopes and the like). Microscopy is well described, though curiously the interference microscope is not mentioned, while the still experimental proton microscope is. Description of cytoplasmic organelles occupies a large part of the remainder of the book. The emphasis here is on how their structure and function are interrelated. In the case of the mitochondrion, a good deal of space is devoted to Green's proposals on this subject. I felt that some areas of organelle biology merited a fuller discussion. For instance, rather more is known about the structure and function of lysosomes, flagella, plasmodesmata, microtubules (subunits are not mentioned) and the Golgi apparatus than the author admits. The description of the function of the nucleolus is rather out of date, and one experiment described in detail in this connexion is of equivocal value.

The details given of the genetic code are quite obsolete; it is a pity that Heller in his discussion of nucleic acid metabolism does not give more information on this point as the few RNA codons and their corresponding amino-acids that he mentions are correct. These omissions give the impression that the writing of *Cytologie* was completed some years ago; but nevertheless it is an excellent volume, especially if the reader wants a general description of cell components.

Heller, in *Nutrition et Métabolisme*, presents a thorough account of the physical chemistry and thermodynamics encountered in cell physiology and of the biosynthesis of cell constituents. In the section dealing with the biochemistry of respiration and photosynthesis, the exposition becomes splintered by quantities of short chemical equations without there being a good scheme which shows how they are related to one another. I felt that rather better summaries of these subjects could be found in Nougarède's book.

Champagnat contributes a chapter on "Croissance, Morphogenèse et Développement" in volume three. His principal theme seems to be that growth is regulated by growth substances. Unfortunately, the real problem of the relation of these substances and growth regulation is hardly considered: in what form do auxins and the like exist in plants; where are they located; how do they interact with the machinery of cell growth ?—these questions are never raised. The problem of growth is continued by Baillaud with a section on "Les Mouvements". Here again I felt that the subject had not been done justice, especially the section on photo- and geotropism. As in Champagnat's chapter, a lot of words are needed to describe relatively trivial observations, while there is too little discussion of the major topics.

The third volume is concluded by Ozenda, who, in two hundred pages, neatly covers flower and seed biology, embryology, life cycles of algae to archegoniates and the physiology and genetics of sex in fungi and algae. Neither Nougarède nor Ozenda describes meiosis; it seems as though each author thought the other was going to cover it. Even Nougarède's description of the multitudinous varieties of mitosis cannot save the authors from being awarded a black mark for this omission.

References to original papers are kept to a minimum in volume one, and there are none at all in volumes two and three. The quality of printing is high and these volumes are a handsome and useful set. Whether they will find a market in English speaking countries I cannot say; there are rival volumes in English, *Plant Cells* (by F. A. L. Clowes and B. E. Juniper) and *Plant Biochemistry* (by D. D. Davies, J. Giovanelli and T. Ap Rees) in Blackwell's "Botanical Monographs" being the closest in outlook and content. The price of the set is high, but I hope that libraries will give biologists the opportunity to read these books.

PETER BARLOW

ANTIMITOTIC AND ANTIBIOTIC

Rubidomycin

A New Agent against Cancer. Edited by J. Bernard, R. Paul, M. Boiron, Cl. Jacquillat and R. Maral. (Recent Results in Cancer Research, No. 20.) Pp. xiv + 181. (Springer-Verlag: Berlin, Heidelberg and New York, 1969.) DM 48.

THIS compact summary of all aspects of the antimitotic antibiotic rubidomycin, or daunomycin, has been compiled through the efforts of more than ninety listed authors, guided by five editors. Most published work on the substance, from the time of its first isolation simultaneously in France and Italy in 1962 to late 1968 when the monograph went to press, is included in the 274 bibliographic references and mentioned somewhere in the text, but the work is essentially a compendium of French and Italian studies on the chemistry and pharmacology of rubidomycin, and of French experiences with its clinical use.

In spite of its comprehensive character the monograph is of doubtful value. If it had been published as a series of review papers in appropriate specialist journals, the complex details of structure analysis and physico-chemical properties, the mass of *in vitro* and *in vivo* work on biological activity and pharmacological characteristics, and the clinical therapeutic trials could each have been received with interest and profit by an informed specialist readership. But the monograph as now presented is likely to be partially indigestible to all save the most erudite; the clinician will not appreciate the chemistry, nor the chemist the therapeutics. And for each man with his expertise the book has little new to offer that has not already been published.

Nevertheless, as a source of references and illuminating case data the monograph should come down often enough from the library shelves not to collect dust for the next year or two.

F. G. J. HAYHOE

HEREDITY OR ENVIRONMENT?

Social Status and Psychological Disorder

A Causal Inquiry. By Bruce P. Dohrenwend and Barbara Snell Dohrenwend. (Wiley Series on Psychological Disorders.) Pp. xiii + 207. (Wiley (Interscience): New York and London, October 1969.) 90*s*.

FOR many years there has been reasonably consistent evidence that psychological disorders occur more frequently among the "lower" socio-economic classes than among the "higher". To anybody who looks for simple explanations there are two possibilities. First, psychological disorders may be the result of genetic factors: those affected drift into the lower classes because of their disabilities, and swell the proportion of psychological illness in these classes. Alternatively, living conditions among the underprivileged may often cause stress, and this greater exposure to stress raises the incidence of psychological disorders. The simple choice is between an explanation in terms of heredity and environment.

Social Status and Psychological Disorder on balance comes down on the side of the "environmental" explanation, but only after showing that the choice is not easy, that the evidence is confused and inadequate, and that the issue cannot be resolved simply by attributing all the evidence to either environment or genetics. The authors make two contributions of their own. First, they provide a most useful review of the literature, distinguished by a very lucid analysis of the conceptual and methodological problems which bedevil studies of the etiology and epidemiology of psychological disorders. This alone makes their book an intelligent and a convenient source of reference. Second, and more important, they suggest a novel and rather elegant approach towards resolving the controversy about the role of heredity and environment. Their central assumptions are the existence of a norm which makes upward social mobility desirable, and of social barriers which limit mobility for certain subgroups of the population; for example, ethnic minorities. They suggest that comparisons between rates of disorders for such subgroups within a given class could help to decide between the genetic and environmental types of explanations. If rates of disorder in any one class are caused by social pressures, then these rates would be higher for, say, Negroes and Puerto Ricans than for other ethnic groups. If, however, disorder is caused by genetic factors, they would be lower; the able would rise irrespective of ethnic group. But there are barriers which block the rise of Negroes and Puerto Ricans: therefore, there should be more able (healthy) people among Negroes and Puerto

Ricans, diluting the overall rates of disorder within their particular class/minority combination.

Evidence from the authors' own research is still exploratory and ambiguous; the conceptual refinement of their approach nonetheless warrants careful consideration by anybody concerned with these problems.

PETER KELVIN

RADIATION PROTECTION

Basic Radiation Protection

Principles and Organization. By C. W. Easley. Pp. 132. (Gordon and Breach: New York and London, October 1969.) 125*s*; $15.

IT is very difficult to decide what basis was used for the selection of the material for this little book. In his preface, the author states that the book contains the information which has been useful to him and that he felt the desire to pass it on. The publishers obviously thought very highly of this collection of notes and happily charge accordingly.

The author covers the field in ten chapters and there are thirty pages of appendices. The equipment described is usually commercially available in the United States. The style of writing is certainly not verbose and the layout of the chapters is systematic. The brevity in the technical descriptions is good, but any background information suffers from it. Appendix A contains about thirty entries of two to three lines each. One finds definitions, for instance, for the atom, dosimetry, electron, molecule, roentgen, rem, but not for the rad, which I would have thought was most important. Appendix B contains sixteen full-page facsimile reproductions of forms of the type used in many laboratories handling radioactive material and usually designed by the laboratories according to their own needs. The literature of this large field is covered by five sources of information; the most recent one is a textbook published in 1960. It is surprising that the publishers believe they can find a market among scientists for this expensive little book and that they believe readers of *Nature* might be at all interested.

M. EBERT

CHOQUET'S THEORY

Lectures on Analysis

By Gustave Choquet. Edited by J. Marsden, T. Lance and S. Gelbart. Vol. 1: Integration and Topological Vector Spaces. Pp. xix + 382. Vol. 2: Representation Theory. Pp. xix + 336. Vol. 3: Infinite Dimensional Measures and Problem Solutions. Pp. xix + 341. (W. A. Benjamin: New York and Amsterdam, 1969.) Cloth $12.50 each; paper $4.95 each.

THIS book is an edited account of a graduate course of lectures delivered at Princeton by Professor Choquet in the fall term of 1967. It is a notable addition to the large number of books on functional analysis, chiefly because it provides the most complete account to date of what is justly called Choquet representation theory.

The first half of the course was an introduction to general topology, integration and topological vector spaces. The reader is supposed to know the rudiments of topology and conventional (set-function) measure theory: what is needed here is summarized without proof. The course proper opens with some lectures on selected topics from general topology: nets, filters, uniform spaces, embedding and metrization, and Baire spaces.

The treatment of Baire spaces is original in its use of the notion of a winning tactic from the theory of games. Two players, α, β, play a certain game with the open sets and points of a topological space. The latter is called α-favourable if α has a winning tactic: this is sufficient for the space to be a Baire space. Besides being elegant, the notion of an α-favourable space is more pliable than that of a Baire space. For instance, it survives the formation of topological products, which is more than can be said (it is an open question) of the property of being a metric Baire space. The use of Baire spaces in analysis is illustrated by a wide variety of examples.

There is a substantial introduction to Bourbaki integration theory in later lectures. This opens with an account of Choquet's theory of capacities, which, with the Reisz representation theorem, serves *inter alia* to link conventional measure theory and the Bourbaki theory.

There follows an introduction to topological vector spaces, with some emphasis on convexity and partially ordered vector spaces.

Having thus introduced the reader to many of the basic tools and concepts of functional analysis, the author has also set the stage for Choquet representation theory, which occupies most of the second half of this work.

According to the Krein–Milman theorem, each nonempty compact convex set K in a Hausdorff locally convex space is the closed convex hull of its extreme points. Every point of K then is a limit of convex combinations of extreme points. Can the limit always be realized by an integration ? This is the question that Choquet theory, in its original form, answered affirmatively: every point of K is the barycentre of a probability measure that is carried, in a certain sense, by the extreme points of K. Early versions of this theorem appeared in 1956, and the basic theory was perfected (by the joint efforts of a number of mathematicians) by the early 1960s. Since then it has been much developed, and this account shows us the state of the subject in 1967.

The theory is applied here to prove Bochner's theorem, Bernstein's theorem on completely monotone functions, and the ergodic decomposition of invariant measures. Other applications to potential theory and operator theory are sketched (much work has been done in these two fields, and in simplex theory, since these lectures were given).

The work concludes with a treatment of measures in infinite-dimensional vector spaces which includes a proof, by use of Choquet theory, of the Umemura–Choquet theorem that every rotation-invariant affine measure in an infinite-dimensional pre-Hilbert space is an average of Wiener measures. There is an appendix on potential theory.

The book omits certain proofs that were omitted by the lecturer, but in such cases references to standard works are usually given. There is a large collection of problems, many of them solved in an appendix. There are a good many minor misprints. All foreign words are printed without diacritical marks.

D. A. EDWARDS

CLASSIC ASTRONOMY

Celestial Mechanics

Vol. 5. By the Marquis de la Place. In French. Pp. ix + 508. (Chelsea: Bronx, New York, 1969. First published 1825.) $17.50.

THE reprint of Bowditch's English translation of Laplace's *Mécanique Céleste* has now been completed by a reprint of the fifth volume of Laplace's great work, which Bowditch did not manage to translate. In this volume, which was published many years later than the main body of his work, Laplace collected various supplements to the matters treated in the previous volumes. For us, however, the chief interest of the fifth volume lies in the historical sections, in which Laplace gives, for each topic, a critical account of the work preceding his own. Taken together, these sections present an outline of the whole progress of theoretical astronomy, distinguished by exquisite precision and unequalled mastery in bringing out the essential points. They are not made superfluous by the historical part of the *Exposition du Système du Monde*, in which Laplace had given a more continuous narrative of the same history, treated with less technical detail and a greater display of eloquence; they still deserve to be consulted for any deeper study. Their first-hand assessment of the extremely active and fertile period extending from Newton's *Principia* to Laplace's own researches is particularly valuable. The reprint is made, not from the original edition of 1825, but from the typographically neater and more correct edition of Laplace's collected works, sponsored by the Académie des Sciences (1882). It is not only a useful addition to Bowditch's translation; it will also afford those many collectors and librarians whose set of the original work lacks the fifth volume a welcome opportunity of completing it.

L. ROSENFELD

Correspondence

DDT in Milk

SIR,—In the biased review on DDT by T. H. Jukes (*Nature*, **225**, 301; 1970), I was personally attacked and misquoted. The fact is that I was invited to the DDT hearing in Madison, Wisconsin, in May 1969 by the Assistant Attorney General of the State of Wisconsin, Robert McConnell, who was the public intervenor in the case.

The presence of DDT in human milk was first reported in 1951. The general public was, however, not told about DDT in human milk until I and others in 1968 and 1969 pointed it out. It was then also pointed out that breast-fed babies ingest more DDT compounds than the acceptable daily intake (ADI), and that the ADI was set without considerations to the long-term effects of DDT compounds on the metabolism of endogenous and xenobiotic compounds as well as to the carcinogenic action in mice of low DDT doses.

The review by Jukes is not even modest—it is meagre. He apparently thinks that possible negative effects of DDT only are acute toxicity symptoms—exactly as the arguments of those who have a vested financial interest that DDT is used indiscriminately—completely disregarding the possible late, long-term effects.

We know now some of the late effects of DDT compounds on wildlife; partly from multi-generational studies. Man has not yet been exposed to the organochlorine pesticides for a full lifetime. As we have very few anamnestic data, we will probably never be able to acquire the epidemiological variables which are necessary for an evaluation of the possible actual hazards.

The use of DDT and other organochlorine compounds has been so ingeniously advocated that we no longer have any proper controls. This is a sufficient reason to ban these almost persistent compounds completely. We may argue about the time to complete the de-escalation, be it one year, two or at most five years, but no other discussions are necessary.

Yours faithfully,
GÖRAN LÖFROTH

University of Stockholm,
Stockholm, Sweden.

Enzymes in Crystals

SIR,—In response to the recent article by your Molecular Biology Correspondent on "Enzymes in Crystals" (*Nature*, **225**, 225; 1970), we would like to clarify his statement concerning the "pardonable satisfaction" with which Professor Lipscomb is said to "contradict with confidence" the amino-acid sequence of carboxypeptidase A as deduced in our laboratory (Bradshaw *et al.*, *Proc. US Nat. Acad. Sci.*, **63**, 1389; 1969). While the interpretation of the X-ray data by Professor Lipscomb and co-workers (Lipscomb *et al.*, *Proc. US Nat. Acad. Sci.*, **64**, 28; 1969) may contradict three points in the chemical sequence, we, in turn, take a certain degree of satisfaction in being able to supply unequivocal chemical evidence in support of our reported assignments.

It has been established that there are two forms of the enzyme differing at three sites in the polypeptide chain (Pétra *et al.*, *Biochemistry*, **8**, 2762; 1969). Professor Lipscomb believes—solely on the basis of X-ray assignments—that the enzyme used in his studies was of the valine type (Pétra and Neurath, *Biochemistry*, **8**, 2466; 1969). Taking this report at face value, we have prepared pure valine enzyme, by chromatographic means, in order to avoid the allotype controversy. An analysis of the tryptic peptides was conducted specifically to re-examine the residue in position 151. In agreement with our previous report, we have found this residue to be phenylalanine, whereas Professor Lipscomb and co-workers interpreted it to be tryptophan. Although re-examination of the other two sites (residues 93 and 245) in genetically pure enzymes has not been made, there is no question that in the pooled enzyme preparations used in the original studies the chemical identification is correct. At worst, these discrepancies may be due to allotypic variations not previously uncovered.

We do not enjoy a polemic of the relative merits of X-ray and chemical methods for the determination of the structure of an enzyme since, in the final analysis, a combination of both of these methods is the most expeditious approach to the total determination of structure. We feel compelled, however, to set the record straight, to avoid misinterpretation of the report of your Molecular Biology Correspondent.

Yours faithfully,
HANS NEURATH
KENNETH A. WALSH
RALPH A. BRADSHAW

Department of Biochemistry,
University of Washington,
Seattle, Washington,
and
Department of Biological Chemistry,
Washington University School of Medicine,
St Louis, Missouri.

Our Molecular Biology Correspondent writes:

It was certainly not my intention to whip up from the ringside a contest between the sequencers and the crystallographers. And to be fair, Lipscomb and his colleagues did firmly make the point that sequencing by X-ray structure analysis is not on, and probably never will be. It is interesting, just the same, to see how many errors in sequences have been uncovered by crystallographic studies, and indeed how few sequences since insulin have proved correct in every detail—which proves merely that sequencing is still an arduous trade. Professor Neurath's interesting letter shows that there can be other problems than the purely chemical to make life hard for sequencers and crystallographers alike. At the same time there is clearly a conflict still to be resolved between the chemical and crystallographic data. Meanwhile, to set the record straight, Professor Neurath's satisfaction is no whit less pardonable than that of Lipscomb *et al.*

Sternglass's Assumptions

SIR,—Lindop and Rotblat state in their article in *Nature* (**224**, 1257; 1969) that they see no reason why the logarithm of infant mortality should decrease linearly with time; in fact, such a law follows from the simplest of hypotheses. The probability S of an infant surviving a given year is assumed given by the formula $S = \underset{i}{\pi}(1-p_i)$, with p_i the probability of succumbing to the ith hazard in that year; thus $\ln S = \underset{i}{\Sigma}\ln(1-p_i)$. It is now reasonable to assume that a given fraction k of the existing hazards is removed each year, so that $d(\ln S)/dt = -k \ln S$, from which $\ln\ln S = A - kt$, with A another constant. Since the annual infant mortality I is equal to $1-S$, we find that $\ln\ln(1-I)$ varies linearly with time. When I is small compared with unity (the case considered by Sternglass) a linear variation of $\ln I$ itself is predicted; for larger values of I a departure from linearity occurs.

Yours faithfully,

PHILIP L. TAYLOR

Case Western Reserve University,
Cleveland, Ohio 44106.

View that may Upset Scientists

SIR,—Your editorial of January 31 (*Nature*, **225**, 397; 1970) attacked the Thames documentary "And on the Eighth Day" as a symptom of a current "sense of hysteria" over pollution. I will happily defend the programme both in detail and overall, but I think the most important point for your readers is that you misinterpret the message of the film. It is not at all "an attack on rationalism and the pursuit of scientific knowledge". Quite the contrary. What the film queries is whether what today passes for scientific method is a sufficiently sophisticated instrument to cope with complex environmental problems.

In the programme we were arguing that pollution was certainly a cost that ought to be taken into account by industry and had not been. But we were also warning that scientific research could be just as narrow and misleading as old fashioned accountancy. Laboratory results may be incontestable in the laboratory but utterly misleading if too readily applied to the more complex circumstances of the environment. The interaction of variables may mean that the picture as a whole is quite different from the sum of its parts. I am not a scientist, but it seems to me that scientists ought to be examining (more humbly than your editorial) the appropriateness of their methods and outlook to the solution of complex problems.

I think they will also have to accept that if these problems affect all of us then non-scientists will have views on them. If in some respects the world is now a laboratory in which we are all experimental animals, we are unlikely to be reassured by a scientific outlook (or an industrial one) that says that because a danger is not proven with full scientific rigor nothing should be done about it. We cannot wait for proof if the proof may simply confirm that harm is already happening to us.

This view may upset some scientists. Let me make it clear that I do not suggest that a scientist as a scientist should deviate from the most rigorous search for scientific proof. But scientists are also citizens and if, before they have definite proof, they begin to fear that what they are investigating may point to widespread and long term dangers for everyone, they have a duty as citizens to say so. This should not then influence the scientific investigation of these possible dangers, but it most assuredly should influence public policies that may be creating dangers. It should transfer the onus of proof from the critics of hazards to the creators of them—the industrialist, the Atomic Energy Commission, the food manufacturers, the local authorities, the fertilizer producers, the drug manufacturers and so forth.

We cannot continue to assume that any scientific or technological innovation is fine until someone has spent 10 years proving otherwise. That is what the argument is about. You ask confidently, "Must man really be forbidden to use fire?" Your choice of metaphor is unwise. There are many occasions when men are forbidden to use fire, and rightly. And there are few things more terrifying than fire carelessly used and out of control.

Yours faithfully,

IAN MARTIN

Thames Television Limited,
Kingsway,
London WC2.

University News

Dr Baruj Benacerraf has been appointed Fabyan professor of comparative pathology and head of the Department of Pathology at **Harvard Medical School. Professor Ralph Mitchell** has been appointed Gordon McKay professor of applied biology in the Division of Engineering and Applied Physics at **Harvard University.**

Professor H. Jones has been appointed Pro Rector of the **Imperial College of Science and Technology, University of London,** and **Professor J. D. Smyth** has been appointed to the chair of parasitology at the College.

Dr Eric R. Bryan has been appointed professor of structural engineering in the Department of Civil Engineering, **University of Salford.**

Appointments

Mr Reginald G. Voysey has been appointed deputy director at the **National Physical Laboratory.**

Professor Lloyd Motz, Columbia University, has succeeded Dr Irving J. Selikoff as president of the **New York Academy of Sciences.**

Professor Walther Manshard, Justus Liebig University, has been appointed director of **Unesco's Department of Environmental Sciences and Natural Resources Research.**

Dr Alan L. Pinkerson has been appointed assistant chief of the **Myocardial Infarction Branch of the National Heart and Lung Institute, US National Institutes of Health.**

Captain A. A. Murphy has been appointed director of the **Guided Weapons Research and Development (Naval) Branch, Ministry of Technology,** in succession to **Captain K. A. W. Pilgrim.**

Dr L. Rotherham, vice-chancellor of Bath University of Technology, has been appointed chairman of the **Advisory Committee for Scientific and Technical Information, Department of Education and Science,** in succession to **Dr F. S. Dainton.**

Announcements

Professor B. L. Horecker, Albert Einstein College of Medicine, has been elected vice-chairman of the **Pan-American Association of Biochemical Societies** for 1971, and will succeed **Dr D. R. Whitaker,** National Research Council of Canada, as chairman, in 1972.

Professor F. S. LaBella, University of Manitoba, has been awarded the **1969 Steacie Prize** in natural science.

The third **Clark Kerr award** for extraordinary and distinguished contributions to higher education will be presented to **Sir Eric Ashby.**

The managing trustees of the **Drummond Trust** invite applications for a senior Drummond fellowship for research in nutrition. The fellowship will normally be tenable for 2 years and preference will be given to biochemists with interests in the field of inherited metabolic disease. Further details can be obtained from the Honorary Secretary, Drummond Trust, University College London, Gower Street, London WC1.

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Wanted: Pharmacologist, wife and boy of 3, en route from Addis Ababa to Hong Kong, wish to rent small furnished house for 2 weeks only from June 27 to July 11, 1970. Preferably W, NW or SW London suburban area. Please contact Dr M. Roberts, PO Box 30015 MA, Addis Ababa, Ethiopia.

Wanted: Small furnished flat in London for American academic couple, June through August. Please contact J. K. DeVries, Department of Microbiology, New York University Medical Center, 550 First Avenue, New York, NY 10016, USA.

Wanted: 2 or 3-bedroomed furnished house or flat for visiting fellow at Clare Hall, Cambridge, June 1 to August 31, 1970. Children aged 14 and 20. Please contact J. M. Smithells, White Cottage, Godstone, Surrey.

Wanted: 4-bedroomed furnished house in or near Cambridge for 1 year from either July 1 or August 1, 1970 (by arrangement), by Canadian professor, wife and family of four children aged 9–15. Garage desirable but central heating essential. Please contact Professor G. H. Dixon, Department of Biochemistry, University of British Columbia, Vancouver 8, Canada, or Mr and Mrs Derek Anderson, 11 Spring Lane, Bottisham, Cambridgeshire (telephone Bottisham 583).

Wanted: Small house or flat for married couple in Pittsburgh, Pennsylvania, from summer 1970 for 12 months. Please contact J. L. Finney, Department of Crystallography, Birkbeck College, Malet Street, London WC1.

Vacant: Small, furnished 3-bedroom 1930s style house in West London to let for 6 months from March 27, 1970. Near Hanger Lane underground and shops, 30 min to West End by train. £65 a month. Please contact Dr A. Goldsworthy, Botany Department, Imperial College, London SW7.

Vacant: From June 1 until August 31. 4-bedroom ranch-type house in select location, fully furnished, with air-conditioning and air purification, 2 bathrooms, 2 fireplaces, full basement, garage. Rent reasonable. Owner going abroad for summer. Please contact Dr D. F. Moore, 327 Engineering Sciences Building, West Virginia University, Morgantown, West Virginia 26506, USA.

Vacant: Furnished house in London, with 4 bedrooms, central heating. Available June to September 1970. Please contact Dr L. V. Crawford, 63 Swain's Lane, Highgate, London N6.

Vacant: 3-bedroomed, centrally heated house in Blackheath, SE London. Newly decorated and fully furnished. Large garden, and close to woodland and the river. Frequent train service to Charing Cross (25 minute journey) or easy drive. Available between July 1970 and September 1971. Please contact J. L. Finney, Department of Crystallography, Birkbeck College, Malet Street, London WC1.

British Diary

Monday, March 2

Developments in the Use of Plastics in Railway Rolling Stock (6 p.m.) Mr B. J. Hawthorne, Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Physics Exhibition (four days) Institute of Physics and the Physical Society, at Alexandra Palace, London.

Stereophonic Transmission (7 p.m.) Mr G. J. Phillips, Institution of Electrical Engineers, at the Royal Star Hotel, Maidstone, Kent.

Structural Enzymology (1): Conformations of Enzyme Molecules (5 p.m.) Professor D. C. Phillips, University of London, at the Middlesex Hospital Medical School, Mortimer Street, London W1.

The Competition for Cash between Innovation and Extension (6.30 p.m.) Dr D. S. Davies, Society of Chemical Industry, at 14 Belgrave Square, London SW1.

The First Steps Into Space (8 p.m.) Professor R. A. Lyttleton, FRS, University of Hull, in the Middleton Hall. (The St John's College, Cambridge, Lecture.)

The Value of Polymer Blends (7.30 p.m.) Mr P. W. Milner, Institution of the Rubber Industry, at the Midland Hotel, Burton-on-Trent.

Tuesday, March 3

Banking and Automation (5.30 p.m.) Mr P. J. V. Ashurst, Institution of Electrical Engineers, at Savoy Place, London WC2.

Indirect Pressure Measurement (5.30 p.m. discussion) Institution of Electronic and Radio Engineers, Joint IEE/IERE Medical Biological Electronics Group, at the Institution of Electrical Engineers, Savoy Place, London WC2.

Pulse Code Modulation (7.15 p.m.) Mr G. H. Bennett, Institution of Electrical Engineers, at the Aylesbury College of Further Education, Aylesbury, Bucks.

Safety in the Design of Process Plant (6 p.m. discussion) Institution of Mechanical Engineers, Process Engineering Group, at 1 Birdcage Walk, London SW1.

Some Bronze Age, Iron Age and Early Medieval Settlements in the Netherlands (5.45 p.m.) Professor H. T. Waterbolk (Groningen), University of London, at the Institute of Archaeology, 31–34 Gordon Square, London WC1. (Further lectures on March 5 and 10.)

The Biology of Dolphins (5.30 p.m.) Professor R. J. Harrison, Royal Institution, at 21 Albemarle Street, London W1. (Lecture for Sixth Form Pupils from Schools in London and the Home Counties. To be repeated on March 4, 10 and 11.)

The Development of the Hypothesis of Continental Drift (5.30 p.m.) Professor H. Martin (Göttingen), University of London, at Senate House, London WC1. (Further lecture on March 5.)

Ultrastructure of Blue-Green Algae (5.30 p.m.) Professor Norma J. Lang, University of London, in the Botany Lecture Theatre, University College London, Gower Street, London WC1. (Further lecture on March 4 at King's College Department of Botany, 68 Half Moon Lane, Herne Hill, London SE24.)

Wednesday, March 4

Electroluminescence Solid State Devices (5.30 p.m. Joint IEE/IPPS Conference), at Savoy Place, London WC2.

Interferometric Determination of EHL Contact Pressures (6 p.m.) Mr D. L. Cope and Mr D. J. Haines, Institution of Mechanical Engineers, Tribology Group, at 1 Birdcage Walk, London SW1.

Mathematical Notation (1 p.m.) Dr H. R. Calvert, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Measurement of Stress Cracking Resistance (2.30 p.m.) Mr R. A. Horsley, Plastics Institute, at 11 Hobart Place, London SW1.

Recent Developments in Test Sieving (5.30 p.m.) Dr J. E. English, University of London, at King's College, Strand, London WC2.

Some Other Turbid Medium Theories (3 p.m.) Mr S. Orchard; **The Optical Variables in White Paint Films** (4.05 p.m.) Mr D. F. Tunstall, The Colour Group (Great Britain) in the Physics Department, Imperial College, Prince Consort Road, London SW7.

Structural Enzymology (2): Enzyme Substrate Interactions (5 p.m.) Professor D. C. Phillips, University of London, at the Middlesex Hospital Medical School, Mortimer Street, London W1.

Superconductors: With Us Today or Tomorrow? (7.30 p.m.) Dr W. T. Norris, Institution of Electrical Engineers, at the Martineau Hall, Dorking Halls, Dorking, Surrey.

Surface Area Determination (2 p.m.) Mr I. C. Edmundson, Mr A. S. Joy, Mr R. W. Lines and Mr N. G. Stanley-Wood, Society for Analytical Chemistry, Particle Size Analysis Group, in the Chemical Engineering Department, University of Technology, Loughborough.

The Continuing Education and Development of Professional Electronic Engineers (6 p.m.) Dr K. G. Stephens, Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Toxicity in Polymers (6.45 p.m.) Institution of the Rubber Industry, at John Dalton College of Technology, Chester Street, Manchester.

Why Sociology? (1.15 p.m.) Dr Ernest Krausz, City University, at the Graduate Business Centre, Gresham College, Basinghall Street, London EC2.

Thursday, March 5

Automation of Thermal Power Stations in the USSR (6 p.m. discussion) Institution of Mechanical Engineers, and the Institution of Electrical Engineers, at 1 Birdcage Walk, London SW1.

Direct Digital Control Without a Computer (6 p.m.) Mr C. C. Lawson, Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Effective Incentives for Productivity (all-day discussion) Institution of Mechanical Engineers, Manufacture and Management Group, at 1 Birdcage Walk, London SW1.

Psychological Aspects of Short-Term and Long-Term Memory (4.30 p.m.) Dr D. E. Broadbent, FRS, Royal Society, at 6 Carlton House Terrace, London SW1.

Radar Meteorology (5.30 p.m.) Dr E. Eastwood, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Influence of Freezing Practice on the Quality of Fish and Meat (6 p.m.) Dr C. L. Cutting, Institute of Refrigeration, at the National College for Heating, Ventilating, Refrigeration and Fan Engineering, Southwark Bridge Road, London SE1.

Friday, March 6

How Plants Grow (9 p.m.) Professor F. C. Steward, FRS, Royal Institution, at 21 Albemarle Street, London W1.

Photochemistry of Quinones in Solution (1 p.m.) Mr D. Kemp, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

Saturated Leakage Reactance of Cage Induction Motors (5.30 p.m.) Dr B. J. Chalmers and Mr R. Dodgson, Institution of Electrical Engineers, at Savoy Place, London WC2.

Structural Enzymology (3): The Evolution of Enzymes (5 p.m.) Professor D. C. Phillips, University of London, at the Middlesex Hospital Medical School, Mortimer Street, London W1.

Monday, March 9

Management by Objectives (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Myrtos: an Early Bronze Age Settlement in Crete (5.45 p.m.) Dr P. Warren, University of London, at the Institute of Archaeology, 31–34 Gordon Square, London WC1.

Training—a Systems Approach (5.30 p.m.) Captain G. Huggett, Institution of Electrical Engineers, at Savoy Place, London WC2.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Royal Society. Report of Council for the eleven months ended 31 August, 1969. Pp. 97. (London: The Royal Society, 1969.) [112

Northern Ireland, Ministry of Agriculture. Leaflet No. 99: Cabbage and Kale for Stock Feeding. Pp. 7. (Belfast: Ministry of Agriculture, 1969). [112

Ministry of Transport: Road Research Laboratory. RRL Report LR 285: Note on the Costs, Lives and Effectiveness of Various Road Markings. By F. G. James and J. A. Reid. Pp. 28. (Crowthorne, Berkshire: Road Research Laboratory, 1969.) [112

University of Nottingham. Report of the School of Agriculture, 1968/1969. Pp. 168. (Sutton Bonington, Loughborough: University of Nottingham, School of Agriculture, 1969.) 10*s*. [112

Judging the Unborn. By Professor J. H. Edwards. (Inaugural Lecture delivered in the University of Birmingham on 4th February, 1969.) Pp. 12. 3*s*.; $0.36. The Era of Molecular Pathology. By Professor K. W. Walton. (Inaugural Lecture delivered in the University of Birmingham on 27th May, 1969.) Pp. 15. 3*s*.; $0.36. (Birmingham: The University, 1969.) [112

Electronics and the Quantum. By Professor A. L. Cullen. (Inaugural Lecture delivered at University College London, 30 May 1968.) Pp. 13. (London: H. K. Lewis and Co., Ltd., 1969.) 5*s*. net. [212

University of Sussex. Science Policy Research Unit—Third Report 1969. Pp. 36. (Falmer, Brighton: University of Sussex, 1969.) [312

London School of Economics and Political Science. Higher Education Research Unit—The First Five Years—1964–1968. Pp. iv + 43. (London: LSE, 1969.) [312

Department of Health and Social Security. The New Pensions Scheme—Latest Facts and Figures, with Examples. Pp. 30. (London: HM Stationery Office, 1969.) 1*s*. (5*p*) net. [312

Bulletin of the British Museum (Natural History). Entomology. Vol. 24, No. 2: The Type-Material of Tachinidae (Diptera) Described by N. Baranov. By C. W. Sabrosky and R. W. Crosskey. Pp. 27–63. 25*s*. Vol. 24, No. 3: Bombyliidae and a First Record of Nemestrinidae from Sokotra (Diptera). By D. J. Greathead. Pp. 65–82. 11*s*. (London: British Museum (Natural History), 1969.) [412

Politics and the Multi-National Company. By Louis Turber. (Fabian Research Series, No. 279.) Pp. 32. (London: Fabian Society, 1969.) 5*s*. [512

Building Research Station. Current Papers. No. 42/69. Gardens on Housing Estates—a Survey of User Attitudes and Behaviour on Seven Layouts. By J. A. Cook. (Reprinted from *Town Planning Review*, 1969, Vol. 39 (3), October.) Pp. 16. No. 45/69: The Effect of Manganese, Iron and Fluorine on the Properties of Tricalcium Silicate. By W. Gutt and G. J. Osborne. (Reprinted from *Trans. of the British Ceramic Soc.*, 1969, Vol. 68 (3), May.) Pp. 8. No. 46/69: The Response of a High Frequency Moisture Probe to Soluble Salts. By V. Laws and R. Sharpe. (Reprinted from *British Journal of Applied Physics*, 1969, Ser. 2, Vol. 2 (9), September.) Pp. 7. (Garston, Watford: Building Research Station, 1969.) [512

International Association for the Exchange of Students for Technical Experience, 22nd Annual Report 1969. Pp. 93. (London: IAESTE (UK), c/o Imperial College, 1969.) [512

The Zoological Record, 1967, Vol. 104, Section 5: Echinodermata. Compiled by A. M. Clark. Pp. 46. (London: Zoological Society of London, 1969.) UK and Eire 20*s*.; overseas 24*s*. ($US 2.90). [1112

The Shortage of Mathematics and Science Teachers in Schools. Pp. 18. (London: The Royal Society, 1969.) [1112

Fringe Benefits for Pensions: a Study of Company Post Retirement Schemes. By F. Le Gros Clark and Carole Faubert. (Information Report 2, New Series.) Pp. 32. (London: Institute of Personnel Management, 1969.) 9*s*. [1112

Annual Report and Accounts of the British Broadcasting Corporation 1968–69. Pp. 220 + 16 photographs. (Cmnd 4216.) (London: HM Stationery Office, 1969.) 22*s*. 6*d*. [1112

Nuclear Energy in Britain. (Central Office of Information Reference Pamphlet, No. 28.) Pp. 51. (London: HM Stationery Office, 1969.) 6*s*. net. [1112

Ministry of Agriculture, Fisheries and Food, Fisheries Radiobiological Laboratory. Technical Report FRL 5: Radioactivity in Surface and Coastal Waters of the British Isles 1968. By N. T. Mitchell. Pp. 39. (Lowestoft, Suffolk: Fisheries Radiobiological Laboratory, 1969.) [1112

Other Countries

US Department of the Interior: Geological Survey. Professional Paper 625-A: Distribution of Gold and other Metals in the Cripple Creek District, Colorado. By Garland B. Gott, J. Howard McCarthy, Jr., Gordon H. Van Sickle, and John B. McHugh. Pp. iii + 17. Professional Paper 634: Diagenesis of Tuffs in the Barstow Formation, Mud Hills, San Bernardino County, California. By Richard A. Sheppard and Arthur J. Gude, III. Pp. iv + 35. $0.50. (Washington, DC: Government Printing Office, 1969.) [2411

National Museum of Canada. Anthropology Papers No. 22: Historic Site Archaeology in Canada. By Kenneth E. Kidd. Pp. 47. Publications en Zoologie, No. 1: Les Oiseaux de l'Ile Anticosti, Province de Québec, Canada. Par Henri Ouellet. Pp. x + 79. $1.75. (Ottawa: Queen's Printer, 1969). [2511

US Department of the Interior: Geological Survey. Bulletin 1262: General and Engineering Geology of the Northern Part of Pueblo, Colorado. By Glenn R. Scott. Pp. vi + 131 + 2 plates. (Washington, DC: Government Printing Office, 1969.) [2511

Publications of the Observatory of the University of Michigan, Vol. 9, No. 9: Spectrophotometry of B Stars. By Lawrence H. Aller and Jun Jugaku. Pp. 203–273. (Ann Arbor: University of Michigan, 1969.) [2611

This is Creation, Universe, Motion and Life!—The Law of Universe: 366 Discoveries and Theories. By Z. K. Rezavi. Pp. 36. (Tehran, Iran: Z. K. Rezavi, Technical Department of the Ministry of Justice, 1969.) [2611

Annals of the New York Academy of Sciences. Vol. 165, Article 2: Cyclitols and Phosphoinositides; Chemistry, Metabolism, and Function. By F. Eisenberg and 56 other authors. Pp. 509–819. (New York: New York Academy of Sciences, 1969.) $18.50. [2711

US Department of the Interior: Geological Survey. Bulletin 1276: Geology of the Sandy Hook Quadrangle in Monmouth County, New Jersey. By James P. Minard. Pp. iv + 43 + 1 plate. Bulletin 1279: Use of Seismic Intensity Data to Predict the Effects of Earthquakes and Underground Nuclear Explosions in Various Geologic Settings. By Patrick J. Barosh. Pp. v + 93. $0.45. Bulletin 1292: The Geologic Story of Mount Rainier. By Dwight R. Crandell. Pp. vi + 43. $0.65. (Washington, DC: Government Printing Office, 1969.) [2811

The International Hydrological Decade. Water and Man: a World View. By Raymond L. Nace. Pp. 46. (Paris: Unesco, 1969.) [2811

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Tribophysics, 1968/1969. Pp. 22. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1969.) [2811

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Irrigation Research 1968/1969. Pp. iv + 75. (Griffith, NSW: Commonwealth Scientific and Industrial Research Organization, 1969.) [2811

Commonwealth of Australia. Seventeenth Annual Report of the Australian Atomic Energy Commission. (Being the Commission's Report for the year ended 30 June 1969.) Pp. 115. (Coogee, N.S.W.: Australian Atomic Energy Commission, 1969.) [312

Institute of Medical and Veterinary Science—30th Annual Report of the Council, July 1967—June 1968. Pp. 71. (Adelaide: Institute of Medical and Veterinary Science, 1969.) [312

The Moon, Vol. 1. No. 2, November 1969. (International Journal of Lunar Studies.) Pp. 1–168. Subscription price per volume of 4 issues (about 500 pages) Dfl. 114.80 (US $32.15) including postage. Private persons (no institutes, laboratories, libraries, etc.) may subscribe at the reduced rate of Dfl. 49.80 (US $13.95) per volume, including postage. Published quarterly (Dordrecht, Holland: D. Reidel Publishing Company, 1969.) [412

HOW TO BUY NATURE

Volumes start in January, April, July and October, but subscriptions may begin with any issue.

The direct postal price per subscription for one copy of NATURE each week is:

12 MONTHS (52 issues and 4 indexes)

Great Britain and Eire	**£14.0.0** each
United States of America........(by air freight)	**$48** each
Canada........(by air freight)	**$52** each
Elsewhere overseas by surface mail	**£14.0.0** each

(Charge for delivery by air mail on application)

•Shorter periods *pro rata*. (Minimum three months.)

Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
4 LITTLE ESSEX STREET, LONDON WC2
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2
Subscription Department
MACMILLAN (JOURNALS) LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 5431
Advertisements only should be addressed to
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2
Telephones 01-242 6264/01-405 4743
Telegrams: Textualist London WC2
Registered as a newspaper at the Post Office
Copyright © Macmillan (Journals) Limited, February 28, 1970

New Fabri-Tek 1010

True Signal Averaging

at a new low price . . .

$4,400*

FEATURES

■ Low Cost ■ True Signal Averaging ■ Ease of Operation ■ Input Filter ■ Sweep Speeds From 20 Microseconds/Address to 200 Seconds/Sweep ■ Visual Sweep Counter ■ Autostop After Selected Number of Sweeps ■ Pushbutton Calibration for Oscilloscope and X-Y Recorder Outputs

The Fabri-Tek Model 1010 Signal Averager is not a stripped down version of a more versatile unit. This low cost computer utilizes the most up-to-date technology (it's made almost entirely of integrated circuits) and sacrifices none of the essentials of a complete signal averaging system. It is a completely digital system with a 256 word magnetic core memory.

The unique circuitry does not resort to unequal weighting of individual inputs (weighting techniques which emphasize more recent data), but rather presents the user with a true average of the input signal regardless of the number of sweeps.

Automatic data normalization is one of the key features of this single input signal averaging computer. A completely new approach automatically provides the user with normalized data regardless of the sweep speed or the number of sweeps selected. At the same time this computer is easier to use than most laboratory oscilloscopes. Pushbutton programming, for instance, greatly simplifies and speeds up the operation.

The input signal can be viewed for set-up purposes while adjusting the sensitivity and trigger threshold. External address advance is included. A pre-filter is provided for optimizing the signal-to-noise ratio improvement as well as avoiding "aliasing" errors due to high frequency noise. Calibration of X-Y plotter or oscilloscope outputs is simplified by means of pushbutton controls. A meter is provided to indicate the number of pre-set sweeps completed. "Real-time" readout is available for comparing averaged signal to raw signal or for spectral analysis.

*U.S.A. and Canada

For more information, write

FABRI-TEK Instruments, Inc.

Dept. **N-9** 5225 Verona Rd., Madison, Wis. 53711 Phone: 608/238-8476
In Europe: Bruker-Physik Ag, 7501 Karlsruhe-Forchheim,
Postfach 40 10, Karlsruhe, West Germany

CENTRAL ELECTRICITY GENERATING BOARD

South Western Region

Scientific Services Department

HATCHERY SUPERVISOR

Applications are invited for the above post at the Board's established Salmon Hatchery and Smolt Rearing Station situated on the River Cynrig, near Brecon. The Hatchery is part of the Environmental Science Section of the South Western Region Scientific Services Department which is based at Portishead, near Bristol.

The main function of the Hatchery is to incubate and rear salmon from the ova to the smolt stage but there is scope for experimental feeding programmes and fish husbandry methods to optimize smolt production.

In addition, there could be opportunities within the Environmental Science Section for general activities concerned with River Boards and fish farming projects within the Region.

Applicants should hold a qualification in Biology, or have considerable practical experience in hatchery operation, or alternatively have worked for some time in a fisheries laboratory. The successful applicant will be responsible for running the Hatchery with one full time assistant.

Salary will be within the range £1,479–£2,139 plus £60 per annum allowance.

Point of entry will depend on qualifications and experience. The supervisor is required to live in a modern house on the Hatchery site for which a rent will be charged.

Good holidays, sick pay and superannuation schemes are in operation.

Applications on form SF/1 obtainable from the **Personnel Manager, Central Electricity Generating Board, 15-23 Oakfield Grove, Clifton, Bristol BS8 2AS,** *should be completed and returned to him (quoting vacancy number 95/70) by not later than 30th March 1970.* (868)

IMPERIAL CANCER RESEARCH FUND

Department of Tumour Virology

POST-DOCTORAL BIOCHEMIST required to study cell membranes, especially the function of their contained lipid. Three- or five-year appointment. Starting salary in the range £1,700 by £115 to £2,275 plus £60 London Allowance p.a.

Applications accompanied by a curriculum vitae and the names of two referees should be sent to:

The Secretary, Imperial Cancer Research Fund,
P.O. Box 123, Lincoln's Inn Fields, London W.C.2.,

to arrive by March 21, 1970.

(880)

CITRUS RESEARCH

Applications are invited for the post of AGRONOMIST, Citrus Research, University of the West Indies. Appointment will be made by the British Caribbean Citrus Association on a salary scale: $J2,800 by $J180 to $J3,160 by $J200 to $J4,560 by $J220 to $J7,200 ($J2 = £1 sterling). The post is tenable in Jamaica. Preference will be given to persons with qualifications and/or experience in pathology.

Applications (four copies), giving brief details of education, experience, personal information and nominating three referees, should be submitted on or before March 31, 1970, to: Head, Citrus Research, University of the West Indies, St. Augustine, Trinidad, W.I., from whom further particulars can be obtained. (696)

UNIVERSITY OF SHEFFIELD

DEPARTMENT OF HUMAN BIOLOGY AND ANATOMY

LECTURER required commencing October, 1970. Applicants should be capable of teaching topographical anatomy to medical students. Excellent research facilities are available. An interest in neurological anatomy or experimental haematology would be desirable but not essential. Initial salary according to qualifications and experience on the scale £1,240 by £115 to £2,850 with F.S.S.U. provision. Candidates should write informally in the first instance to Professor R. Barer giving details of training and interests. Quote Ref. R.2/G. (808)

For further Classified advertisements see page va

THE BRITISH COUNCIL

LEBANON

THE LEBANESE UNIVERSITY, BEIRUT, requires a LECTURER IN THERMODYNAMICS by mid-October, 1970. Candidates should have a Ph.D. degree, or its equivalent from a university in Britain. Some teaching and research experience at university level is desirable.

Salary: £2,335 to £2,910 per annum according to qualifications and experience. Terms for a two-year period as follows: Married overseas allowance within the following ranges: accompanied £593 to £418, unaccompanied £373 to £198. Children's and home education allowances. Income tax refunded if levied. Free furnished accommodation. Outfit grant. Employer's superannuation contribution paid. Medical and insurance schemes. Fares paid for Lecturer and family, including mid-tour passages for children in Britain. Assistance with freight and import duty on approved car. Contract guaranteed by the British Council.

Write quoting reference number 9PU61 to Appointments Division, The British Council, 65 Davies Street, London W1Y 2AA, for further particulars and application form to be returned completed as soon as possible. (804)

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF PHYSIOLOGY

Applications are invited for two LECTURESHIPS IN PHYSIOLOGY from October 1, 1970, or an earlier date to be arranged. Salary within the range £1,240 by £115 to £2,850 with F.S.S.U. benefits: initial salary according to qualifications and experience. For medical or dental graduates this will normally be not less han £1,585 per annum.

Medical or dental graduates who wish to have one or more years' experience of preclinical work are eligible to apply, or to make preliminary inquiries from either Professor R. C. Jordan or Professor V. R. Pickles (Head of Department). One post may be of interest to applicants preparing for the **Primary F.R.C.S.** examination, who would be given appropriate facilities. In such cases the post would be limited to the period October 1970-June 30, 1971, and the salary would be at the rate of £1,355 per annum.

Applications, with curriculum vitae and the names of 2 referees, should be sent by March 16 to the Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further details can be obtained. (817)

WOOLWICH POLYTECHNIC

LONDON, S.E.18

DEPARTMENT OF BIOLOGY AND CELL SCIENCE

A vacancy exists for the post of

LABORATORY TECHNICIAN

Preference will be given to applicants in the field of animal physiology; experience of animal house maintenance would be an advantage. Day release by arrangement.

The work of the Department includes courses leading to a B.Sc. in Applied Biology and to M.Phil. and Ph.D. by research.

Salary scale: £745 (unqualified) to £1,125 (qualified) plus £75 London Weighting Allowance.

Commencing salary according to age and qualifications.

Further particulars and application form, to be returned by March 17, 1970, from the Clerk to the Governing Body, Woolwich Polytechnic, Wellington Street, London, S.E.18 (01-854 9624, Ext. 12). (832)

Physical Organic Chemist

The Medicinal Chemistry Department of our expanding Research and Development Group in Loughborough has a vacancy for an Organic Chemist with experience and a pronounced interest in all physical techniques required in modern drug research. This will include a sound familiarity with all types of spectroscopy and a basic knowledge of molecular-orbital theories.

The successful candidate will be in charge of a group of staff concerned with the establishment of routine physico-chemical data related to compounds designed in the Medicinal Research Laboratories. In the context he will also provide a service to other departments.

His main function will be to participate in and supervise all investigations of a physico-chemical nature related to problems arising from drug research. In this responsibility he will liase closely with the Project Leaders and research personnel both inside and outside the department. He will be expected to keep himself informed on all new developments in his area of interest and he will be required to introduce new and useful techniques when they become available. As a senior member of our research staff he will be encouraged to attend scientific meetings and to publish scientific papers.

The man appointed will work in a modern, well-equipped department at our new Research and Development Laboratories at Loughborough.

We are a member of the Fisons Group providing a first class pension scheme, generous holidays, assistance with removal expenses where applicable and other welfare benefits which come from being part of a large internationally known British Group. Applications quoting job reference No. 308 should be addressed to Divisional Personnel Manager, Research Laboratories,

FISONS

Fisons Pharmaceuticals Limited,
Bakewell Road,
Loughborough,
Leics.

(814)

The STATE UNIVERSITY of LEIDEN

invites applications or suggestions for a

FULL PROFESSOR in GENETICS

He will be charged with lectures and courses in general genetics and with the direction of the research in population genetics and development genetics.

Applications, including curriculum vitae and references or suggestions, should be sent as soon as possible to: The Faculty of Science, University of Leiden, Rapenburg 46, Leiden, The Netherlands.

(836)

THE UNIVERSITY COLLEGE OF WALES

ABERYSTWYTH

DEPARTMENT OF BOTANY

Applications are invited for the post of LECTURER. Candidates should have a special interest in Physiological Ecology (preferably Microclimatology) and/or Whole Plant Physiology.

Application forms and further particulars are available from the Registrar, quoting reference LEB/3, and should be returned as soon as possible. (873)

TECHNICIAN

For the Department of Microbiology and North West London Public Health Laboratory, Central Middlesex Hospital, Park Royal, London, N.W.10. This post offers progressive training in both public health and clinical bacteriology, providing excellent opportunity for further study in microbiology.

Applications to Dr. C. E. D. Taylor, Director, at the above address. (874)

IMPERIAL COLLEGE
(Physics Department)
LECTURER OR RESEARCH ASSISTANT

A vacancy exists for a Lecturer or Research Assistant in Solid State Theory in the Physics Department at Imperial College (for Lectureship some post-doctoral experience would be required). The person appointed will work in a small but growing solid state theory group with strong links with the experimental group working in superconductivity and magnetism.

Salary will be in the range £1,240 by £115 to £2,850 plus £60 London Allowance, depending upon age and experience.

Applications, with full details and names of two referees to Professor B. R. Coles, Physics Department, Imperial College, London, S.W.7, by March 10, 1970.

(736)

INSTITUTE OF OPHTHALMOLOGY
(UNIVERSITY OF LONDON)
JUDD STREET, LONDON, W.C.1

DEPARTMENT OF PATHOLOGY

An opportunity now occurs for an experienced TECHNICIAN to join the research staff of the above Department in studying the retinal vasculature in health and disease.

Preferably candidates should have at least O.N.C., I.M.L.T., or equivalent. A knowledge of immunology, histology or animal experimentation would be an advantage.

Please apply in writing, stating age, sex, qualifications, experience, and giving the names of two referees to The Director, Department of Pathology, at the above address. (887)

AGRICULTURAL RESEARCH COUNCIL

FOOD RESEARCH INSTITUTE

An industrial grant has been made to the Food Research Institute for research on the chemical nature of the compounds responsible for the fishy taint in poultry meat and on the relationship of the compounds to the lipids in the diet. The grant, which is for two years in the first instance and which may be extended for a third year, is for the support of one POST-DOCTORAL RESEARCH WORKER and an assistant.

Applications are invited from CHEMISTS/BIOCHEMISTS with post-doctoral experience to work on this subject. A suitable candidate would be a lipid chemist with experience of instrumental analysis of fatty acid oxidation products. The successful candidate will work in the lipid section of the Chemistry Division.

Salary will be according to experience in the £1,600 to £2,000 per annum range. Superannuation under F.S.S.U.

Further information may be obtained from the Secretary, A.R.C. Food Research Institute, Colney Lane, Norwich NOR 7OF, to whom applications, in the form of a curriculum vitae, a bibliography and the names of three referees, should be sent by March 1, 1970. (810)

THE SCHOOL OF PHARMACY

UNIVERSITY OF LONDON

LECTURESHIP IN PHARMACOGNOSY

Applications are invited from suitably qualified persons for an appointment as a Lecturer in the Department of Pharmacognosy, commencing on September 1, 1970. The successful candidate will be expected to carry out teaching and personal research in a field acceptable to the Head of the Department.

Salary on an incremental scale from £1,240 per annum to £2,850 per annum, plus London Allowance of £60 per annum. Point of entry on the scale will depend on qualifications and experience. Membership of F.S.S.U. and normal University terms of service apply to this appointment.

Applications in the form of a letter accompanied by a curriculum vitae and names of at least two persons to whom references may be made, should be submitted not later than March 31, 1970, to the Clerk to the Council, The School of Pharmacy, University of London, 29/39 Brunswick Square, London, W.C.1, from whom further details may be obtained. (877)

GOVERNMENT OF NORTHERN IRELAND
MINISTRY OF AGRICULTURE

Parasitologist

Applications are invited for a permanent and pensionable post in the Veterinary Research Laboratories, Stormont, Belfast.

The laboratories are of recent construction and are equipped for a wide range of techniques. A total of 120 professional and technical staff are employed in the major disciplines of pathology, bacteriology, virology, biochemistry and parasitology. The work of the laboratories consists of routine diagnosis and research on diseases of farm animals.

QUALIFICATIONS: MRCVS or veterinary degree. Postgraduate experience in parasitology is essential.

DUTIES: The officer appointed will take charge of the Parasitology Department and will be required to provide a routine diagnostic service but the major portion of his time will be available for research on parasitic diseases of farm animals.

SALARY SCALE: Senior Veterinary Research Officer (Grade II) £3,043–£3,883

Veterinary Research Officer (Grade I) £2,349–£2,846

Grading and starting salary will be related to qualifications and experience. Application forms and further information, obtainable from the Secretary, Civil Service Commission, 45 Chichester Street, Belfast, BT1 4JU, must be returned by March 23, 1970. Please quote SB.37/70/64.

(830)

SALMON AND FRESHWATER FISHERIES LABORATORY

Ministry of Agriculture, Fisheries and Food
Fish Pathology Unit, Weymouth, Dorset.

VIROLOGIST/BACTERIOLOGIST (graded Experimental Officer/Assistant Experimental Officer) required for research on viral and bacterial diseases of fish with special interest in those affecting salmon. This entirely new field of work offers the opportunity for applied research in relation to a specific and urgent problem the solution of which is of considerable economic importance.

This post will involve mainly laboratory work, though some field work will also be undertaken, and there will be collaboration with other Laboratories in Scotland and Ireland working on similar problems.

Qualifications: Degree HNC or equivalent in appropriate subject. It is desirable that candidates should be **either** Fellows of the Institute of Medical Laboratory Technology with experience in virological techniques and tissue culture methods, **or** Associates of the Institute, with Final Virology, preferably with some experience in bacteriology: or that they should have a degree in Microbiology. Age: AEO under 28. EO normally 26–30. Non-contributory pension; prospects of promotion.

Salary: AEO £940 (at 22) to £1,208 (at 26 or over) to £1,454; EO £1,590 to £2,006.

APPLICATION FORMS are obtainable by writing to the Civil Service Commission, Savile Row, London W1X 2AA, or by telephoning 01-734 6010, ext. 229 (after 5.30 p.m. 01-734 6464 "Ansafone" service). **Please quote S/94/MAF/8.**

Closing date March 17, 1970.

(812)

UNIVERSITY OF NEWCASTLE UPON TYNE

SECOND CHAIR OF ELECTRICAL ENGINEERING

APPLICATIONS are invited for the newy created Second Chair within the Department of Electrical and Electronic Engineering. Candidates should have a special interest in the field of light electrical engineering or in electrical materials technology. The successful candidate will be required to take up duty at a date to be arranged as early as possible in the academic year 1970/71. Salary in accordance with the professorial scale (£3,780 by £140 to £4,480 per annum) with membership of F.S.S.U.

Further particulars may be obtained from the Registrar of the University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU, with whom applications (15 copies), giving the names of not more than three referees, must be lodged not later than March 3, 1970. (678)

THE UNIVERSITY OF LEEDS

Applications are invited for posts in the following Departments:

HOULDSWORTH SCHOOL OF APPLIED SCIENCE

RESEARCH STUDENTSHIPS

for a variety of interesting investigations into the preparation, structure and properties of high-temperature non-metallic materials, including oxides, silicon nitride, silicates (both crystalline and vitreous), and chrome-magnesite and other refractories. The research areas include mechanical properties, electrical conduction, optical transmission, kinetics and mechanisms of chemical reactions, chemical aspects of friction, crystal structure and microstructure, computer work with mathematical models and fabrication science of ceramics and glasses. Grants will be in accordance with S.R.C. rates.

Applications (two copies), stating age, education, qualifications and experience, together with the names of three referees, should reach Professor J. P. Roberts, Department of Ceramics, The University, Leeds, LS2 9JT (from whom further particulars may be obtained) as soon as possible.

MECHANICAL ENGINEERING

RESEARCH MATHEMATICIAN or ENGINEER

on the new phase of the hydrofoil research programme planned for the coming three years. Applicants should possess a good honours degree in applied mathematics, engineering or physics and preferably be experienced in hydrodynamic theory and experimentation. In contact also with the Applied Mathematics Department of the University, the appointment would aim at further improvement of understanding of ventilating and cavitating flows round hydrofoil systems. Salary dependent on age and qualifications, which could rise to £2,235 a year.

Applications (one copy), with names of at least two referees, should be addressed to **Professor B. N. Cole, The University, Leeds, LS2 9JT** (from whom further particulars may be obtained). (851)

POSTDOCTORAL RESEARCH POSITION available as soon as possible. Minimum salary $US7,000. Temple University School of Medicine, Philadelphia, Pa., U.S.A. Research of immunological tolerance and immunological aspects of myeloma tumours.—Further details from Dr. H. Francis Havas, Department of Microbiology, Temple University School of Medicine, 3400 N. Broad Street, Philadelphia, Pa., U.S.A. (621)

NATURE

MACMILLAN (JOURNALS) LTD

No. 5236 SATURDAY MARCH 7 1970 Vol. 225

CONTENTS

POLLUTION IS ALWAYS WITH US—PREMATURE BIRTH OF TEST TUBE BABY—WHAT ECLIPSES ARE WORTH 885

Old World—Battle for Atlantic Salmon—Conservation—Science Research Council — Computers — Dogs — Technology — Soviet Union — Physics Exhibition — Chemical Industry—Countryside—Natural History—Parliament in Britain—Miscellaneous Intelligence 887

TRANSITION TO THE SEVENTIES 892

New World—DOWNBEAT AT THE OFFICE OF NAVAL RESEARCH—Molecular Biology—Oceanography—Labour Market—High Energy Physics 895

News and Views—MYSTERIOUS SATELLITES—Minerals—Solid State—Band Structure—Variable Stars—Hearing—Land Management—Antibiotics—Protein Synthesis—Amphibia 899

HIGH-ENERGY GAMMA-RAY ASTRONOMY by G. G. Fazio 905

CHROMOSOMAL AND NUCLEAR LOCATION OF MOUSE SATELLITE DNA IN INDIVIDUAL CELLS by K. W. Jones 912

VARIATIONS IN THE DNA FROM TWO RODENT FAMILIES (CRICETIDAE AND MURIDAE) by W. Hennig and P. M. B. Walker 915

POSITION OF THE INITIATOR AND PEPTIDYL SITES IN THE *E. coli* RIBOSOME by Ernst Kuechler and Alexander Rich 920

ORIGIN AND DYNAMICAL BEHAVIOUR OF THERMAL PROTONS IN THE VENUSIAN IONOSPHERE by P. M. Banks and W. I. Axford 924

Letters to the Editor (see overleaf) 927

Book Reviews by John Vaizey, C. S. Whewell, Barry J. Garner, R. C. Jennison, R. S. Harwood, R. M. Shackleton, M. K. Wallis, R. W. Carter, G. Lowe, N. Shaw, D. C. Smith and G. A. Gresham 973

Obituaries—Dr G. D. Greville; Dr I. A. Galloway 977

Correspondence—Sternglass's Assumptions by Patricia J. Lindop and J. Rotblat; Information Explosion by F. Bruce Sanford; Keep Off the Grass by F. Bergel; SI Units by David Davies 979

University News—Appointments—Announcements—International Meetings—Sabbatical Itinerants 980

Nature in North America: The *Nature* office in Washington DC has opened and contributors in the United States and Canada are invited to submit manuscripts for publication either to the address given below or to the present London office. The intention is that each office will be able to answer inquiries about all manuscripts and other matters. This is one of several steps by which it is hoped to provide a better service for readers and contributors.

London Office
4 Little Essex Street,
London WC2.
01-836-6633

Washington Office
711 National Press Building,
Washington DC 20004
(202) 737-2355

(Continued overleaf)

Unicam SP1800 Ultraviolet Spectrophotometer
Power
Deuterium Lamp
Scale divisions
Absorbance or Concentration
Meter Range
Zero
Concentration
Unicam SP 8000 Ultraviolet Spectrophotometer

Unicam UV Spectrophotometers make light work

What does this mean to you? In research, results you can trust and repeat. In routine applications, error-free operation by the least experienced member of your staff.

When you come to Pye Unicam, even the selection of your UV spectrophotometer is simple. There is a choice of instruments broad enough to meet your every requirement and to match your budget, with a staff of experienced application specialists to back them up.

Just look at what is available . . . **SP500 Series 2** and **SP600 Series 2** quantitative single beam spectrophotometers, **SP700** for research, **SP1800** for low-cost double-beam operation, **SP3000** automatic digital instrument, **SP8000** for the ultimate in flexibility, and th **SP1300 Series 2** low-cost colorimeter.

Write or 'phone for free information today. That's simple too, isn't it.

Pye Unicam Ltd
York Street Cambridge, CB1 2PX, England
Telephone (0223) 58866 Telex 81215

1966 1969

PYE UNICAM

LETTERS TO THE EDITOR

Page

PHYSICAL SCIENCES

Quasar redshifts—Peak at $z=0{\cdot}060$ significant?—WESSELINK (Yale) 927
Comets — Fluorescent excitation of emission spectra —MYER and NICHOLLS (York University, Toronto) 928
Transient lunar phenomena — From glow discharges in dust clouds ?—MILLS 929
Glasses—Dielectric and anelastic effects in inorganic glasses—HALE (NPL) 930
Maxwell's equations—Charge moving with the speed of light—BONNOR (London) 932
Spectroscopy—Direct detection of hydrogen atoms in atmospheric flame—BENNETT, MILE and SUMMERS (Shell Research Limited) 932
Catalysis—Catalytic inhibition by adsorbed oxygen —GASSER and VAIGHT (Oxford) 933
Drag reduction—Effect of polymeric drag reducing additives on pressure loss across transitions—PISOLKAR (Hendon College of Technology) 936
Radiocarbon dating — The ages of mortars — BAXTER and WALTON (Glasgow) 937

BIOLOGICAL SCIENCES

Haptoglobin—A sequence possibly showing relationship with serine proteases—BARNETT, LEE and BOWMAN (Texas) 938
Haemoglobin—Unstable variant with acidic residue in haem pocket — WHITE, BRAIN, LORKIN, LEHMANN (MRC) and SMITH (London) . . . 939
β-Lipoproteins—Polymorphism in pigs—RAPACZ, GRUMMER, HASLER and SHACKELFORD (Wisconsin) 941
Vitamin B_{12}—Organo-cobalt compounds as analogues —STAGNI, DE BERNARD, COSTA and MESTRONI (Trieste) 942
Antitumour polysaccharide—Side chain cleavage transforms natural glucan into active product—CHIHARA, HAMURO, MAEDA, ARAI and FUKUOKA (National Cancer Centre, Tokyo) 943
Myxovirus RNAs—Presence of plus and minus strands in virion RNAs—ROBINSON (Stanford) . 944
Virus particles—Three RNA viruses isolated from *Penicillium stoloniferum* — BUCK and KEMPSON-JONES (London) 945
RNA-DNA hybridization—Autoradiographic detection of hybrids in tissue — BUONGIORNO-NARDELLI and AMALDI (Rome) 946
DNA methylation—5-Methylcytosine in the DNA of various animal species and tissues—VANYUSHIN, TKACHEVA and BELOZERSKY (Moscow State) . 948
Lymphomas—Tumours in mice attributed to murine leukaemia virus — LEVY and HUEBNER (US National Institutes of Health) 949
Immunology—Histocompatibility and human placentation—SEPPÄLÄ and TOLONEN (Helsinki) . . 950
Isoantigen typing—Heparin has no effect on immunological reactions of human lymphocytes — OREN, HERBERMAN and ROGENTINE (US National Institutes of Health) 951
L-Asparaginase—Inhibition of primary antibody response in C3H mice—PRAGER and DERR (Texas) 952
Carcinogenesis — Pyridoxine starvation and liver cancer — FOY, KONDI (Wellcome Trust Research Laboratories, Nairobi), LINSELL (International Agency for Research on Cancer, Nairobi), PARKER (University College, Nairobi) and SIZARET (International Agency for Research on Cancer, Lyon) . 952
Hepatitis—Failure to detect antigen—MOSLEY (US National Communicable Disease Center), BARKER, SHULMAN (US National Institutes of Health) and HATCH (US National Communicable Disease Center) 953
Lysosomes—Stabilized by a substance in serum of pregnant females—HEMPEL, FERNANDEZ and PERSELLIN (Texas) 955
Synovial fluid—Under pressure the polymer separates from the plasma—WALKER, SIKORSKI, DOWSON, LONGFIELD and WRIGHT (Leeds) . . . 956
Trabecular bone — Scanning electron microscope results—DYSON, JACKSON and WHITEHOUSE (AERE) 957
Cyclic behaviour patterns — Periodicity in human oral intake — OSWALD, MERRINGTON (Royal Edinburgh Hospital) and LEWIS (Napier College of Science and Technology) 959
Central Nervous System — Effects of 6-hydroxydopamine — EVETTS, URETSKY, IVERSEN and IVERSEN (Cambridge) 961
Parkinson's disease—Alleviation by chloramphenicol supports hypothesis of protein synthesis abnormality—STEFANIS and ISSIDORIDES (Athens National) 962
Learning — Retention through metamorphosis in mealworms — BORSELLINO, PIERANTONI and SCHIETI-CAVAZZA (Genoa) 963
Gregarines—Effect on growth of desert locust—HARRY (Birmingham) 964
Invertebrate behaviour—Cycloheximide and actinomycin *D* decrease the ability of headless cockroaches to acquire avoidance behaviour — GLASSMAN, HENDERSON, CORDLE, MOON and WILSON (North Carolina) 967
Insect immunology—Production of a lytic substance by insect cells during culture *in vitro*—LANDUREAU and JOLLÈS (Paris) 968
Plant pigments—Identification in the common blue butterfly—FELTWELL and VALADON (London) . 969
Aphids—Lateral abdominal tubercles in the peach-potato aphid may be associated with insecticide excretion—EASTHOP (British Museum (Natural History)) and BANKS (Rothamsted Experimental Station) 970
Vegetative hybridity—Investigated in potato blight fungus — MALCOLMSON (Scottish Plant Breeding Station) 971

GENERAL

Logic of discovery—Abstraction and generalization not beyond analysis—MELTZER (Edinburgh) . . 972

The titles on this page are prepared by the staff of *Nature* on the basis of the titles submitted by authors which appear in full on the pages indicated

The History of Difco Quality Control...

and what it means to you today

Difco was developed by producing better products through control of quality.
Now there is a history of more than seventy years of dedication to accuracy and dependability, specialized equipment, exacting materials control.
It's a history of a unique kind of control. Difco tests each ingredient before it is accepted for use. Testing throughout preparation assures compatibility.
The finished product is then tested for dependable performance under the conditions for which the product is intended. Result? Perfectly standardized products that give reproducible results every time.
What does it mean to you?
Assured reliability. Savings in time and money.
Dependable microbiological results in your laboratory.

DIFCO
B&T

B&T are the sole concessionaire for DIFCO products.
A complete line of microbiological reagents and media.

Baird and Tatlock, *Postal Address:* P.O. Box 1, Romford, RM1 1HA.
Situated at: Freshwater Road, Chadwell Heath, Essex.
Telephone No: 01-590 7700 (25 lines). *Telex:* 24225 Bairhop. *Cables:* Burette Romford.

TAS.DO.A

NATURE Volume 225
MARCH 7, 1970

Pollution is Always with Us

ONE cynical but not necessarily misguided view of the wave of concern about the condition of the environment is that it has served marvellously to concentrate the interest of university students on issues other than student power or the supposed lack of it. A year ago, the young people at San José State College in California, who buried a brand new motor car in a hole in the ground as a symbol of their devotion to clean air, might well have been scrawling obscenities on the walls of university administration buildings or pelting administrators themselves with various kinds of food. It is true, of course, that the young people's passion may simply be another way of protesting that older generations have made a mess of things, but even this is probably irrelevant. And the fact remains that young people are not alone in their devotion to environmental causes. Whatever public policies eventually emerge from all the present excitement, it is an important question to know why there has been such a rapid growth of concern about the environment.

The notion that the photographs of the Earth returned by the Apollo astronauts have served to emphasize what a fragile place the Earth must be has been widely canvassed, most eloquently perhaps by Professor Fred Hoyle at the Houston conference about the Moon in January. No doubt there is something in this, although the Apollo photographs have probably done more to rationalize and make explicit a tendency which must already have been well under way. Indeed, both in western Europe and the United States, the origins of the present concern about the environment have been evident for several years. The smog over Los Angeles became in the sixties what the cloud of smoke over Pittsburgh used to be in the days before clean air legislation, and served as a focus for legislation about motor vehicle exhausts. In Britain, the condition of the Thames became first a public scandal and then an incentive for the kinds of administrative actions which have now helped to make the river almost tolerable. In the same way, the Rhine has been the chief stimulus to the development of regulations for international waterways. It is, in circumstances like these, something of a surprise that public anxiety of the kind which has now led to the creation of a standing Royal Commission in Britain, a Federal Council on the Environment in the United States, and a permanent organization within OECD should have come to a head in such a dramatic way. Is it that there really is a crisis around the corner ?

The importance of a level headed appraisal of the threats to the environment is that exaggeration either way is likely to be damaging. This, for example, is what seems to have happened with the hasty ban on cyclamates at the end of 1969—exaggerated solicitude for the environment has engendered disbelief. In exactly the same way, the talk now widely to be heard of "environmental crisis" is likely to do more harm than good. The truth is that the environment is a good deal better now than it used to be for those generations which were decimated by tuberculosis and even scarlet fever as recently as the 1930s. And even now, a great many populations live in circumstances in which the most serious scourges—malaria, for example—must be fought with means such as DDT which are now considered to be polluting in Britain, the United States, Canada and Sweden. One man's potential crisis is, in other words, another's potential salvation. Exaggeration of the threat to the environment, however valuable as a means of concentrating public energy, will therefore smack of what used to be called "neo-colonialism" if advanced societies are not careful. Worse still, too simple a view of what the danger is may seriously hamper attempts to get the cleanest environment for the least money.

In these circumstances, there are a great many things which responsible societies could do to improve their environment by simple and comparatively cheap devices. In Britain, the Clean Air Acts have done a lot to improve conditions in the large cities, even though their enforcement leaves a lot to be desired. There is obviously plenty of scope for clean water legislation. On the mainland of Europe and in the United States, geographical size has complicated many problems, but it is still a mystery that opportunities for straightforward remedial legislation are so consistently ignored. In the United States, for example, raw sewage is still discharged into open rivers either in defiance of regulations or with impunity. Although research is at once necessary and interesting, there is no reason why advanced societies should not be able to make respectable progress towards a more carefully controlled environment by the use of simple administrative devices. The international problem is more serious, if only because international agreements are inherently hard to achieve. Governments will doubtless try to negotiate, for cases such as the Torrey Canyon wrecking, agreements similar to those which regulate insurance against radioactive accidents at nuclear power plants. But issues like that are bound to take years to resolve. The institutions, both national and international, which will eventually be needed for regulating the environment will similarly be slow to emerge. This is why it would be best if some of the red hot passion about the environmental crisis could be converted into a more durable form even if one result might be a resurgence of the interest of students in conditions on the campus.

Premature Birth of Test Tube Baby

THE test tube baby has struck again. Several years, perhaps even decades, in advance of it being even theoretically possible to grow mammalian embryos to full term outside the uterus, the test tube baby has been forcibly delivered and morally agonized over. "The era of the test tube baby has begun" was the annunciation made by one newspaper to its readers; "One step nearer to the super race" was the *Nunc dimittis* of another. It seemed to be generally felt that the test tube baby had arrived trailing clouds of thorny ethical problems in its wake.

The genesis of the affair was the announcement last week on the BBC television programme *Horizon* that a technique of *in vitro* fertilization was shortly to be attempted at Oldham General Hospital as a treatment for infertile patients. The procedure was first outlined a year ago by Edwards, Bavister and Steptoe (*Nature*, **221**, 632; 1969). It involves removing oocytes from a woman's follicles, fertilizing them with her husband's sperm and replacing an embryo in the uterus for it to implant in the usual manner. By all accounts the technique is a courageous and imaginative way of overcoming a blocked Fallopian tube, which is the commonest single cause of infertility in women.

What has all this to do with the test tube baby? In terms of scientific fact, almost nothing at all. The test tube baby, as this phrase is usually understood, refers to the growing of a human embryo to full term outside the body and the chief obstacle to this feat (not that anybody has proclaimed it as a goal) is the formidable problem of maintaining the embryo after the stage at which it would normally implant in the uterus. The Oldham procedure concerns only the pre-implantation embryo which, except in the trivial sense that fertilization is carried out *in vitro*, can hardly be equated with the test tube baby. Moreover, it is difficult to see that the wastage of embryos occasioned by the procedure raises moral problems any knottier than those to do with the IUCD, a device that probably prevents the embryo from implanting in the womb.

What then was all the fuss about? Part of the reason, it seems, was that the implications of the Oldham procedure were genuinely misunderstood in some quarters, and publicists with scientific doctorates who spoke of genetic engineering did not help to clarify the issue. It may also be a side-effect of the present concern about pollution that people are prepared almost by reflex to equate the new knowledge with new dangers. Public interest in scientific and technical developments is all to the good, but it would be a pity if a wail of premature jeremiads were allowed to drown the occasional eurekas before they had a chance to be heard. This, of course, is one of the dangers prompted by the overreaction to the Oldham procedure; the other is the obvious peril of crying wolf too soon and too often.

A curious feature of the public debate is that the letter writing segment of the public, at least, seemed to believe that human life was about to be created from nothing in the test tube. For example, a correspondent in *The Times* voiced the fear that "The ability of scientists to develop the technique of creating life in a test tube is so serious that I feel human beings should be given the opportunity to express their views on whether or not this line of research should be pursued. . . . Personally I find the idea of creating life at man's will terrifying". These are indeed dark atavistic fears which have been nurtured, perhaps, by the views of Dr Edmund Leach that scientists have usurped the creative powers and should assume the moral responsibilities formerly attributed to gods. Whatever the merit of Dr Leach's thesis, those who are engaged in research that is at all liable to be misinterpreted will doubtless take the present episode as a warning of the misunderstandings that can arise, particularly if the true facts are not readily available from authoritative sources. There is always the danger that lack of information or misinformation may convert legitimate public concern about new knowledge into a paranoia that impedes research.

What Eclipses are Worth

THE eclipse on Saturday in North America has brought to the surface a number of important and interesting issues in the organization of research. Although this week's phenomenon will by sheer good luck pass near several of the best equipped laboratories for observations of this kind, it is being followed with a programme of observation which is modest compared with the effort that would have been spent on it ten years ago.

Indeed, the National Science Foundation, which has coordinated the programme of observation, has no separate allocation of money for this work, which is being financed out of the ordinary budget for solar and terrestrial physics. One way and another, the foundation is likely to spend a good deal less than $500,000 on its own work. It is improbable that the total cost of all the work being carried out will exceed $3 million. A good many worthwhile plans have been abandoned for lack of money. The most spectacular casualty is the scheme which NASA had been canvassing for flying a supersonic aircraft along the track of totality, thus extending the duration of observation from 3 to 90 min, so allowing a longer study of motion in the corona. The cost of modifying the aircraft would have been $10 million.

But how much is it worth spending to observe an eclipse with extra care? The most important objective is of course the observation of the corona and the chance of filling in gaps in present understanding of what happens in the high atmosphere of the Sun. A century ago, when *Nature* was constantly at loggerheads with the British government for what then seemed to be parsimony in supporting observations of eclipses, an opportunity such as that on Saturday to observe such a favourable eclipse would have been invaluable. It is only fair to say that times have changed a little. For one thing, the high atmosphere of the Sun is now much better understood and there is every chance that in time the data which are accumulating about the solar wind will support accurate and complete inferences about the Sun's outer atmosphere.

But how is it possible to give this chance a numerical value, in dollars or pounds? Unhappily, the yardsticks which are available for comparison are probably misleading. The fact that the first Apollo applications satellite, with its steerable solar telescope, will be equipped for direct observation of the corona is hardly any guide—that is a much more expensive operation than any eclipse observer would expect to participate in. In the long run, in any case, it may well be that eclipses are to be valued most as occasions when people make collective and simultaneous observations in a way that evokes some of the old spirit of the international geophysical year (which is why nobody should weep too much if it is cloudy in Mexico on Saturday). But this is why it is a pity that this week's occasion is not more fully equipped. The real worry will be to make sure that the next eclipse, in 1972, is properly observed in the parts of Africa from which it will be visible.

100 Years Ago

M. Radau falls into the schoolboy blunder of making the *ratio between the friction and pressure constant throughout the motion*, confounding the actual friction with its limiting maximum value! It is, indeed, surprising that such a perversion of the facts of the case should have found insertion in a serious journal, such as that published by the Ecole Normale Supérieure, and I might fairly have expected from M. Radau the courtesy habitual with his adopted countrymen, of applying to me for information on anything in my paper which might have appeared to him obscure or erroneous, before rushing into print with such a *mare's nest.*

In a future number of your esteemed journal (as time at present fails me) I propose to show how, by the simplest contrivance in the world, a downright material top of ellipsoidal form may be actually made to roll, with its centre fixed, on a fixed plane and so exhibit to the eye the surprising spectacle of a motion precisely identical *in time*, as well as in its successive displacements of *position*, with that of a body, turning round a fixed centre, but otherwise absolutely unconstrained.

From a letter from the mathematician J. J. Sylvester whose conclusions about the motions of a free rotating body had been criticized by M. Radau. From Nature, **1**, 482, *March* 10, 1870.

OLD WORLD

Battle for Atlantic Salmon

THE antipathy of Denmark, Sweden and West Germany to a ban on salmon fishing in international waters of the North Atlantic has now prompted Mr Harold Wilson to send a personal message to their heads of government—an appeal the Danish Premier has already rejected. Last summer the delegates of these countries were the only people to oppose the call for a ban on salmon fishing made at meetings of the North East and North West Atlantic Fisheries Commissions, which have no powers to enforce their recommendations. The present disagreement has been caused by the rapidly growing exploitation of salmon on their feeding grounds on the high seas, which several countries (especially Britain and Canada) believe is frustrating their efforts to breed and conserve salmon for coastal and freshwater fishing.

There is a strong case for countries in whose rivers the salmon breed to have a prior claim on the harvest. In Britain, for example, more than £1 million a year is spent on salmon protection and conservation, and on top of this sum there is a large "invisible" expenditure by industry in terms of effluent treatment and by power stations in keeping young migrating fish out of their water intakes. Fishing is also strictly controlled by by-laws and close seasons, and the number of licences available for fishing in estuaries can be restricted as a conservation measure within river authority areas, while only about a dozen licences have been issued for coastal fishing elsewhere in England and Wales. Coastal fishing off Scotland is prohibited. In Canada and the state of Maine, according to Sir Hugh Mackenzie, director of the Atlantic Salmon Research Trust, the annual cost of protecting salmon runs to about $5 million.

On the other hand, Denmark, where there are only a very few rivers in which salmon are found, gets the lion's share of the high seas catch. The chief fishing area is off the west coast of Greenland, where drift netting began in 1965. By 1967 seven or eight boats were fishing there, in 1968 eighteen, and in 1969 at least thirty-seven. Eighteen of the fishing vessels came from Denmark, six from the Danish-owned Faroes, eleven from Norway and two from Sweden. Table 1 (top row) shows the growth in catches since 1965. But claims that the coastal fisheries of Greenland or Britain are suffering as a result are not directly supported by the available figures. In Greenland a drop in the 1968 catch was reversed in 1969 (Table 1, second row); for England, Wales and Scotland there is so far no clear effect on the total catches that can be

Table 1. SALMON CATCHES, GREENLAND AND GREAT BRITAIN (1965–69), IN METRIC TONS

	1965	1966	1967	1968	1969
Greenland (international waters)	36	119	318	548	1,300*
Greenland (coastal fishery)	825	1,251	1,283	579	900*
Great Britain (coastal and freshwater)	1,922	2,011	2,553	1,845*	2,315*

* Provisional figure.

All figures from the Ministry of Agriculture, Fisheries and Food.

attributed to the high seas fisheries (Table 1, third row), although reductions in the number of salmon found in certain rivers are hard to explain otherwise.

The justification for a ban on salmon fishing in the high seas seems to be a matter of principle rather than fact. German fishermen, who fish for salmon to the north of Norway, are opposing a ban on the grounds that it would amount to a restriction of the freedom to fish in the open sea. Abolitionists contend that because the salmon is not a "free" fish, arguments of this kind are irrelevant. They also have the support of people who fear that the salmon may be overfished if restrictions are not forthcoming. Sir Hugh Mackenzie warned that "the situation could become calamitous very quickly", and there is a body of opinion believing that it would be prudent to call a halt until more is known.

Britain, Canada and Denmark have been running a research programme in the West Greenland area for five years. Salmon caught in the coastal and offshore regions are being tagged to give information about the size of the exploited population, the proportions returning to home rivers, and the increase in weight during the return journey. But it will take some time to collect enough data, for the chances of recapturing tagged fish are small.

The next attempt to protect the inshore fisheries will presumably be made at the meeting later this month of the ministers for fisheries of Denmark, Sweden and Norway. Denmark may be willing to make concessions if international opinion demands them—for example, larger net meshes could be used, or there could be a close season. But Sir Hugh Mackenzie has pointed out that there is, in effect, an automatic close season, for the salmon are only fished off Greenland from August to November; he believes that anything short of a complete ban will be useless in preventing further increases in exploitation. The Fisheries Commissions in which the ban was originally recommended meet again in May and June.

CONSERVATION

No Votes in European Sewage

THERE have been enough conferences encouraging the breast-beating type of conservationist. Their cries of wolf are already having an immunizing effect, and the danger is that the topic of pollution will have become a bore before any worthwhile action has got under way.

For this reason the Council of Europe's February conference at Strasbourg on "The Management of the Environment in Tomorrow's Europe" is to be welcomed. This was an intergovernmental conference and its object was action. Three hundred participants came from 27 countries (17 Council of Europe countries, plus 11 more with observer status). Members were drawn from the people who actually structure the European environment from one decade to another—planners, government and local government servants, industrialists, landowners, politicians. Four hefty reports were tabled and discussed, on urbanization, industry, leisure, farming and forestry, each written by a single rapporteur. Each concluded with a list of conclusions and recommendations. These provided the groundwork for a declaration that was drawn up at the end of the conference and was accepted unanimously. It contained 30 policy guidelines for Europe's decision makers.

A conference of senior government ministers is called for by the parent body, the Council of Europe, "as a matter of urgency"—that is, it should meet within a year. This will provide an opportunity to put European governments on the spot as to what they are actually doing or proposing to do about the policies and measures agreed last month by their representatives.

The introductory declaration of the document clarifies the issues:

"(1) Rational use and management of the environment must have high priority in national government policy and be adequately financed, and clear ministerial responsibility must be established for the planning and use of land and other natural resources and the conservation of nature.

"(2) Policies should be strengthened or introduced to control pollution of air, water and soil, and internationally agreed standards for those purposes should be devised as soon as possible.

"(3) Legislation and regulations introduced to safeguard the environment and its quality should be harmonized to the extent necessary at European level."

Five points are referred for the attention of the European ministerial conference in particular. The numerous intergovernmental bodies nibbling at these problems need to be sorted out; for instance, both NATO and OECD are flirting with pollution and environmental matters. Neither seems appropriate, especially in view of the interest in the Strasbourg plans displayed by East European countries, three of which took part in the conference.

Point two is that there is a need for internationally agreed standards to be adopted for European industry, especially in the sphere of pesticides, car exhausts, the noise and pollution products of aircraft engines. Steps should also be taken to "harmonize" national legislation relating to the environment.

The two final points for ministerial attention are a proposal for "a political authority at European level" with the task of supervising and guiding the management of the European natural environment, if possible irrespective of political frontiers; and a proposal (sponsored by the French minister of agriculture) for a European fund to fight pollution.

The most significant, perhaps, of the guidelines for national governments is an imperative to establish an "infrastructure" (as they say) and a chain of command. Otherwise, most of the national guidelines relate to the use and treatment of land—rational use, reclamation and re-use of derelict land, unspoilt coasts to stay unspoilt and spoilt coasts improved, upkeep of landscape, classification of all land for its most appropriate use, and so on. Five guidelines are directed at industry. Here cooperation is emphasized and the point made that both sides of industry should appreciate that "the rational use of natural resources is in their long-term interest".

This is one aspect of a theme that was emphasized by the chairman of the Strasbourg conference, Mr R. E. Boote of the Nature Conservancy. This is, that the individual pays, as consumer, taxpayer and ratepayer, for despoiling the environment and for pollution.

Perhaps the underlying concept of the Strasbourg conference is in line with another of Boote's main preoccupations, that as long as society gives certain terms of reference, these get fulfilled. "The need now is for society to get these terms of reference changed." Most politicians would agree with this, though what they are going to do about it remains very much an open question in a European and indeed a British context. The assumption that there are no votes in sewage seems still to hold both government and local government in thrall.

SCIENCE RESEARCH COUNCIL

Boost for Ion Implantation

THE bombardment of solid surfaces by ions is a technique which is providing new devices in electronics and a new tool for physicists and chemists as well as being a fascinating phenomenon in its own right. An admirably comprehensive survey of British work in the field has just been published by the Science Research Council (*Ion Implantation Panel Report*, available from the SRC free of charge). The report recommends that the universities should receive £200,000 of grants over the next two years, but that thereafter support should return to its present level of about £60,000 a year.

Ion implantation has developed over the past ten years, encouraged by the realization that because of the phenomenon of channelling, ions penetrate deeper into the lattice along certain preferred directions than along random directions. Although comparatively few people are deeply concerned with ion implantation, the subject is of marginal interest to many more. The report necessarily describes a hotch-potch of work on the physics of ion implantation, its use in making new materials and electronic devices, and the development of implantation equipment. The thinking behind the two-year boost in expenditure is to identify the most promising areas for university research. The development of new electronic devices based on the alteration of the characteristics of materials by ion bombardment is best left to government and industry, for example, but there is room for university work to improve implantation machines—ion sources are a particular problem—and on future applications of ion beams in the electronics industry.

It ought to be possible, for instance, to replace the conventional photographic and chemical method of manufacturing microcircuits by using electron beams and vacuum evaporation to deposit the masks, and ion beams for doping and mask removal, in a process that would be amenable to computer control. It would be more difficult but nevertheless feasible to dispense with the masks and use the steerable property of an ion beam to "write" the circuit. There is also the technique of machining with ion beams, and two companies in the United States are using argon ion beams for the figuring of topical components.

The report recommends that Professor M. W. Thompson's group at the University of Sussex should remain the chief group investigating the fundamental processes of ion implantation. Equipment for implantation is comparatively expensive—typically £30,000 for a 200 keV machine, for example—and few universities have accelerators designed for ion implantation. The report recommends that the SRC should add to the equipment already available at the universities of Salford, Surrey and Sussex, which will become the chief centres, on condition that the beams will be available to other groups with particular expertise in the study of the various effects of implantation—radiative emission, production of defects and so on.

A major problem seems to be the definition of areas where the technique of ion implantation is going to prove most valuable, particularly since support is dispersed through university, government and industrial laboratories. This is where the exploratory programme recommended by the panel may help.

COMPUTERS

IBM's Handicap

DESPITE the fact that International Business Machines UK bestows many benefits on the British economy, it has constantly to overcome feather bedding by the government of British computer manufacturers. This was the impression that emerged when representatives of IBM UK met sub-committee D of the Select Committee on Science and Technology last week. Mr Edwin Nixon, the company's managing director, told the sub-committee that "the government will give an order to ICL if it possibly can. To gain an order we have to be 25 per cent better, or provide a service that ICL cannot". Mr Nixon, who declined to give the source of his information, said that he did not believe this to be just a journalistic guess. This, he said, is the guidance given by the Ministry of Technology for purchase of computers by government departments and by nationalized industry.

Nevertheless this alleged government policy does not seem to be too great a handicap to the activities of IBM UK. The sub-committee was told that the company's revenue in 1968 amounted to £125 million, of which £33 million was in exports. Although imports in that year amounted to £38 million, preliminary figures for 1969 show that the export deficit was reversed; the company exported £38 million worth of goods, and imported £35 million during that year. Mr Nixon also offered no comment on Mr Leadbitter's estimate that IBM and ICL control about 30 per cent of the UK computer market each, and the remainder is divided among about twenty other companies. He did say, however, that IBM hopes at least to retain its share of the market.

Mr Nixon also disclosed that the company's factory at Havant, Hampshire, is being almost doubled in size, but when asked by Mr Lubbock what will be built there, he said "unfortunately it is a product that we've not yet announced". Such an expansion should go a long way to ensure that the company fulfils its intention to maintain a balance between imports and exports, and IBM UK's research and development capacity will continue to be the largest of the IBM empire outside the United States.

The sub-committee was also treated to a discourse on how IBM helps the British economy. The company employs about 11,000 people, about a quarter of whom are qualified scientists and engineers, and Mr Nixon emphasized that the manufacturing plant in Greenock is situated in a technological "grey area" (unlike the plant at Havant). Besides helping the export drive and paying a lot of taxes to the exchequer, IBM UK has a large stake in computer education. Last year the company claims to have trained some 20,000 people.

Wider implications of the project should then be examined by an interdisciplinary group whose membership should be chosen according to the particular problem under discussion. Such studies, said Mr Benn, are already being considered by the Ministry of Technology for major projects. The final stage, opening up the issue to full public debate, represents the demand that the power of technology be brought under democratic control but, said Mr Benn, it would require a different attitude among members of the public. Many people feel that they are not qualified to comment on technological questions but little technological knowledge is required to suggest that some of the money devoted to space research should be spent on health research, for example.

Mr Benn pointed out that these suggestions are not new but they have not previously been heard from the Ministry of Technology. If the Ministry does change its decision-making process in this way, then perhaps issues such as Concorde, which Mr Benn firmly supports, would be opened up to public debate before a final decision is taken.

DOGS

Rabies at the Races

For the second time in five months a case of rabies has slipped through the British quarantine regulations. A mongrel that was imported from Pakistan in May, and released from quarantine in November, died of rabies at Newmarket on February 27. It had been vaccinated against rabies some eighteen months before it reached Britain. There is apparently no danger to local wildlife or to racehorses stabled in the Newmarket area, for the only animals that the mongrel bit were three dogs which have been well isolated, but the case is significant because it represents the first time since 1922, when Britain became free of rabies, that the disease has appeared more than eight months after importation. The present quarantine period was lengthened to eight months with effect from last December, presumably because it would have covered all the earlier cases.

The Ministry of Agriculture is not yet prepared to say whether the period will be extended yet further, although last year the minister, Mr Cledwyn Hughes, promised to review the quarantine arrangements at the end of 1970. He has, however, asked for an official report on the Newmarket case. Clearly there are practical limits to lengthening the quarantine period; a year's quarantine, for example, would double the demand on the space of the quarantine kennels. Even this would not guarantee against a further outbreak, because the rabies virus has sometimes incubated for more than a year (M. M. Kaplan, *Nature*, **221**, 421; 1969). Is there a case for banning the import of dogs altogether, at least from countries where rabies is established ? The trouble is that giving the quarantine regulations too savage a bite would encourage the smuggling of dogs into the country.

The Newmarket outbreak adds weight to the view that compulsory vaccination would be a poor substitute for quarantine. If the vaccine works it usually gives immunity for three years, but it seems to be only 60–80 per cent effective. It is too soon to know whether the two-day slaughter of wildlife at Camberley last October has been successful, but so far no evidence of rabies has appeared.

TECHNOLOGY

Sermon to Mancunians

Greater participation by the public in technological decision-making and more thorough analysis of the social and economic implications of technological projects were called for last week by Mr Anthony Wedgwood Benn, Minister of Technology. Speaking at a meeting of the Manchester Technology Association in London, Mr Benn said that "increasing fear that technology is being misused, that we are pursuing increased production of goods and services too single-mindedly and without regard for the general quality of life" may lead to a campaign against technology. The answer to this, he suggested, is to open up major technological decisions to public debate, and to encourage research teams to look at the social implications of their work.

Technology can be brought under democratic control in a three-stage decision-making process, said Mr Benn, and his speech was liberally sprinkled with hints that the Ministry of Technology is at present trying to find a way to incorporate such a process into its own decision-making machinery.

For the first stage of the process, Mr Benn suggested ten questions that should be put to research workers and to initiators of technological projects. To answer these questions, the whole research team, board of management or sponsoring agency would have to discuss the likely costs and benefits of the project, to assess its effects on manpower resources and on training, and to evaluate alternative projects. These "ten commandments" are clearly intended to stimulate discussion among technologists of the implications of their activities—a discussion that Mr Benn indicated does not always take place. Instead of saying, for example, love thy neighbour as thyself, Mr Benn suggests that technologists should ask themselves, "Would your project—if carried through—promise benefits to the community, and if so what are these benefits, how will they be distributed and to whom and when will they accrue ?".

SOVIET UNION

Plant for Flower Power

Heavy industry is not always the apple of a Russian planner's eye. A new branch of agriculture has recently been inaugurated in the Azerbaijan SSR—the growing of flowers for the perfumery industry. Roses, narcissi, mint, orange and lemon blossom and other ether-bearing flowers have become the raw material of the latest venture in Soviet production. The State Plan of Azerbaijan calls for the organization of state farms that are to be devoted entirely to this new crop. Processing factories are to be set up, with research laboratories and experimental centres. Present research is concerned with the active principle of saffron (obtained from the stamens of the yellow crocus); the most satisfactory achievement to date has been the isolation of an especially valuable ether oil from the scented narcissus 'Bulgarian Beauty'.

PHYSICS EXHIBITION

Computing to the Fore

SUFFICIENT of the flavour of modern physics seeped through the barrage of electronics at this year's physics exhibition at Alexandra Palace, London, last week to allay the visitor's suspicion that he may have accidentally strayed into an instruments' trade fair. The dominating role of computers in physics was one theme widely in evidence. Exhibits included the computer simulation of the neutron flux of an advanced gas cooled reactor, carried out by the Berkeley Nuclear Laboratories, and on-line monitoring of plasma dynamics experiments by the computational group of the Institute of Physics and the Physical Society.

The three lectures traditionally held at the exhibition were devoted this year to the environment. Professor A. H. Cook of Edinburgh University explained why continental drift has become such a talking point among physicists, and how variations in the Earth's magnetic and gravitational field are providing such rich sources of information about the Earth's history and interior. This was followed by talks on the physics of the oceans, by Dr G. E. R. Deacon, the director of the National Institute of Oceanography, and on buildings and noise by Mr W. E. Scholes of the Building Research Station, both of whom set out to show how physics is increasingly enlarging its traditional frontiers.

Lasers were once again strong among the exhibits. The Atomic Weapons Research Establishment at Aldermaston had on display a rotating mirror hologram camera which uses Q-switched pulses from a ruby laser to give superimposed hologram exposures. This means that the object is seen in what may be thought of as 4-dimensional space. Queen's University of Belfast had on show a new type of dye laser system which is being applied to flash spectroscopy, the optical Stark effect and studies of self-induced transparency.

Mullard's have developed an acoustic surface wave filter. The frequency selective properties of transducers are used, and the waves are launched and received by gold electrodes evaporated on a piezoceramic. Standard Telecommunication Laboratories had on display an optical fibre communication system, on which work is also being done at the Post Office Laboratories in Dollis Hill. The STL system uses a cooled GaAs laser as the light source and a glass fibre as the transmitting medium. Pulse code modulation at about 100 million bits a second is used. Fibre optical communication systems are being vigorously investigated at present as they offer the chance to transmit many hundreds of times as much information as conventional coaxial cables.

CHEMICAL INDUSTRY

No Time for Dragging Feet

A REMINDER of why the British government wishes to join the European Economic Community has been provided by the Chemicals Economic Development Committee, one of the standing committees set up to oversee critical sectors of the British economy. In a report on the chemical industry (Industrial Report by Chemicals EDC on Economic Assessment to 1972; HMSO, no charge), the committee says the chief problem has been low capital investment because of the relatively small size of the British market and the sluggish growth of the national economy. At the same time the demand for larger chemical plants is growing steadily. Ten years ago an ethylene plant would have been designed to produce 50,000 tons a year; now it must yield 500,000 tons to be a commercial success and requires a correspondingly greater capital investment. Joining the EEC would ease the problems of market size, the committee concludes, but the government could give more immediate help by reducing fuel tax, lowering the cost of electricity to large users and easing credit restrictions.

GROWTH IN CHEMICAL AND INDUSTRIAL PRODUCTION
1958–67
(Growth rate, per cent a year)

	Industrial production	Chemical production
United Kingdom	3·1	6·6
Germany	5·1	11·0
France	5·1	9·9
Italy	8·9	13·4
United States	6·0	8·3

Although the chemical industry increased its share of manufacturing output in Britain from 9·3 to 10·1 per cent between 1963 and 1968, its rate of growth has been smaller than that of other countries in the EEC. The report cites the excessive time taken to build plant in Britain as one of the factors behind the slow growth rate. EEC countries also benefit from a favourable costing system for high load electricity, which is important in the production of many chemicals, including polyvinylchloride.

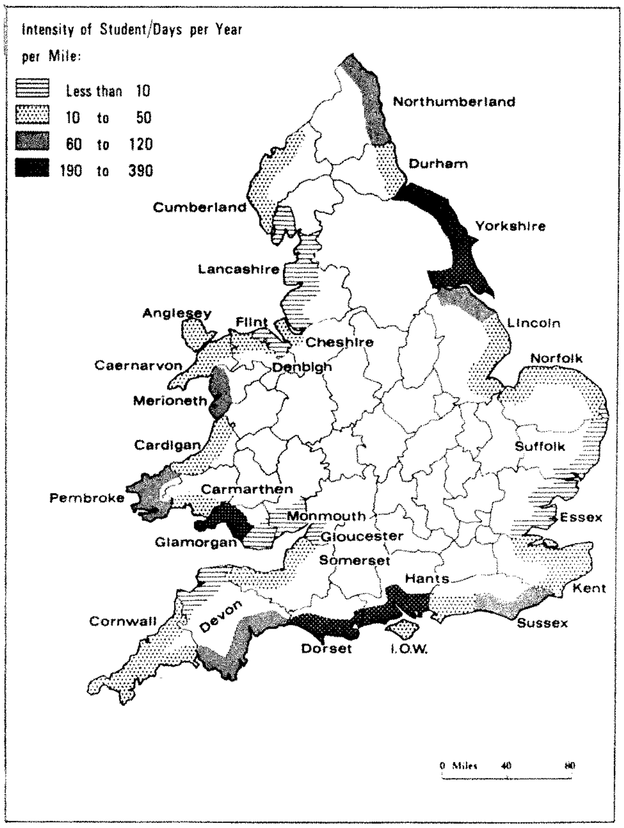
The committee estimates that about 7 per cent of the world's chemical research is done in Britain, although only 5 per cent of chemical industry is in Britain. Britain is still the third largest exporter of chemicals in the world (after West Germany and the USA), and in 1967 had a positive balance of trade of £200 million in chemicals.

COUNTRYSIDE

Safeguarding the Coast

A PLEA that policy decisions about the use of Britain's coast should be made on a national level is the burden of a new report from the Countryside Commission (*Nature Conservation at the Coast*. HMSO, £1 12*s* 6*d*) which is based on a study by the Nature Conservancy. The commission looks specifically at the 3,847 miles of coastline in England and Wales—a resource that it describes as "scarce, finite, valuable and vulnerable"—to see how the conflicting demands on the coast by housing, caravans, motor cars, defence structures, industry and so forth can be reconciled with the need to preserve the coast's unique natural features.

The committee warns that pressures on the use of the coast should not be of concern only to teachers and scientists—the richness and variety of habitat and the associated wildlife are the basis of the amenity and economic value of the coast just as much as of the scientific interest, and anything that affects these basic features will degrade the coast as a whole. A view must therefore be taken of all aspects of the coast, and all planning, management and development of it, whether at the local, regional or national level, must be based on ecological research. Here the commission believes the Nature Conservancy has a special part to play in undertaking and sponsoring research through its National Nature Reserves (of which there are 120 miles in coastal areas); the reserves should continue to be used primarily for research, wildlife conservation and education.



Results of a survey by the Nature Conservancy of the use made by students of the coastline of England and Wales.

Besides making various suggestions for safeguarding the scientific interest of the coast, the new report contains much factual information on the geology and wildlife of the British coastline and on the present administrative measures by which sites of scientific importance are conserved. The various conflicting demands on the coast are usefully tabulated in a "Chart of Human Impacts on Coastal Wildlife and Scientific Interest" which traces the incidence and effects of an activity or operation whether by industry, tourism, recreation and including the over-use of areas by scientists and students on field trips. The report also provides a classified catalogue of 371 sites of scientific importance that lie along the coast of England and Wales.

NATURAL HISTORY

Behind the Scenes

Last week the small back rooms of the British Museum (Natural History) were unlocked to two hundred or so academics and government scientists. This was the first open day at the museum, and many visitors may have been surprised to see the labyrinth of rooms and corridors in which the museum's zoologists, botanists, palaeontologists and mineralogists carry out their labours.

One of the chief duties entrusted to the museum is the cataloguing of the thousands of new animal and plant species that are described each year. To handle so much taxonomic data the museum has installed data processing machinery and is already preparing catalogues of the spider and primate collections in machine readable form.

Another useful piece of equipment is a scanning electron microscope which is revealing the microstructure of pollen grains, diatoms, sponges and mites and is also helping with the identification of mites of the order Astigmata.

Fishermen who hanker after a mention in the *Guinness Book of Records* will be glad to know that the museum has taken their problems to heart. Freshwater fish specialists are sorting out the characteristics of hybrids of the British members of the Cyprinidae, the carp family, so that they can be easily identified before hopes of a record are raised too far—hybrids are, it seems, just not good enough for therecords league table.

The botanists have been striking out in a new direction with their survey of Mull, now in its fifth and final year of field work. The object of the exercise is to produce a comprehensive flora of the area which will describe the whole range of vegetative life from flowering plants to marine algae. The variety of expertise available in the museum has made it possible to mount this project, without the need to concentrate only on flowering plants and ferns, as most writers of floras must. The team from the museum have had the services of a Royal Navy helicopter to take them to outlying islands. The first fruits of the project were on show, including news of a hitherto unknown hybrid fern.

Transition to the Seventies

A quarter of a century after the first nuclear reactor at Harwell went critical the British nuclear power industry now finds itself on the threshold of a new era. The last of the Magnox power stations conceived in the first nuclear programme will be opened this year, four of the second generation advanced gas cooled reactors are already under construction, with several more in the pipeline, and the high temperature and fast reactors are far enough along the design and testing path to keep the electricity generating authorities planning well into the nineteen eighties. But how far is British industry a prisoner of its early advances in nuclear power, and how imaginatively have the essential elements of continuity and innovation been combined in defining a reactor policy appropriate to the last two decades of the century ?

Table 1. NUCLEAR GENERATING CAPACITIES, MARCH 1969
(Excluding communist countries)

Country	MWe capacity
Britain	4,200
United States	1,981
France	1,580
W. Germany	707
Italy	597
Canada	223
Japan	168
Spain	153
Netherlands	52
Belgium	10
Sweden	9

The extent to which Britain has forged ahead in nuclear power can be seen from Table 1. By 1985 it is expected that up to a third of Britain's electricity generating capacity will be nuclear, and by the end of the century more than 100,000 MW will be nuclear generated. But an increase in the variety of reactor systems and in the number of countries embarking on nuclear power programmes has sharpened the need for a more widely international view of reactor policy—spotlighted by the continuing rumpus over the future of the steam generating heavy water reactor—and has emphasized the ease with which British industry could waste away its lead in this field through administrative or technical confusion.

The early reactors were all graphite moderated with natural uranium as the fuel. Both British and French reactors were predominantly of this design, although the French authorities have recently transferred their allegiance to light water reactors, developed principally in the United States. The two most important types of heavy water reactor are the Canadian CANDU reactor and the steam generating heavy water reactor (SGHWR), in which light water is the coolant with the steam having direct access to the turbines. Different technologies are needed for graphite and water moderated systems, which explains the somewhat anomalous role of the SGHWR in Britain's reactor programme.

The most exciting long term prospect is the fast breeder reactor. This uses plutonium as fuel, but can generate its own supply by converting uranium 238, which is non-fissile, into plutonium. Moreover, the very high energy of the neutrons—10^{11} m/s compared with 3,000 m/s in normal thermal reactors—means that fission can be induced in isotopes of plutonium that are stable to thermal neutrons, and no attempt need be made to produce fuel rich in plutonium 239. The advantages of the fast breeder reactor are compelling, both in reducing fuel requirements and core size. The prototype fast reactor at Dounreay is due to reach full power (250 MW) in 1972, but there is unlikely to be a commercial model ready for the generating boards until the end of the decade.

Table 2. MAIN NUCLEAR POWER STATIONS IN BRITAIN

Location	Type	Year of commissioning	Capacity MWe	Generating cost d/kWh
Berkeley	Magnox	1962	276	1·25
Bradwell	Magnox	1962	300	1·14
Hunterston A	Magnox	1964	320	
Hinckley A	Magnox	1965	500	1·07
Trawnsfynydd	Magnox	1965	500	0·96
Dungeness A	Magnox	1965	550	0·78
Sizewell	Magnox	1966	580	0·74
Oldbury	Magnox	1967	600	0·74
Wylfa	Magnox	1970	1,180	0·70
Dungeness B	AGR	1972	1,200	0·56
Hinckley B	AGR	1972	1,250	0·52
Hunterston B	AGR	1972	1,250	
Hartlepool	AGR	1974	1,250	0·52
(Dounreay)	PFR	1972	250	
(Winfrith)	SGHWR	1967	100	

In the intervening period two new types of gas cooled reactor will be brought into operation, namely, the advanced gas cooled reactor and the high temperature reactor, which is strongly tipped as the Central Electricity Generating Board's choice for Connah's Quay and Oldbury B. Many new concepts distinguish the AGR from the Magnox design, including a new fuel element—slightly enriched uranium—and a new type of reinforced pressure vessel which further enhances the safety of the design. This has allowed the CEGB to site its latest power station near a densely populated area at Hartlepool. The economic advantage of the AGR is also evident from Table 2.

The high temperature reactor has been developed under the auspices of the OECD in the Dragon project. It is designed to operate at around 800° C, compared with 650° C for the AGR, achieved by using helium coolant, and the core has been made homogeneous by combining the fissile and fertile components of the fuel in the moderating material. This allows the whole of the core to be removed at the end of the fuel cycle, avoiding excessive fast neutron doses and permitting higher thermal neutron densities. Another novel feature of the HTR is the use of graphite and ceramic to contain the fuel elements, eliminating the need for metal canning.

Table 3. NUCLEAR POWER STATIONS IN THE EUROPEAN ECONOMIC COMMUNITY
(January 1970)

Type	Country	MWe in service	MWe under construction
Gas-graphite	France	1,310	1,055
	Italy	200	
Boiling water	Germany	407	1,410
	Italy	150	
	Netherlands	52	
Pressurized water	Germany	283	1,780
	France/Belgium	266	
	Italy	257	
	Belgium	10	1,650
	Netherlands		450
Heavy water	Germany	50	100
	France	70	
High temperature	Germany	13	22
Na/Zr hydroxide	Germany		19
Nuclear superheat	Germany	22	

A prototype steam generating heavy water reactor is already in operation at Winfrith in Dorset, but the success of the HTR has diminished the chances of the SGHWR being adopted by the British generating boards. Table 2 shows the British nuclear power stations completed or under construction, and Table 3 gives a résumé of nuclear generating capacity throughout the European economic community. The most significant change since January 1969 is the growth in the number of pressurized water reactors under construction in the EEC, from 630 MW to 3,880 MW capacity.

Parliament in Britain

BAC 3-11

Mr Wedgwood Benn refused last week to be rushed into making a firm decision on whether to give financial support to the British Aircraft Corporation for development of the BAC 3-11 airbus. Mr John Corfield and Mr Michael McNair-Wilson both asked if any decision has been taken, and Mr Corfield suggested that delay in reaching a decision could be disastrous to the project. He also pointed out that there are very few British aircraft in the pipeline at the moment. Mr Benn denied that there has been delay in arriving at a decision. He said that discussions are still taking place between Ministry of Technology staff and the company, and a full economic analysis must be made before any decision is taken. BAC has requested a government contribution of 50 per cent of the estimated initial costs of £150 million and for some help with production. (Oral answers, February 23.)

Government Research Establishments

Mr David Howell accused the government of being on the wrong tack in the Green Paper on government research and development. He suggested that the effort spent in drumming up more work for government research establishments might be better spent in putting industrial research back in industry. Mr Benn said that the government hopes to announce its decisions on the Green Paper in the early summer, provided that no major alterations to the proposals are necessary and that the earliest possible date for establishing the proposed British Research and Development Corporation is 1972. (Oral answers, February 23.)

CS Gas

Lord Chalfont, Minister of State, Foreign and Commonwealth Office, refused to give an undertaking that the government will reconsider its decision to regard CS as being outside the Geneva Protocol. The decision, he said, was based on evidence that CS is not significantly harmful, except in exceptional circumstances, and the Geneva Protocol was formulated specifically to exclude significantly harmful agents. Lord Brockway, however, pointed out that the British government has always stood for the inclusion of lachrymatory agents within the terms of the Geneva Protocol, and he asked Lord Chalfont why there has been a change of attitude. Both Lord Brockway and Lord Ritchie-Calder also pointed out that the decision to regard CS as being outside the Geneva Protocol might affect the credibility of British intentions in relation to the Disarmament Conference. Lord Ritchie-Calder also asked for the evidence relating to the effects of the gas when it was used in Northern Ireland to be made public, to which Lord Chalfont replied that much of it is already public, and it shows that CS is harmless unless used in exceptional circumstances, such as prolonged use in confined spaces. (Oral answers, Lords, February 26.)

VTOL Aircraft

Research and development connected with vertical take off aircraft for both civil and military use has so far cost more than £110 million and a further £10 million will be spent in 1969–70. Mr Neil Carmichael, who gave this information in reply to a question from Mr Eric Lubbock, also said that £50,000 has been spent on civil market research and a further £50,000 will be spent this year. (Written answers, February 23.)

Miscellaneous Intelligence

Astronomers in the United States may grumble from time to time at their present financial straits, but times have been harsher for the profession. The prediction of today's eclipse, for example, was probably governed by regulations less draconian than the imperial Chinese prescript set forth in the *Shu Ching*: "Being before the time, the astronomers are to be killed without respite; and being behind the time, they are to be slain without reprieve". The first recorded eclipse was that of October 22, 2137 BC, but on that unhappy occasion the Chinese astronomer-royals, Hi and Ho, had drunk too much wine and neglected to scare off the dragon that was eating the Sun, an error for which they very properly lost their heads.

Like the Rector of Stiffkey, Dr James Shapiro of Harvard University seems to have acquired a more lion like reputation after leaving his profession. According to the London *Evening Standard* (February 24) he is now "one of the world's most brilliant scientists". The *Evening Standard*, of course, is in its own way equally distinguished for having produced what was perhaps the least sensible headline of 1969, "Now the Pill Contaminates London's Water" (December 23).

A vial of Moon dust was stolen this week from a charity dinner at Los Angeles where it had been put on display without official permission. With the cost of the lunar rocks being $500 million per pound, according to one estimate, and the stolen sample weighing 2·3 grams, it seems that the thief made off with $5 million. Never can so much loot have been lifted so lightly. Luckily the thief repented his crime and the stolen vial was later returned.

Mr Arthur C. Clarke, the inventor of the communications satellite, was told the following story by Mr Wedgwood Benn when the latter was Postmaster General. A parliamentary commission, convened to examine the decline in gas company shares occasioned by Edison's invention of the electric light, asked one of its witnesses if he had any comments to make on another American invention, the telephone. The witness, Sir William Preece, who was chief engineer of the Post Office, replied, "No, Sir. The Americans have need of the telephone, but we do not. We have plenty of messenger boys" (*Unesco Courier*, March 1970, p. 32). Last week Mr Benn told the same story to Terry Coleman of the *Guardian*, adding that in 1898 Preece said there was no need for dialling because girl operators were cheap, and in 1911, when he retired, Preece was called the father of the British telephone service. Mr Benn's next instalment may tell of a certain spectre recently seen stalking the GPO tower.

An imaginative way of killing two birds with one stone has been seized upon by G. D. Searle, the company that produced the world's first oral contraceptive. Its most recent product, known as Ornitrol, is a contraceptive pill with a farinaceous coating that can be scattered in the highways and byways of cities with a pigeon problem. The company appears to believe that the pill will be too large for song birds to swallow, but perhaps it should not count its linnets before they are hatched.

NEW WORLD

Downbeat at the Office of Naval Research

THE most enduring monument to the postwar growth of basic science in the United States is the Office of Naval Research, by common consent the first embodiment of the doctrine that only a national government is large enough to support scientific research effectively. The Office of Naval Research came into being immediately after the Second World War in 1946, partly because of the urging of Dr Vannevar Bush that continued support for scientific research had become a necessity and partly because of the willingness of the US Navy's managers to let some unspent money be spent on basic research. The setting up of the National Science Foundation in 1951 is a proof of how quickly the pattern established by the Office of Naval Research was accepted as a good model for sustained financial support for research projects.

It seems to be no accident that the navy should have become the service to recognize the civilian need for research support—its own operations during the war were sustained by an exceedingly distinguished group of men recruited from the universities. By all accounts, lobbying for the postwar establishment of a civilian research agency seems to have been vigorous from 1943 on, and detailed plans were made at a series of meetings within the Navy Department in the second half of 1944. In the end, the publication of the report by Dr Bush, *Science, The Endless Frontier*, seems to have persuaded the navy and the Administration that the national interest required arrangements to make sure that there would be a good supply of trained scientists in future emergencies.

The Office of Naval Research came into being with a budget of $44 million and responsibility not merely for the granting of money to university researchers but also for a laboratory of its own—the Naval Research Laboratory—and for providing advice on research as a whole to other parts of the US Navy. In its heyday, in the fifties, the Office of Naval Research was a great benefactor of all kinds of research projects. In those years, it seemed as if quite flimsy connexions with the future strength of the navy were sufficient to open the pursestrings. Thus the Office of Naval Research once explained its involvement in molecular biology by saying, possibly with its tongue in its cheek, that experiments on feeding RNA to rats may well have paved the way for a means of teaching navy pilots their jobs by a suitable choice of nucleic acid in the diet.

For the most part, however, the office's objectives were as serious as the work which it sponsored. It can now claim that it has helped the early development of masers (and therefore lasers), a great deal of behavioural research and the evolution of an understanding of the chemistry and physiology of vision as well as the mounting of a programme of rocket research which has contributed greatly to the understanding of high frequency radiation from the Sun and X-ray radiation from the galaxy as a whole. It tends to be forgotten that it was the US Navy that sponsored the disappointing Vanguard satellite project.

In the years immediately after the war, the Office of Naval Research set what was then a new pattern for the sponsorship of research by means of contracts with individual investigators for carrying out particular tasks in basic research. To begin with, the method seems to have been regarded with some suspicion by the principal universities in the United States. No doubt the success of the years since the war would not have been possible if universities such as Harvard, the California Institute of Technology and the University of California at Berkeley had not been persuaded that no damage would be done to their independence by this device.

In the fifties, some of the first successes of the Office of Naval Research stimulated other agencies to steal its thunder. Other services—the air force, for example—followed suit, although it seems to be accepted that, in the fifties and early sixties at least, the navy remained more imaginative than its competitors in its choice of projects to sponsor. Much of the success of the Office of Naval Research seems to stem from the manner in which contracts are awarded. Rear-Admiral Thomas B. Owen, the chief of Naval Research and director of the Office of Naval Research, explains that responsibility for the choice of contracts still remains with individual programme directors, and that formal advisory boards are not used in the making of decisions with the regularity with which they intervene in other organizations. No doubt the fact that ex-programme directors from the office are frequently found in important positions in the administration of other research agencies is probably closely linked with the way in which responsibility for choice tends not to be diffused through the membership of a committee.

Inevitably, with the passage of time, the pattern of work has changed and now the criteria for relevance of sponsored research are much more rigorously applied than they seem to have been ten or fifteen years ago. Admiral Owen says that with the economic stringency of the past few years, relevance has been increasingly insisted on, with the result that the Mansfield amendment forbidding the Department of Defense to sponsor basic research which has no "direct and apparent relationship" with the navy's chief function may be more a symptom than a cause of change. As things are, standing instructions of the Department of Defense require that the office should let contracts for research only when it is assured of the quality of the research proposal, its relevance to the navy's general if long term interests, the ability of the investigator, the availability of equipment and the reasonableness of the cost. Admiral Owen says that it is bound to be a subjective matter deciding when each of these criteria is satisfied and that, indeed, research sponsorship necessarily boils down to "gut feeling". Even so, the chances are that in the current climate, the point at which a programme sponsor is satisfied by a proposal will be a more exacting one.

This said, the range of the interests of the Office of Naval Research remains impressive. In the coming fiscal year, if Congress grants what has been asked of it, roughly $50 million will be spent on the three laboratories which the office maintains, two of them by contract, and a roughly equal amount will be spent in universities on research contracts. At the same time, it is likely that basic research in industry will cost $8 million in the year, that $4 million will find its way to non-profit agencies under research contracts and that between $1 and $2 million will be spent in federal contract research centres. Admiral Owen says that in the process of contraction by about 20 per cent which has been forced on the office in the past three years, care has been taken to keep support for universities and for domestic laboratories more or less in balance, so that there has been no undue concentration of effort on the places at which the office would itself be most immediately affected.

Naval Research Laboratory, Washington.

In the universities, the range of work supported is still quite substantial (and in value is roughly 3 per cent of all federal money for basic research in universities as well as about a fifth of what the National Science Foundation spends in this way). Thus the ONR has been the chief sponsor of the development of the supersonic particle accelerator at Stanford University, chiefly on the grounds that the technology of superconducting machinery is essential to its long term interests. (The National Science Foundation now shares in this development.) Naturally enough, oceanography takes a large and growing share in the office's budget—nearly a quarter of what was spent on basic science in 1969. Over the years, the ONR has been responsible for the development of submersible research vehicles, including the deep submersible Alvin which was lost in 1968 after having helped to locate the hydrogen bomb which went astray off the coast of Spain, and also the up-endable research vehicle used widely in the BOMEX programme of meteorological and oceanographic observations carried out a year ago in the eastern Atlantic.

Admiral Owen says that his office is at present particularly interested in a number of general themes which bear on the US Navy's long term interest. The development of techniques for surveillance under water and above it is understandable enough, as is the interest in techniques for command and control. The navy has its own special interest in materials technology, being as often concerned to make hollow objects which will withstand pressure of deep water as air forces are to make lightweight lifting surfaces. Energy conversion is another field of interest (responsible for some 5 per cent of the budget in the past few years). Increasing attention is being given to problems of the management of people, chiefly on the grounds that the questions of how best to get a good service out of 650,000 people (not counting the marines) have not previously been given the weight that they deserve.

The principal laboratory is the Naval Research Laboratory, with interests in thermonuclear fusion, radio astronomy and rocket astronomy as well as the more obvious functions of a naval research establishment. (The two smallish contract laboratories are the Naval Biological Laboratory at Oakland, California, which is largely concerned with aerosols, and the Arctic Laboratory in Alaska which is concerned with physiological problems.)

The part played by the Office of Naval Research in the years since the Second World War is thus not simply measured by the amount of money spent on research grants. At the same time, however, there is no doubt —this is simple arithmetic—that the scale of operations has been declining in real terms for the past three or four years, with the result that the involvement of the ONR in the direct sponsorship of basic research in the universities has necessarily become a smaller proportion of the total. In the long run, no doubt, this may be not merely inevitable but desirable—everything will depend on whether the budget of the National Science Foundation grows at such a pace as to take up the slack. But the ONR seems still to retain some of the daring that endeared it to university scientists in the years after the Second World War.

MOLECULAR BIOLOGY

Elites and Labels

THERE are now almost too many labourers in the vineyard, in the view of Dr James Watson, director of the Cold Spring Harbor Laboratory of Quantitative Biology. "Until recently all of us have thought of (the world of molecular biology) as a small group, containing most of our best friends, plus a few dissidents whom, nevertheless, we wanted to talk with if not exchange preprints." In the annual report of the laboratory Dr Watson estimates that world wide "the number of intelligent people now doing sensible, if not important, work in molecular biology" is probably at least 2,500, of whom two-fifths passed through Cold Spring Harbor last summer. The existence of the Symposium now has to be under-advertised because there is room for less than half the applicants. But despite having to turn away the crowd from his doors, Dr Watson intends to see that Cold Spring Harbor "remains an institution where graduate students are as welcome as their professors, where bad science is so labelled, and good science encouraged with every resource at our disposal".

OCEANOGRAPHY

Few Friends for NOAA

No influential supporters have been found for a proposal to set up a federal agency for oceanography and ocean engineering. This, at least, has been the experience in the past few days of the subcommittee on oceanography set up by the Senate Committee on Commerce, which has been trying to put flesh on the bones of the proposal that there should be a National Oceanic and Atmospheric Agency (NOAA). The commission, known after its chairman, Dr Julius Stratton, provided an optimistic but detailed prospectus for the exploitation of the oceans as a "national effort", and urged that an agency linking together organizations as varied as the United States Coast Guard and the US Weather Bureau would be needed to seize all the opportunities which exist. In the past year, however, interest in oceanography as such has decreased as that in the environment has grown, which is one reason why the subcommittee has found most of its witnesses lukewarm towards the concept of NOAA. At the same time, it has become known that the Ash commission set up last May to advise President Nixon on the organization of those parts of the federal government responsible for domestic affairs has the problem of organization in oceanography and for the environment very much in mind. In the circumstances, the subcommittee's proceedings have been chiefly an opportunity to know what people have been telling the Ash commission. Last week, for example, Dr L. DuBridge, the President's science adviser, said that the Ash commission's report was due to reach the President on April 15. He said that he was against the proposals of the Stratton commission because they were at once "too radical and too conservative". By this, he meant that their repercussions on government organizations would be drastic, but that even the organization called NOAA would not adequately link problems of oceanography with problems of pollution. To the assertion that "there is enough in the oceans to feed and sustain mankind more or less indefinitely, but that man is in danger of ruining this by pollution", he replied that research is necessary to test the assumptions in this view. "It has been asserted that the ocean is being polluted, but I don't believe that's true"—there is instead pollution in coastal waters which will be mitigated by the Administration's proposals for dealing with water pollution.

Dr W. D. McElroy of the National Science Foundation also opposed the setting up of NOAA in his statement to the subcommittee last week. He thought that the proposed organization would be so unwieldy that it would create more problems than it solved. He also argued that there is a logical need for organization around four functions—environmental forecasting and services (weather, for example), the development of resources, scientific research and the development of technology. He argued that the agencies now responsible for these functions should continue to look after them and he stoutly defended the interest of the National Science Foundation in oceanography through what is called the Sea Grant Program (on which the foundation spent $6 million last financial year, nearly a third of its entire support for oceanography).

The Secretary of the Interior, Mr Walter J. Hickel, also turned out to be against the proposal to set up NOAA, perhaps predictably on the grounds that the Department of the Interior could manage quite well enough with its present responsibilities for management of resources and for organizations such as the Coast Guard. At the same time, he admitted that there is much room for better coordination between the federal agencies at present responsible for various aspects of oceanography and the environment.

The extent to which the Ash commission will affect the arrangements that now exist remains to be determined. On the face of things, it would take exceedingly clever administrators to think of devising a single agency responsible for, say, "the environment"—Dr DuBridge said in his evidence that such an agency would be as hard to create as a single agency for all scientific research.

LABOUR MARKET

Bread Lines for Physicists

THE controversy whether there is actual unemployment among PhD physicists in the United States has been intensified as details have become known of the government plans for cutting back on some activities in federal laboratories. Although Dr Philip Handler has claimed that a survey carried out by the National Academy of Sciences has shown that tales of unemployment stem from "anecdotes about a few theoretical physicists" (see *Nature*, **225**, 786; 1970), the American Institute of Physics is making much of the experience of its job finding service at the Chicago meeting at the end of January this year. The institute says that the people putting down their names on the register of those wishing to move have increased over the years from 567 in 1964 to 677 in 1966, 984 in 1968, 1,285 in 1969 and 1,347 in 1970. The numbers of vacancies cannot easily be inferred from what is known about potential employers registering with the placement service, although it is held to be significant that the number of employers in 1970 was only 101, compared with 167 in 1969 and a high figure of 272 in 1967. By the same test, the numbers of vacancies registered has also fallen from 617 in 1967 to 121 in 1970, but this comparison is complicated by the way in which some employers may decide not to make publicly known details of all the jobs available. Of the physicists seeking jobs in 1970, it appears that only 70 out of 1,347 were actually unemployed, that 252 were academics and 647 were graduate students or the equivalent. More than a half of those seeking jobs were in their twenties, 439 were between 30 and 40. A further difficulty in making this comparison is that the anxiety of which physicists now speak is as much a fear of what the labour market will be like when the impending cuts in public expenditure on research take their full effect. Many of those due to leave the NASA Electronics Research Center at Cambridge, Massachusetts, have not yet resigned in the hope that something will turn up to save their jobs at the last minute, while it appears that the Atomic Energy Commission will be having to dispense with some 1,500 people from its laboratories at the beginning of the next financial year.

The American Institute of Physics is hoping that it may not be too late to influence the course of events. Dr Lewis Slack said last week that the institute was planning to carry out a survey among universities of the extent to which the new budget would make it necessary to curtail the scale of operations. He said that similar surveys were carried out among physics departments after the budget cuts of the past two years, and that the results had been found to be of some value to agencies of the federal government in their battles with Congress.

The extent to which physics typifies other scientific disciplines is not at this stage clear—given that its involvement in the new environmental programmes is less direct than that of, say, biology, it would be no surprise if physics turned out to be a particular hard luck story. Some of the features of the labour market in physics have, however, been documented in a report for the American Institute of Physics by Susanne D. Ellis (*Work Complex Study*, to be had from the American Institute of Physics, New York). On the basis of interviews with industrial physicists, employers and employees, the report points to a sharp change in the balance between industrial laboratories on the one hand, and universities and government laboratories on the other; since 1967, when the growth of university research was first halted, industrial laboratories have apparently found it easier to recruit people. At the same time, the employers interviewed seem to have been convinced that "physics education has become too specialized and graduate training, especially, is strong but narrow". A part of the problem in industrial laboratories seems to be the need to fit physicists into disciplinary teams within which communication should be easy. Mrs Ellis, in her survey, found that universities were inclined to reply with "if the universities do not train specialists, where else can one find a research atmosphere conducive to the pursuit of a narrow topic in great detail ?" The industrial employers seem also to complain, however, that those seeking jobs as physicists in research laboratories without PhD degrees tended to be too inexperienced, and that physicists were less practically minded than engineers when engaged on similar work. Mrs Ellis concludes that universities should make more effort to produce broadly trained physicists but that many potential employers were not aware of the changes in curriculum which there have been in the past few years.

Some of these issues will no doubt be raised in the study of physics that is now being carried out by the National Research Council of the National Academy of Sciences under Professor D. Allan Bromley of Yale University. The intention is to extend the work of an earlier committee under Professor George E. Pake which argued the case for continued support for university physics in a report published in 1966. On this occasion, the intention is that the committee will "necessarily consider a broader range of topics", including the relationship between physics and other branches of science in "the current national and social context". A statement by the National Research Council says that the new committee will try to discuss the criteria which should guide financial support for physics rather than attempt to suggest precisely what programmes should be followed. The committee says that it will welcome constructive advice on the task which it has undertaken. The chances are that it will find itself having to pay close attention to the character of physics education at universities.

HIGH ENERGY PHYSICS

Robbing Peter to Pay Paul

THE Joint Committee on Atomic Energy seems reluctantly to be presiding over the shrinking of high energy physics. In his evidence to the committee on Tuesday this week, Mr Paul McDaniel of the Atomic Energy Commission explained some of the consequences of closing down the Princeton–Pennsylvania accelerator, and of restricting work at the Cambridge electron accelerator to that concerned with electron beams. Between now and June 1971, he estimates, there would be a reduction of ten in the manpower involved in high energy physics in the United States. The most spectacular decrease would be associated with the Princeton–Pennsylvania accelerator where the work force consists at present of 180 scientists, but more than sixty people would also leave the Cambridge operation. At the same time, he said, there has been a reduction in all the contracts let to the operators of the accelerators which will continue working.

One of the ironies of the situation that has developed is that nobody is quite clear what will now happen to the Princeton–Pennsylvania accelerator, the facilities at which are worth $39 million. By all accounts, the fixed equipment now belongs to the universities collaborating in the venture, and Professor D. H. White, director of the laboratory, told the Joint Committee that he is hoping to raise enough money to keep the machine in operation either from the National Science Foundation, private foundations or even the universities themselves. Dr White said afterwards that the position of many research students engaged on work at the accelerator is very much in doubt, and that many of them will no doubt be "ruined". He reckons that as little as a million dollars a year might be enough to keep the machine in being for two or three years, in the hope that by then the climate will have improved a little. Dr McDaniel agreed that "with the Princeton–Pennsylvania accelerator, we've cut too deeply—one can't turn on and off scientific activities like these". The new 200 GeV machine in Illinois is by contrast pushing ahead, although it seems now to be clear that the machine that eventually delivers the first beam of protons in 1972 will be as spartan as any machine could be. This year, the AEC was apparently hoping to persuade the Administration to let it spend $112 million on the machine, but it was given authority for only $65 million. The result is that equipment for using the beam is being deferred.

If high energy physics has taken the brunt of the Administration's economies in the Atomic Energy Commission's parish, however, two parts of the research programme will prosper. First, the Los Alamos meson production facility seems to have raised everybody's hopes that the negative pi meson will be exceedingly useful in radiotherapy. At the same time, the control thermonuclear research teams have been so heartened by the progress of the Russian Tokamak device that they are proposing to spend $5·5 million, roughly half as much again as in the present year, on new experimental devices which are frankly intended "to go into this field in a big way". Plainly the Joint Committee, one of the most avuncular in congress, will still have plenty to be avuncular about even if there was only the thinnest logic in one member's suggestion that gravitational waves should also be within its purview.

NEWS AND VIEWS

Mysterious Satellites

ANY biologist told that 10 to 12 per cent of the total DNA genome of an animal is sequestered in a chemically distinct fraction would find it hard to escape the conclusion that such DNA has some crucial cellular function. That explains why the so-called satellite DNAs are exciting so much interest and why the reports in this issue of *Nature* by Jones (page 912) and Hennig and Walker (page 915) will be widely welcomed.

Satellite DNAs were discovered some six years ago when several groups independently, and more or less simultaneously, found that the sheared DNA of higher organisms can be fractionated on the basis of its buoyant density. During density gradient centrifugation of the total cell DNA, satellite DNA is resolved as a distinct band with a different buoyant density from the bulk of the DNA of the main band. This difference in buoyant densities means, of course, that the nucleotide base composition of satellite DNA is quite distinct from that of main band DNA. Moreover, when the two strands of a satellite DNA are separated and then allowed to reassociate, they do so very much faster than does the main band DNA. Satellite DNA must therefore contain many copies of very few base sequences which means its potential as a repository for information specifying the structure of proteins is severely limited. This restriction of base sequences usually, but not inevitably, results in satellite DNA having a different overall base composition and therefore buoyant density from the rest of the DNA.

What are the other salient properties of satellite DNA and what possible function can it serve ? First, a glance at Hennig and Walker's article shows that the amount of satellite DNA varies widely between even closely related species. Twelve per cent of the DNA of mouse cells is satellite, enough for four mouse chromosomes, while another rodent, *Microtus agrestis*, has less than 2 per cent satellite DNA, and *M. arvalis* has 6 per cent satellite DNA. Second, as Hennig and Walker and others have shown, the kinetics of the renaturation of satellite DNAs are consistent with the idea that they are made up of tandem repeats of identical sequences about 150–300 base pairs long. Sequence analyses still in progress in Walker's laboratory apparently suggest that this is an overestimate of the length of satellite sequences and that they may be families of sequences only about ten or fewer base pairs long. Third, the overall base compositions of satellite DNAs from different species differ as widely as the relative proportions of satellite DNA present. Finally, the attempts reliably to detect RNAs complementary to satellite DNA have all failed. It seems that satellite DNA plays no part in coding for proteins or RNAs.

Jones (page 912) and Gall's group in the United States (*Nature*, **225**, 414; 1970) have exploited the technique of intracellular nucleic acid hybridization, which they have developed recently, in order to localize satellite DNA in interphase and mitotic nuclei. As Jones's autoradiograms show, satellite DNA seems to be located in the condensed chromatin of interphase nuclei. This is not surprising because current theory holds that condensed chromatin is not active as a template for RNA synthesis and satellite DNA is not transcribed as far as anyone knows. Localization of satellite DNA in the centromere regions of mitotic chromosomes, however, is most interesting.

It is early days yet, and Jones says that he cannot be certain that every chromosome contains satellite DNA in the centromere region, though if that proves to be the case it may explain both the function and chemistry of satellite DNAs. Their restricted and highly reiterated base sequences might be the molecular basis of chromosome pairing between both homologous and non-homologous chromosomes. Moreover, the apparent association of satellite DNA with nucleoli begins to make sense because cytogeneticists have often commented on the association of centromeres and nucleoli.

A host of experiments and speculations leap to mind. Perhaps satellite DNA plays some part in the assembly of the mitotic spindle, for example, by influencing polymerization of spindle protein or the attachment of chromosomes to the spindle. Hybrid cells might be useful in studying the specificity of a putative interaction between satellite DNA and components of the mitotic spindle. And the chromosomes of organisms with diffuse centromeres might be useful for further testing the relationship between satellite DNA and centromeres.

The localization of satellite DNA in centromere regions may suggest explanations of some of its bizarre properties, but many questions remain. What is the quantitative distribution of satellite DNA in any chromosome set, why do some species have five or six times as much satellite DNA as other species, and how does satellite DNA evolve ? One possible explanation is that it arises by the duplication or amplification of a short sequence of base pairs followed by, or concomitant with, the translocation of some of these duplicates to every chromosome in the cell. Once several million copies of the sequence had become established, and assuming some sequences have selective advantages over others, it might well take long times, even on an evolutionary time scale, to change radically the overall composition of satellite DNA. During speciation from a common ancestor perhaps different short sequences are selected for amplification from the ancestoral sequence pool, so that two closely related species end up with very different satellite DNAs. It is becoming increasingly clear that gene amplification is not all that uncommon, and Gibson and Hewitt (*Nature*, **225**, 67; 1970) in their work on the DNAs of geographically separated populations of grasshoppers may have found an example of the evolution of something very similar to satellite DNA.

MINERALS

Extraction, not Destruction

THE extraction of minerals in Britain is an economic necessity, but conservationists need not despair because dereliction on the scale left by nineteenth century industry is avoidable. And, according to speakers at a symposium on mineral extraction and the countryside held in London on March 3, it is not even a very expensive job.

Each year 480 million tons of minerals are produced in Britain and, as Dr K. C. Dunham (Institute of Geological Sciences, London) implied, there is scope for expansion in terms of available reserves. In Cornwall, for example, there are still valuable veins of tin, some stretching out under the sea, which are expected to contribute to a doubling of the Cornish output of this mineral. And although there is a tendency to import high grade iron ore there may still be a need for the considerable British reserves of a lower quality. Dunham predicted increasing extraction of fluospar and barites (CaF and $BaSO_4$) in the Pennines and further discoveries of metalliferous minerals in the north of Scotland. The potassium salts to be extracted from north-east Yorkshire represent the largest discovery of minerals made under land in Britain this century.

But all this does not mean that the countryside need suffer, for it is now possible to cover up the traces of mining by filling up holes, smoothing out the land and replanting it with vegetation. The story is not of course that simple, and Mr J. Taylor (Blue Circle Group and Confederation of British Industry Minerals Committee) explained some of the reasons why. Local authorities, he said, are unwilling to give planning permission on a time scale sufficiently long to allow extractors to plan for restoration work. Transport costs often increase the price of filling in holes, and Taylor suggested that there might be more enthusiasm on the part of local authorities to use abandoned mineral sites as deposits for domestic and industrial refuse. Local authorities, he implied, could also do more to cope with sites that are already derelict. In 1969 the British Government provided £5,000,000 in grants for reclamation, but only fifty-eight local authorities applied for help, and most of the money was unused.

The local authorities were defended by Mr D. J. Davison (National Coal Board Open Cast Executive), who pointed out that even with a 95 per cent grant impoverished councils cannot afford the burden on the rates that reclamation would require. A new organization is needed, he said, to formulate a national programme to eliminate dereliction. The money is available and the technical problems involved are not enormous. The size of the problem and some of the efforts made to tackle it are illustrated in a small exhibition on mineral extraction and the countryside which was opened on March 3 at the Geological Museum, London, under the auspices of the Institute of Geological Sciences and the Geological Section of the Nature Conservancy.

SOLID STATE

Trends in Diffusion

from a Correspondent

ALTHOUGH measurements of substitutional diffusion in metals are well documented, diffusion in ceramics and semiconductors, and even interstitial diffusion in metals, is far less clear. Several new techniques aimed at these more difficult systems were described at a conference on trends in diffusion sponsored by the Institute of Physics and the Physical Society in collaboration with the Institute of Metals and the National Physical Laboratory at Teddington on February 25 and 26. The most novel of the new techniques was probably the use of centrifuge methods to study the diffusion of noble metals in the alkali metals. Dr L. W. Barr (Atomic Energy Research Establishment, Harwell) described experiments confirming the interstitial nature of the process for gold in sodium and potassium. Studies of oxygen diffusion in TiO_2 were described by Dr D. G. Lees (University of Manchester), who told how the α-particle yield from the diffusing ^{18}O was measured as a function of the depth at which the energy of the incident proton beam is attenuated to the resonance energy of 1·78 MeV.

Present interest in thin-film electron microscopy techniques was well represented by contributions dealing with annealing studies of secondary defects. Apart from their relative experimental simplicity, these techniques have the advantage that measurements can be made at much lower temperatures than with conventional radioactive tracer methods, so that information can be obtained about the curvature of the Arrhenius plot. Professor R. E. Smallman (University of Birmingham) made it clear that these experiments have the merit that the process can be observed dynamically while the diffusion data are obtained. This is a valuable feature in aiding interpretation, as shown by the work of Dr R. N. Cornell (Berkeley Nuclear Laboratories) on the growth of fission gas bubbles in irradiated uranium dioxide.

In spite of the lost dimension, surface diffusion studies pose many problems in interpretation. Dr G. E. Rhead (University of Paris) pointed out that when the temperature is normalized to that of melting, the surface diffusion coefficients for clean metal surfaces lie on the same curve, and can be related to solid state diffusion. For surfaces containing adsorbed impurity layers the melting point of the two dimensional adsorbed compound was shown to be important, the high diffusion coefficients obtained being interpreted in terms of diffusion in a two dimensional liquid. A record value of 1 $cm^2 s^{-1}$ was claimed for the diffusion coefficient in the Pb/Cu system.

The application of diffusion in metallurgical processes such as diffusion creep is largely concerned with the efficiency of interfaces as vacancy sources or sinks. This was clearly shown to be related to the effect of surface films and solute absorption at grain boundaries. Although interfaces are usually assumed to be both high diffusivity paths and vacancy sources or sinks, Dr J. E. Harris (Berkeley Nuclear Laboratories) pointed out that experimental evidence suggests otherwise. Clearly interface phenomena are becoming increasingly important and are likely to be a centre of attention in the future.

BAND STRUCTURE

Copper Leads the Way

by our Solid State Physics Correspondent

The complicated band structure of the transition metals may at last be within reach of a solution if a new way of treating the *d* bands of copper proves to be as general as it seems. C. Y. Fong and M. L. Cohen (*Phys. Rev. Lett.*, **24**, 306; 1970) have replaced the tight binding approximation for the *d* bands by a non-local pseudopotential with an angular quantum number of 2, a technique first vented on the simpler case of potassium. By introducing parameters to respond to well known energy gaps in the zone they have obtained encouraging agreement with experiment and with other more fundamental calculations. Fong and Cohen urge that the more intractable transition metals should now be analysed.

Empirical pseudopotentials became fashionable in the wake of Abarenkov and Heine's work in 1964, which established the value of relaxing the pseudopotential formalism in favour of physically sensible model potentials. It was seen as a means of capitalizing on the weakness of the pseudopotential method, namely, the non-uniqueness of the potential due to the pseudo-wave-functions forming an overcomplete set. It soon became clear that a parametrized model could describe alkali metals with fair precision, as might be expected, but was not suitable for the transition metals, where the interaction between the *s* and *d* bands is strong. Attempts to use the tight binding approximation for the *d* bands and a pseudopotential for the *s* and *p* bands have proved of limited value, and so Fong and Cohen decided to dispense completely with the tight binding approximation in copper.

The pseudopotential they use was a square well with a simple damping term. The bands were constrained to fit experimental values at important symmetry points in the zone, and the band structure and the corresponding density of states were computed. Although only limited experimental results are available, the agreement produced by the model was good enough to make Fong and Cohen raise their sights to what may be achieved with other noble metals, and with the transition metals and their compounds, where the richer prizes lie.

There may be room, however, for an improved form of empirical pseudopotential for copper, based on the same approach to the *d* bands. Fong and Cohen could not use their model to reproduce some important details of the density of states curve, in particular the heights of the peaks, and although other calculations seem to have shown similar discrepancies there is no reason to suppose that such an unsophisticated potential should be the optimum form. But it is encouraging that this new representation of the *d* bands produced the sort of agreement that was needed to justify work on the transition metals.

VARIABLE STARS

Fast Pulses from White Dwarf

from our Cosmology Correspondent

Following the discovery by A. U. Landolt that the dwarf star Haro-Luyten Taurus 76 has a rapid variability with a period of about 12 minutes (*Astrophys. J.*, **153**, 151; 1968), B. Warner and R. E. Nather have observed this object over the 1969 observing season, in an attempt to determine the cause of its variability. Their observations seem clearly to favour a pulsational rather than an eclipsing model for HL Tau-76, for the light curves which they have obtained greatly resemble those of dwarf novae of the SS Cygni type, although HL Tau-76 varies on a remarkably short time scale when compared with those of the accepted dwarf novae. Typical quasi-periods for dwarf novae are in the range 20–100 days (*Mon. Not. Roy. Astron. Soc.*, **147**, 21; 1970).

Several important features are clear from the observations of Warner and Nather, notably that although the amplitude variations of the light curves are quasi-periodic with a period of 12·44 minutes, the size of different peaks varies so much that occasionally odd peaks seem to be absent. The identification of this variation with that of dwarf novae must be tentative, if only because the period is so short. Warner and Nather point out that although the usual period–amplitude relation for these novae does not fit HL Tau-76 this is hardly surprising in view of the extrapolation required, which is more than three orders of magnitude. The most important question raised by this discovery must be that of a possible evolutionary sequence from models with periods of about 50 days to those with periods of a few minutes. Observational effects naturally favour the discovery of the longer period variations, but there will now be some incentive to look for variable stars with shorter periods.

HEARING

Auditory Illusions

from our Experimental Psychology Correspondent

Optical illusions have fascinated psychologists for many years, not just as mere curiosities, but because when the visual system fails to interpret a stimulus correctly, this gives valuable clues as to the nature of perceptual mechanisms. Illusions and confusions of an auditory kind are very much rarer. But R. M. Warren has reported recently (*Science*, **167**, 329; 1970) that noises such as coughs can adequately replace speech sounds, with the hearer unaware that any speech sound is missing, and with the median subject in a group of twenty unable to locate the position of the cough to within five phonemes. (A phoneme is the smallest unit of speech that distinguishes one utterance from another.)

Warren's experiment involved erasing from magnetic tape 120 ms of speech sound, thus removing a complete phoneme from a word together with portions of the adjacent phonemes that might give transitional cues to the missing sound. Control subjects who were played the recording of a sentence with 120 ms of silence where the phoneme had been were easily able to notice that there was a sound missing, and correctly indicate on a written version of the sentence which part has been cut out. But nineteen experimental subjects hearing the sentence with a cough replacing the speech sound reported that all speech sounds were present. One subject reported a sound missing but chose the wrong one.

This is the third rather striking example of an auditory illusion discovered by Warren. With Gregory he reported the phenomenon of verbal transformation (reviewed by Warren, *Psychol. Bull.*, **70**, 261; 1968) in which repetition of a single word on a tape loop gave the hearer the impression that the word altered its apparent sound. Throughout a series of a few hundred repetitions the heard sound was reported to change successively into different words. A typical series of transformations heard by a subject listening to the word "tress" repeated continually for 3 min (with the subject reporting whenever a change in apparent sound occurred) was "stress, dress, stress, dress, Jewish, Joyce, dress, Jewess, Jewish, dress, floris, florist, Joyce, dress, stress, dress, purse". An explanation for this is that unaided by context and possibly encouraged by neural adaptation, perceptions break away from the sounds usually associated with a particular physical stimulus.

The second of Warren's illusions (*Science*, **164**, 586; 1969) should more properly be called a confusion. It turns out to be impossible to assign a correct ordering to four sounds, a high tone (1,000 Hz), a hiss, a low tone (796 Hz), and a buzz, each lasting for 200 ms and played repeatedly in the same order. Four spoken digits repeated at the same rate can easily be ordered even when all transitional cues such as usually occur between fluent speech sounds are absent. When listening to the cycle of sounds there is no difficulty in identifying each sound; it is the ordering that is impossible; a subject understands speech sounds which have an average duration of 70 ms and music can be repeated by musical subjects with notes occurring as fast as 20/s.

These three demonstrations indicate a surprising flexibility of the mechanisms of auditory perception. Powerful reconstructive processes may partly account for the ability to understand speech even in the midst of other distracting noises, and for tolerance of the very different accents and sounds produced by different speakers. Warren has shown that speech perception is not a matter of guessing what might have been said. The reconstructive process gives some information about the timing of irrelevant sounds and, aided by context, yields direct impressions of coherent speech.

LAND MANAGEMENT

Conservation on the Farm

from a Correspondent

A MEETING held at Monks Wood Experimental Station on February 19 and 20 should have forged contacts which will prevent future instances of two government bodies, both concerned with the countryside, giving contrary advice. The topic was land management for agriculture and conservation, and it brought together members of the Nature Conservancy and the Agricultural Land Service (ALS) of the Ministry of Agriculture, Fisheries and Food, as well as representatives of the Forestry and Countryside Commissions.

The ALS is the body which can most affect the farming pattern of England and Wales. Its officers advise farmers on all problems of estate management. Equally important they are closely concerned with the administration of the various grant and subsidy schemes which greatly influence the way farming is done, particularly in the less productive areas of Britain. The ALS is sometimes blamed by conservationists for many allegedly undesirable farming practices, including the destruction of hedgerows, factory farming of livestock and the massive use of fertilizers which pollute inland waters. Farmers, of course, complain that conservationists pay too little attention to the economics of agriculture.

The principal task facing the participants was to discover whether efficient farming need harm Britain's native flora and fauna. Because nature reserves only cover a fraction of 1 per cent of the area of lowland England, most wild plants and animals, if they are not to become extinct, must continue to be found on farm land.

There was much common ground, partly no doubt because so many members of the ALS are essentially countrymen. It had to be agreed that in many lowland areas productivity must continue to have priority, and that changes such as the removal of many—possibly most—hedgerows were inevitable. Only when farmers are keen game preservers will they retain all the cover the conservationists would desire. Mr P. J. Huguet (ALS) and Dr M. Hooper (Monks Wood Experimental Station) agreed that nevertheless even on the most intensive farms there are areas which may be set aside for the benefit of wildlife if suitable advice on their location is available. Mr J. B. Rundle (ALS) pointed out that in mountain and upland Britain farming is partly maintained for social reasons, for the subsidies might be spent more profitably (in terms of food production) in the lowlands. Here the conservationists are quite as keen as the agriculturalists that the present farming pattern should continue. Mr I. Nicholson (Nature Conservancy, Scotland) explained that controlled grazing often improves the wildlife interest.

Not all differences of approach can be resolved easily. Water pollution or eutrophication from farm wastes which cannot be economically used as manure will continue to be a problem, and so will the chemical fertilizers in the run-off from arable land, as Mr K. B. C. Jones (ALS) pointed out. Here the ALS and the National Agricultural Advisory Service may still have to give advice which they know may not be in the best interests of conservation. The only solution, said Dr K. Mellanby (Monks Wood Experimental Station), would be a change in government policy to support the use of organic manures with subsidies, something which may happen if present practices are shown to cause the deterioration in British soils which is alleged to occur in parts of North America.

The meeting revealed that clearly the ALS and the Nature Conservancy can often have an agreed policy. Where this is impossible, close collaboration can often prevent much of the damage which would otherwise occur.

ANTIBIOTICS

Sense and Sensitivity

from our Molecular Biology Correspondent

A NEW group of articles concerned with the mode of action of antibiotics of different kinds in blocking protein synthesis follows hard on the work I discussed last week. New light on the site of streptomycin action comes from Strigini and Gorini (*J. Mol. Biol.*, **47**, 517; 1970), in an article which does not yield up its treasures easily.

The burden of the authors' argument is as follows: the agents that suppress nonsense mutations are known to be tRNA species with anticodons that make sense of the nonsense. Mutations of *E. coli* and T4 bacteriophage at streptomycin loci not only vitiate the proper function of the suppressors, but also affect the extent to which translation can occur by misreading. Now the ribosomal protein associated with streptomycin sensitivity resides in the 30S particle, and has been identified by Traub and Nomura. It therefore seems that the well known manifestation of misreading, which the binding of streptomycin at this site engenders, can be simulated by a structural aberration in the same protein. By working with a set of different suppressors containing identical anticodons, Strigini and Gorini have established that the failure of the suppressor is not related to the nature of the anticodon. They have likewise shown that the identity of the aminoacyl group is not involved in the mechanism. The inference is that the ability of the aminoacyl-tRNA to bind productively to the ribosome is determined by the streptomycin-sensitive protein, which therefore evidently plays a central part in polypeptide chain propagation.

Returning now to the mechanism of antibiotic action on the ribosome, Monro and Vazquez and their collaborators have continued their studies by applying an assay related to the so-called fragment reaction (Celma *et al.*, *FEBS Letters*, **6**, 273; 1970). This takes advantage of the strong evidence that it is only the aminoacyl end of the tRNA chain that actually becomes involved with the peptidyl transferase on the 50S ribosome. By restricting the assay system to an aminoacylpentanucleotide fragment and 50S subunits, it is thus possible to evaluate the effects of added antibiotics in terms explicitly of whether or not they molest the peptidyl transferase. The fragment–ribosome interaction requires the presence of considerable concentrations of ethanol, without which this anthropomorphic enzyme will not function.

The fragment reaction itself is simply the transfer of the amino-acid from the fragment to puromycin, sitting in the so-called A site (normally associated with the attachment of the incoming aminoacyl-tRNA) on the 50S particle. This reaction is known to be strongly inhibited, for example, by the antibiotic, streptogramin *A*, which, it is also found, will effectively chase off the aminoacylpentanucleotide in the binding assay. It follows that the primary binding of the fragment is functional, occurring at the peptidyl transferase site. In a range of twelve antibiotics, three strongly and one rather weakly inhibited the binding of the fragment, and all the rest, including chloramphenicol, actually caused varying degrees of stimulation. If, as seems likely from earlier work, chloramphenicol operates at the A site, it seems that the fragment assay pertains to effects at the P site at which the growing peptidyl-tRNA chain resides. Evidence regarding whether the interactions involve direct competition or binding at a linked site is still equivocal.

The activity of antibiotics on eukaryotic protein synthesis is a relatively unexplored field, which Neth *et al.* (*ibid.*, 198) have now approached by application of the fragment reaction to ribosomes from nothing less than human tonsils, and also from yeast. The experimental conditions for amino-acid transfer to puromycin are similar to those with *E. coli* ribosomes. Three antibiotics inhibit the reaction in systems from all three species of ribosome, but others are highly selective. Cycloheximide, long known as an inhibitor of eukaryotic protein synthesis, has no effect on the fragment reaction, and evidently therefore does not attack the peptidyl transferase. Anisomycin is an antibiotic that has no effect on *E. coli* ribosomes, but is an especially deadly inhibitor of the fragment reaction in the eukaryotic species. The authors suggest that the search for antibiotics with narrowly defined cell specificities might produce all manner of rewards.

PROTEIN SYNTHESIS

Making a Start

from our Cell Biology Correspondent

How are the different initiation sequences that signal the start of cistrons in bacterial and phage messenger RNAs distinguished by bacterial ribosomes, which are presumed to be identical? This has become a question of great interest, and work in several laboratories indicates that the F3 initiation factors will prove to have a key role in providing the specificity required for initiation of a protein chain. The transitory association of an F3 factor may temporarily confer on a ribosome the capacity to distinguish one species of initiation sequence from all others.

Much less is known, of course, about the initiation of protein synthesis in eukaryotes, but it is likely that the mechanism will prove to be basically similar to that in bacteria. There have, for example, been several reports of more or less compelling evidence, chiefly from kinetic studies, which suggest that at the end of each round of translation the eukaryote ribosome, like the bacterial ribosome, dissociates into free subunits ready for the initiation of a new cycle of synthesis. In the current *Journal of Molecular Biology* (**47**, 335; 1970) Vesco and Colombo, for example, report that sodium fluoride inhibits protein synthesis in HeLa cells by preventing the dissociation of 80S ribosomes. Once the pool of free subunits is exhausted protein synthesis stops, and after removal of the fluoride the restart of synthesis is preceded by dissociation of the accumulated ribosomes. Apparently the two subunits produced by the dissociation of 80S ribosomes have a greater chance of recycling than entering the pool of free subunits.

A decision about the nature and precise role of initiation factors and the requirements for the formation of initiation complexes in eukaryotes has been hampered by lack of well characterized mRNAs. Nonetheless, it seems that different species of initiation factor(s), each specific for one or more species of mRNA, probably exist in eukaryotes. Recently Heywood (*Nature*, **225**, 696; 1970) claimed that, in addition to tRNAs and GTP, initiation factors washed from chick muscle cell ribosomes are required for the formation of a 70S initiation complex between 40S ribosome subunits and a 26S RNA fraction containing myosin mRNA. Chick reticulocyte ribosomes will also form an initiation complex with myosin mRNA but only in the presence of muscle cell initiation factors. Also recently, Hertzberg *et al.* (*Europ. J.*

Biochem., **11**, 148; 1969) have reported what may prove to be specific initiation factors in rabbit reticulocytes.

RNA virus RNAs are, of course, far more readily characterized than cellular mRNAs, and Baltimore and Huang have described recently (*J. Mol. Biol.*, **47**, 263, 275; 1970) the fate of polio virus RNA made in HeLa cells. Apparently progeny RNA molecules do not associate with ribosomes until they have left the replication complex but the RNA is bound non-specifically to a heterogeneous collection of cell protein. This RNP has a buoyant density of 1·40 g/cm^3 and it passes sequentially through forms with densities of 1·44 and 1·47 which are precursors to polyribosomes. According to Baltimore and Huang these two forms may well be respectively polio RNP associated with a 40S subunit and an 80S ribosome.

Unequivocal rather than suggestive analysis of eukaryote protein synthesis, however, depends on the future exploitation of cell free systems such as that described by Smith *et al.* (*Nature*, **225**, 184; 1970). With such a system it should be possible, for example, to test critically the claim (Tiboni *et al.*, *J. Mol. Biol.*, **47**, 467; 1970) that the G and probably T chain elongation factors from eukaryotes are specific for 80S ribosomes while the factors from prokaryotes and plastids are specific for 70S ribosomes.

AMPHIBIA

Origins of the Frog

from our Vertebrate Palaeontology Correspondent

SOME newly described fossil material from Oklahoma may help to settle the puzzle of the origin of the Amphibia. The common frog is one of the most aberrant vertebrates ever evolved. Most notably the whole skeleton is profoundly modified for jumping, but there are many other curious anatomical and physiological features. Prominent among these is the use of the whole surface of the skin for respiration. This latter character is not unique to the Anura, the group comprising the frogs and the toads, but is shared with the Urodela, or newts and salamanders, which have a shape more like a conventional primitive tetrapod. The anurans and urodeles together with the rare worm-like Apoda (caecilians) comprise the living representatives of the class Amphibia.

The record of fossil Amphibia is very full, but there is little to connect them with the living groups. The first fossil frog is a Triassic form, the first urodeles occur much later and there are no known fossil apodans. The other members of the class Amphibia are fossil forms ranging from the Devonian–Carboniferous boundary to the top of the Triassic. Two groups were present, the labyrinthodonts and the lepospondyls, but neither is obviously related to the living forms.

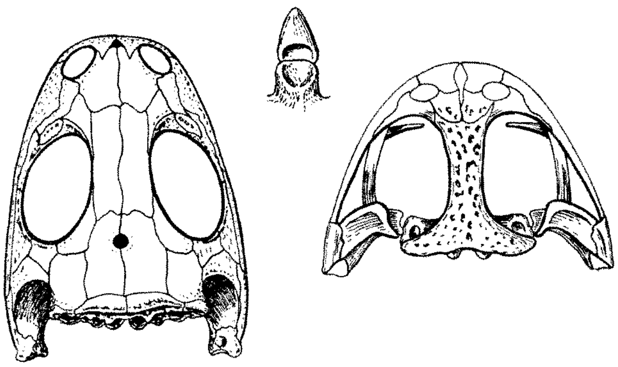
Attempts to relate the living Amphibia to labyrinthodonts or lepospondyls have been numerous and generally unconvincing. D. M. S. Watson in 1941 suggested that several small Upper Carboniferous labyrinthodonts had the reduction in the number of bones of the skull roof and in the number and nature of the vertebrae which would be expected in a frog ancestor. But the urodeles and the apodans were left without putative forefathers. The balance was restored by Romer in 1945 when he assigned the urodeles to the lepospondyls.

The Swedish tradition has been to give frogs on one hand and newts on the other an even longer separate ancestry. In the 1930s Holmgren and Säve-Söderbergh derived the urodeles from the Dipnoi or lungfish, and more recently Jarvik has developed the idea that the Anura and Urodela are derived from separate groups within the Devonian rhipidistian fishes. Unfortunately the Swedish philosophy does not require the tracing of any lineage of intermediate forms to bridge the 350 million year gap. Also later work by Gregory showed that the apparent frog-like character of the skulls of Watson's postulated anuran ancestor was largely due to the misinterpretation of difficult material.

In the past decade interest in the origin of living Amphibia has revived, largely because of the work of Parsons and Williams. In 1962 they cited a unique and unifying character of the three living orders in the structure of their teeth and in the following year discussed the characters to be expected in an ancestor for all three.

Recent speculation has thus tended to be on the ancestry of the Lissamphibia, an old term revived by Parsons and Williams to embrace all living forms. Cox has argued for the lepospondyl ancestry of Lissamphibia, largely because the latter do not use costal respiration to ventilate their lungs, whereas the labyrinthodonts, with well developed ribs, almost certainly did. It seems probable, however, that his favoured ancestors among the lepospondyls also used their ribs to breathe.

Bolt (*Science*, **166**, 888; 1969) has recently proffered the most likely Lissamphibian ancestor yet. He describes a small labyrinthodont from the Lower Permian of Oklahoma which has Parsons and Williams's tooth character. Instead of the characteristic (and diagnostic) labyrinthodont teeth *Doleserpeton*, as the animal is called, has pedicellate teeth (see figure) with a bicuspid crown separate from the root portion by connective tissue. The vertebrae also are unusual for the group of labyrinthodonts to which *Doleserpeton* belongs, and show a possible pre-Lissamphibian condition.



On the left is the skull of *Doleserpeton* (redrawn, after Bolt); in the middle, a pedicellate tooth of *Doleserpeton* (highly magnified, after Bolt). On the right is the skull of the earlier frog-like form, *Triadobatrachus* (redrawn, after Watson).

Thus *Doleserpeton* could be a proto-Lissamphibian anatomically while stratigraphically it appears at about the right geological horizon. Ironically, it is a member of the same family of labyrinthodonts as Watson's original frog ancestors.

The Chemical Physics of Ice

N. H. FLETCHER

A lot has been learned about ice in the last 20 years. The structures of several of the ten allotropes have been elucidated; the freezing process has been intensively studied; the growth and structure of crystals have been investigated, and from a practical point of view, the mechanical properties of ice, so important in climatic areas where ice is a building material, are now much better understood.

Modern theories of quantum mechanics and the solid state have led to this growth of knowledge, and this book is a case study in how to apply such formal principles to explain the structure of a relatively simple material. Professor Fletcher has collected material from many sources for this excellent monograph, which will interest pure scientists and glaciologists, meteorologists and workers in cloud physics. £4 *net*

Random Variables and Probability Distributions

Third Edition

H. CRAMÉR

First published in 1937, this tract develops the purely mathematical side of probability theory.
CONTENTS: 1 Introduction. 2 Axioms, Preliminary Theorems. *Distributions in* R_1: 3 General Properties, Mean Values. 4 Characteristic Functions. 5 Addition of Independent Variables. 6 Normal Distribution, the Central Limit Problem. 7 Error Estimation, Asymptotic Expansions. 8 A Class of Stochastic Processes. *Distributions in* R_k: 9 General Properties. 10 The Normal Distribution. 35*s. net*

Elements of Functional Analysis

I. J. MADDOX

For undergraduates who have completed basic courses on real and complex variable theory.
CONTENTS: 1 Basic Set Theory and Analysis. 2 Metric and Topological Spaces. 3 Linear and Linear Metric Spaces. 4 Normed Linear Spaces. 5 Banach Algebras. 6 Hilbert Space. 7 Matrix Transformations in Sequence Spaces. 50*s. net*

A Second Course in Mathematical Analysis

J. C. and H. BURKILL

Aimed at third year undergraduates, this book presents analysis in the abstract setting of metric spaces, providing a link with topology.
CONTENTS: 1 Sets and Functions. 2 Metric Spaces. 3 Continuous Functions on Metric Spaces. 4 Units in the Spaces R_1 and Z 5 Uniform Convergence. 6 Integration. 7 Functions from R_m to R_n. 8 Integrals in R_n. 9 Fourier Series. 10 Complex Function Theory. 11 Complex Integrals, Cauchy's Theorem. 12 Expansions, Singularities, Residues. 13 General Theorems, Analytic Functions. 14 Applications to Special Functions. £4 *net*

CAMBRIDGE UNIVERSITY PRESS

CARL ZEISS
West Germany

micro?

macro?

New Zeiss
Ultraphot III
Camera Microscope

with
Built-in photomultiplier
Shorter exposure times
Improved optical system
Sturdier base with filter unit
Pre-calibrated exposure setting

New Zeiss
Tessovar
for Macrophotography

with
magnification range 0.8 to 12.6x
Zoom optical system
Special camera exposure control
Automatic diaphragm control
with chosen magnification

New Zeiss
Camera Attachment
For all formats

Available with
CdS cell mounted in viewing
eyepiece unit
High accuracy Ikophot M
exposure meter
Electric shutter for automatic
exposures
Motorised C-35M automatic film
advance camera

Brochures and demonstrations
for all equipment from the sole
UK agents of Carl Zeiss of West
Germany: Degenhardt & Co. Ltd.,
31/36 Foley Street London
W1P 8AP Telephone 01-636 8050
(15 lines)

ZEISS
The great name in optics
Degenhardt

High-energy Gamma-ray Astronomy

by
G. G. FAZIO
Smithsonian Astrophysical Observatory,
and Harvard College Observatory,
Cambridge, Massachusetts

Gamma-ray astronomy above 10 MeV is difficult from the experimental point of view, but promising data are beginning to appear, as this survey progress report shows.

In the late 1950s the detection of cosmic photons with energy greater than 10 MeV seemed to be a most promising new branch of astronomy and a means of obtaining fresh information about the structure of the universe[1]. This was true even though the direct radiation could only be measured above the Earth's atmosphere. There were several reasons for enthusiasm. Theoretical calculations indicated that many known cosmic radio sources contained high-energy protons that could interact to produce detectable γ-ray fluxes. Another fascinating source of γ-rays was matter–antimatter annihilation in a steady state cosmology; the proposal that the radio source Cygnus A could be matter–antimatter galaxies in collision acted as a spur to the experimentalist. In addition, γ-ray photons are very penetrating, and travel in a straight path from the source so that they can be detected from sources at cosmological distances and from sources invisible in other regions of the spectrum. Gamma rays could also penetrate the upper atmosphere just enough to be detected at balloon altitudes. Apparatus for measuring the predicted fluxes already existed in laboratories with high-energy particle accelerators; it needed only to be made rugged and sufficiently compact to be carried by high-altitude balloons and satellites.

Many cosmic ray and nuclear physicists were attracted to this new field, and the search for sources began. Almost ten years of frustration and hard work followed. Scintillation counters, nuclear emulsions, and spark chambers in balloons and satellites were used and a few reports of sources were never substantiated. Only upper limits were published for the most likely radio sources. These did produce some significant facts about sources but hardly had the impact of a direct flux measurement. Finally, in 1968 the most conclusive evidence for a diffuse high-energy γ-ray flux from the galactic plane was published. The experiment, conducted by Clark, Garmire, and Kraushaar[2], consisted of a complex scintillation-Čerenkov detector on the third Orbiting Solar Observatory (OSO 3). In 1969, scientists at Case Western Reserve University and at the University of Melbourne cooperated in joint high-altitude balloon experiments and reported evidence for a point γ-ray source in Sagittarius[3].

Morrison[4] pointed out (with hindsight) that, in general, the cosmic electromagnetic spectrum exhibits a monotonic decline in intensity per unit frequency as a function of frequency, and so experimenters should first investigate the lowest energy photons beyond the visible that can be transmitted through a given galactic region. On this basis, X-rays (~1,000 eV) would disclose the first important results in high-energy astronomy, which indeed was the case. The discovery of cosmic X-rays by Giacconi, Gursky, Paolini and Rossi[5] in 1962, several years after γ-ray astronomy became active, has led to a rich field of research in high energy astronomy. In the short-wavelength region of the spectrum, isotropic diffuse radiation has been detected over the energy range from 0·2 keV–6 MeV, and discrete sources (for example, the Crab Nebula, Cygnus XR-1, and M 87) have been detected to energies of the order of 500 keV. An information gap appears in the spectrum from 1–50 MeV and above 1 GeV (10^9 eV).

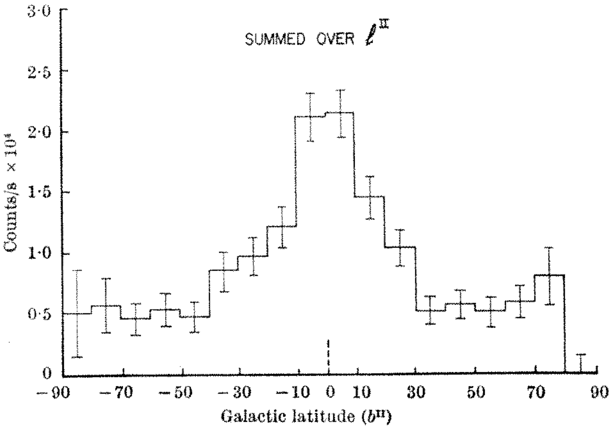


Fig. 1. OSO 3 data showing the dependence of γ-ray intensity on galactic latitude, summed over all galactic longitudes.

Energy Region 10 MeV–1 GeV

The existence of high-energy γ-rays (> 100 MeV) from the galactic plane and particularly from the galactic centre is rather definite. The OSO 3 (refs. 2 and 6) measurements of the directional properties of this radiation are most convincing (Figs. 1 and 2). As the resolution of the detector is ±15°, the angular distribution of the photons is consistent with a line source along the galactic plane, with strongest emission from the galactic centre. The distribution in longitude, however, is much broader than the distribution expected for a point source at the galactic centre. Significant peaks of lesser intensity along the galactic equator are also apparent in the data. The average line intensity in regions more than 60° from the galactic centre is about 0·25 of the line intensity near the centre. What is still in doubt is the absolute intensity that is quoted to be 3×10^{-4} photon cm^{-2} s^{-1} rad^{-1} from a region encompassing the galactic centre. There is still a discrepancy between measurements of the Earth albedo flux from OSO 3 and the albedo measurements of Explorer 11 and high-altitude balloons. The OSO 3 results are a factor of four higher than those of Explorer 11, and a factor of three higher than the results of Fichtel, Kniffen and Ögelman[7]. Several groups[3,7–10] using high altitude balloons have reported supporting evidence for the OSO 3 results but at a marginal statistical level. Recent results of the OGO 5 spacecraft experiment of the University of Southampton[11] have also indicated a maximum γ-ray intensity in the direction of the galactic plane. Table 1 compares the results of two such groups with the OSO data in the region of the galactic centre. Of particular importance are the recent results of Frye *et al.*[3], who can only reconcile their data with OSO 3 if the line source has a half-width of 15° and an intensity less by at least a factor of four. These discrepancies are being investigated by recalibration of the satellite and balloon detectors and by additional balloon flights from Australia.

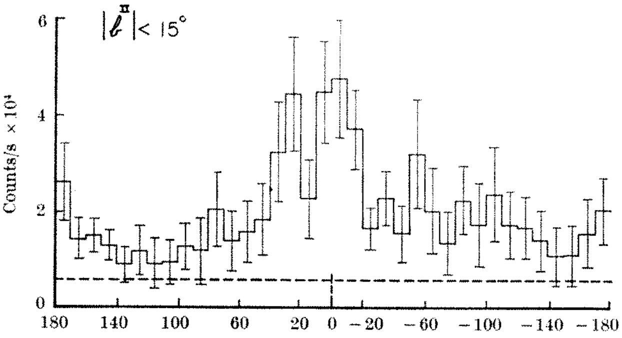


Fig. 2. OSO 3 data showing the dependence of γ-ray intensity on galactic longitudes near the disk. The dotted line shows the average rate at high galactic latitudes.

There is also evidence from the OSO data for an isotropic component of cosmic γ-rays with a background intensity of $(1{\cdot}1 \pm 0{\cdot}2) \times 10^{-4}$ photon cm^{-2} s^{-1} sr^{-1}, but it is difficult to measure and is not so convincing as the galactic flux. It is interesting to note that, within the errors discussed, this flux is in rough agreement with the value predicted by a power-law extrapolation of the isotropic X-ray flux to higher energies. The photon intensity per unit energy interval can be approximated by a spectrum of the form dE/E^2, implying equal amounts of energy per decade of photon energy.

The spectrum measurements, although of rather poor resolution, indicate that γ radiation from the galactic plane is on average more energetic than radiation from the isotropic component at high galactic latitudes.

Above 1 GeV, only upper limits exist for the isotropic γ-ray flux, which have been determined primarily by nuclear emulsion experiments in balloons[12–15]. Large air-shower experiments, based on the detection of showers with a low number of μ-mesons, have given upper limits of 10^{-13} photon cm^{-2} s^{-1} sr^{-1} to the intensity of isotropic γ-rays in the energy region 10^{14}–10^{16} eV (refs. 16 and 17). Recent evidence[18], however, has indicated that these showers were not caused by cosmic γ-rays.

In the energy region from 50 MeV–1 GeV, two important conclusions can be drawn from the upper limit measurements of the isotropic γ-ray flux. (*a*) If matter is being created, as required by steady state cosmology, matter and antimatter cannot be created in the metagalaxy in comparable amounts in the same region. The Explorer 11 and OSO 3 experiments indicate that the density ratio of antiprotons to protons is less than 10^{-6}. Steigman[19] has shown that if a charge-symmetric steady state cosmology is to survive, matter and antimatter must be created in separate regions, or, as Hoyle[20] has proposed, antimatter must be created in dense regions, such as galactic nuclei, where the annihilation γ-rays would be absorbed and never detected. (*b*) These same experiments indicate that the high-energy intergalactic electron density is less than the galactic density by at least a factor of ten. This conclusion is based on the flux predicted by Compton scattering of high-energy electrons on photons of visible light.

Table 1. GAMMA-RAY LINE INTENSITIES MEASURED NEAR THE GALACTIC CENTRE

Experiment	Energy region (MeV)	γ-Ray line intensity (10^{-4} cm^{-2} s^{-1} rad^{-1}) for given galactic latitude interval		Galatic longitude l^{II}
		$b^{II} = \pm 15°$	$b^{II} = \pm 3°$	
Clark, Garmire and Kraushaar[6] (OSO 3 satellite)	> 100	3	—	−30° to +30°
Fichtel, Kniffen and Ögelman[7] (balloon)	30 to 100	$0{\cdot}9 {+2{\cdot}2 \atop -0{\cdot}9}$	—	−10° to +25°
	> 100	2·4 ± 2·4	2·2 ± 1·1	
Frye, Staib, Zych, Hopper, Rawlinson and Thomas[3] (balloon)	> 50	≤0·7*	≤0·3*†	−30° to +30°

* Upper limits are at 95 per cent confidence level.

† $b^{II} = \pm 2{\cdot}3°$.

The evidence for the detection of cosmic γ-radiation above 50 MeV from a discrete source in Sagittarius[3] is not so strong as the evidence for the galactic component. The measured intensity of the source, designated Sgr γ-1, is $(3 \pm 1) \times 10^{-5}$ photon cm^{-2} s^{-1} and its preliminary position is $\alpha = 288° \pm 3°$ and $\delta = -35° \pm 2°$ ($l^{II} = 3°$, $b^{II} = -20°$). There is marginal evidence for the source in each of two high-altitude balloon flights from Australia, performed 21 days apart in February 1969. Two different multiplate spark-chamber detectors of similar design were used on the two flights, and the data were analysed separately, thus eliminating systematic errors.

The detection of a source above atmospheric background is based on two facts. First, in angular resolution bins of equal solid angle, $\Delta\alpha = 4°$, $\Delta \sin \delta = 0{\cdot}08$, the maximum number of events occurred in the same bin on the first flight for pair tracks as well as for single tracks. A γ-ray photon, interacting in the plates of a spark chamber to form an electron–positron pair, leaves visible tracks in the form of an inverted V. At higher energies this pair may not be resolved and a single track, originating in the chamber, results. Second, the maximum signal of the second flight occurred at the same values of right ascension and declination as the maximum signal on the first flight to within one angular resolution bin. The data are summarized in Table 2. The evidence for a source in each flight separately is not significant, but when the two flights are taken together, the effect is seen.

There are in the literature several examples of the possible detection of discrete γ-ray sources above 50 MeV (notably the Cygnus source[21] found in 1966).

These sources were observed on only one flight. In examining these experiments, which were performed by competent groups, no obvious error appears in the data. One explanation, which has never been invoked because it was the easiest way out of the dilemma, is time-variable sources. In view of the recent examples of the sudden appearance of new X-ray sources, the idea of time-dependent γ-ray sources should be considered anew. After a successful balloon flight, usually at least 4–6 months elapse before the results become known to other groups. If a new source is discovered, several more months elapse before another group can verify the results. If the half-life of a source is of the order of 1 month (as with some X-ray stars), then few sources will be verified. The only solution is the long exposure times of satellite-borne detectors.

Table 2. SPARK-CHAMBER DATA FROM FRYE *et al.*[3] ON THE γ-RAY SOURCE IN SAGITTARIUS

	Number of events (pair + single tracks) per equal solid angle bin Source	Background	Poisson probability
Flight I (February 5–6, 1969)	57	36·7 ± 1·0	0·2
Flight II (February 26–27, 1969)	54	33·4 ± 1·0	0·1
Combined	111	70·1 ± 1·4	8×10^{-4}

Astronomy above 10^{11} eV

At photon energies above 10^{11} eV, cosmic γ-rays, interacting within the atmosphere, generate a cascade of high-energy particles and visible Čerenkov light, both of which can be detected with ground-based instruments. Although the expected photon intensity in this energy range is considerably below that expected for the 100-MeV region, the simplicity and relatively low cost of instrumentation encourage experimentation. Again, theoretical predictions, based on Compton-scattering modes in radio sources, have indicated detectable fluxes.

Since 1960, several research groups[22-26] have developed this ground-based technique to search for discrete sources of cosmic γ-rays, particularly from intense radio sources. More recently the emphasis has been on pulsars, because the detection of radiation of this energy would certainly give credence to the theory that pulsars are the source of cosmic rays. Although several indications of positive results have been published[23,26-29] of both continuous and pulsed radiation, they have never been verified[30,31]. Recent upper limits to the flux from the Crab Nebula at 10^{11} eV found by the group at the Smithsonian Astrophysical Observatory[32] are a factor of fifteen below the theoretical predictions[33,34].

Again these negative results have been valuable in interpreting processes, particularly in the Crab Nebula. They have shown that (1) the high-energy electrons in the Crab Nebula could not have been the result of secondary interactions of protons, but must have been accelerated from lower energies[22,35,36], and (2) on the assumption that the continuous radiation, from radio to visible, is synchrotron radiation, the average magnetic field in the Crab Nebula must be greater than $1{\cdot}5 \times 10^{-4}$ G (ref. 37).

Status of Theoretical Calculations

There are two basic ways by which high-energy γ-rays can be produced. First, by particle interactions that convert kinetic energy to photon energy, and second, by matter–antimatter annihilation. In the first process the particular electro-magnetic interactions are bremsstrahlung and Compton scattering by high-energy electrons; the strong interaction is neutral π-meson production in high-energy proton collisions. In the second process protons and antiprotons annihilate into charged and neutral π-mesons. The neutral π-meson decays into two γ-ray photons. A summary of the properties of these interactions is given in Table 3.

Table 3. SUMMARY OF INTERACTIONS THAT PRODUCE HIGH-ENERGY γ-RAYS*

Interaction	Intensity proportional to the product	Characteristic γ-ray energy	Differential γ-ray spectrum
Bremsstrahlung	$(I_e)(\varrho_g)$	E_e	$E_\gamma^{-\Gamma_e}$
Compton scattering	$(I_e)(\varrho_{ph})$	$\gamma_e^2\varepsilon$; $\gamma_e\varepsilon \ll m_ec^2$	$E_\gamma^{-(\Gamma_e+1)/2}$
Neutral π-meson decay	$(I_p)(\varrho_g)$	70 MeV	Peak at 70 MeV; $E_\gamma^{-4(\Gamma_p-1/2)/3}$ for $E_\gamma \gg 70$ MeV
Matter–antimatter annihilation	$(\varrho_p)(\varrho_{\bar{p}})$	70 MeV	Sharp peak at 70 MeV

* Here, $I_e = K_eE_e^{-\Gamma_e}$ and $I_p = K_pE_p^{-\Gamma_p}$ are the differential intensity spectra of electrons and protons, respectively; ε is the low-energy photon energy and m_ec^2 is the rest energy of the electron; ϱ_i is the density of gas (g), low-energy photons (ph), protons (p) or antiprotons ($\bar{p}$), $\gamma_e = E_e/m_eC^2$.

Theoretical activity at present is devoted primarily to explaining the 100-MeV γ-ray flux from the galactic centre and plane. Before the discovery of this radiation, the most optimistic and reliable calculations of a galactic γ-ray flux were based on neutral π-meson production in cosmic-ray proton collisions with the interstellar gas. If the proton flux were assumed the same everywhere in the galactic disk, the γ-ray flux would be proportional to the integrated interstellar hydrogen-gas density subtended by the field of view of the detector. The cosmic-ray proton intensity was assumed to be the same as that measured at the Earth, and the gas density was obtained from 21-cm radio measurements. The present OSO 3 flux is a factor of ten higher than the predicted value and exhibits a greater dependence on galactic longitude than the calculated value. The discrepancy in intensity could be due to (1) a large amount of cool or molecular hydrogen[38] or (2) higher cosmic-ray proton intensity in the galactic disk. The difference would also be reduced by a factor of three or four if the flux measurements were too high, as previously indicated. The theoretical intensity, based on the usual assumptions about gas density and cosmic-ray intensity, could be increased by a factor of two, but not much more. Even if the flux intensity is explained, the distribution in galactic longitude is difficult to explain with present models of π-meson production.

Bremsstrahlung by cosmic-ray electrons in the interstellar gas, under assumptions similar to those used in the proton calculations, gives a flux a hundred times smaller than the OSO 3 results.

Compton scattering[39,40] (inverse Compton effect) by cosmic-ray electrons on known optical stellar photons or the microwave (2·7 K) blackbody radiation cannot explain the flux. But Cowsik and Pal[41] and Shen[42] have shown that Compton scattering on the infrared (8 K) blackbody radiation, reported by Shivanandan, Houck and Harwit[43], can give γ-ray flux values of the measured intensity and angular distribution in the galactic plane. The flux at the galactic centre can only be explained by assuming an additional intense infrared source at the centre or the galactic centre to be a strong cosmic-ray source. The existence of universal or even galactic infrared radiation at such a temperature and density is still in doubt.

It is interesting to note that the measured flux is similar in galactic longitude distribution to the nonthermal radio noise[6] at 1·5 m and to the cumulative flux from discrete X-ray sources within 15° of the plane. Using this latter fact, Ögelman[44] obtains agreement with the γ-ray flux by extrapolating X-ray spectra to 100 MeV (three decades) assuming a power-law spectrum of index two. Although a meaningful extrapolation is doubtful, the idea that the flux arises from a group of unresolved discrete sources (whatever their nature) in the galactic plane is interesting and very probable[2,45].

A review of these theoretical calculations is given by Clark and colleagues[6].

Theoretical explanations of the isotropic and presumably metagalactic γ-ray flux are based on experimental data from the one OSO 3 measurement at 100 MeV and a power-law extrapolation to 100 MeV of the X-ray data at 100 keV–1 MeV. The most popular explanation is Compton scattering by relativistic metagalactic electrons on the universal microwave (2·7 K) radiation[46]. The greatest uncertainty in these calculations is the intensity of relativistic electrons, which are assumed to come from radio galaxies. There have been several attempts to place the source of this radiation at early epochs (redshifts of $z \geq 2$) to ease energy requirements and to match the observed spectrum. Stecker[47] has shown that the radiation above 1 MeV may be due to neutral π-meson decay at $z = 100$. The isotropic γ-ray flux may well be a remnant of an early phase of the universe.

Again, assuming the presence of the universal infrared radiation, which has an energy density more than fifty times greater than the microwave radiation, the isotropic γ-ray flux can be explained by Compton scattering of cosmic ray electrons in the galactic halo. O'Connell and Verma[48] have shown that this same interaction also produces the X-ray background flux. If galactic in origin, however, this X-ray flux will not be isotropic outside the galactic disk, in contradiction to the measured isotropy of ~ 10 per cent. Although the predicted values of background flux seem reasonable in these models, many other consequences of the universal infrared radiation cast doubt on its existence.

Recently Longair and Sunyaev[49] have suggested that the hard X-ray portion of the isotropic radiation is produced by Compton scattering on the infrared photons in the nuclei of galaxies (Seyfert galaxies, for example).

Above γ-ray energies of 100 MeV, neutral π-meson production may become the important source of the isotropic radiation because the electrons needed to produce γ-rays by Compton scattering very rapidly lose energy by radiation.

The isotropic γ-ray spectrum is not expected to continue beyond 10^{14} eV, because of interactions with the universal microwave radiation[50,51]. The photon–photon interaction at these energies results in the production of an electron–positron pair.

The real origins of both the galactic and the metagalactic γ-ray flux still remain a mystery, and at this stage the success of theoretical activity depends on the quality and quantity of the experimental data.

Recent calculations of the γ-ray flux expected from strong radio sources[33,34] are based on Compton scattering by relativistic electrons on their own synchrotron radiation. From the measured synchrotron spectrum (radio to X-ray), and an assumed average magnetic field intensity, the relativistic electron intensity and spectrum as well as the photon density can be calculated. Comparison with experimental data permits the mean value of the magnetic field to be determined. These discrete-source flux predictions are also most useful in designing new detectors.

Apparao[52] has also considered Compton scattering by relativistic electrons in radio sources on the 2·7 K universal radiation. This effect is important in expanded sources, whereas the Compton-synchrotron effect dominates in compact sources.

Greisen (unpublished) has pointed out that in contrast to the difficulty of finding adequate mechanisms to account for the diffuse γ radiation, which has been detected, there are numerous means of producing γ-rays from discrete sources as yet undetected.

Detailed discussions of the theoretical aspects of γ-ray astronomy are given in review articles by Ginzburg and Syrovatskii[53], Garmire and Kraushaar[54], Hayakawa and Matsuoka[55], Gould and Burbidge[36], Fazio[56], and Lüst and Pinkau[57].

Gamma-ray Detectors

In the 100-MeV region a γ-ray photon can be detected only when it interacts with matter, producing an electron and positron. The particle pair branches in the direction of the initial γ-ray photon with an opening angle of the order of $m_e c^2/E_\gamma$, where E_γ is the photon energy. The γ-ray energy and direction can be determined from the kinematics of the pair. Unless the converting material is very thin, the electron and positron undergo considerable multiple scattering. Thus a typical detector has an angular resolution only of the order of 1°. Energy measurements, determined from multiple-scattering measurements or by total absorption of the pair, have only fair resolution (10–50 per cent). The minimum detectable flux, I_m, from a discrete source in the presence of a background flux, B, is given by

$$I_m \propto \left(\frac{B\Omega}{\varepsilon A T}\right)^{1/2}$$

where Ω is the solid angle of the resolution cone of the detector, A the sensitive area, ε the conversion efficiency, and T the observing time.

Table 4. MINIMUM DETECTABLE FLUX OF γ-RAYS FROM THE CRAB NEBULA (UNPUBLISHED WORK OF GREISEN)

γ-Ray energy threshold	Upper limit to the integral flux (photon cm^{-2} s^{-1})	Extrapolated integral flux (photon cm^{-2} s^{-1})
10 MeV	4×10^{-3}	$1·9 \times 10^{-4}$
50 MeV	$1·7 \times 10^{-5}$	
100 MeV	$3·1 \times 10^{-5}$	$1·5 \times 10^{-5}$
500 MeV	5×10^{-6}	
1 GeV	12×10^{-8}	$1·2 \times 10^{-6}$
2 TeV	1×10^{-10}	
10^3 TeV	3×10^{-15}	

The present minimum detectable flux levels, as functions of energy, for a discrete source such as the Crab Nebula are given in Table 4. The fluxes are already quite small (the optical flux from Polaris is ~100 photons cm^{-2} s^{-1}) and the values of I_m must be made considerably smaller if other sources are to be found. The values of B, Ω and ε are relatively fixed. The γ-ray background flux at balloon altitudes is of the order of 10^{-3} photon cm^{-2} s^{-1} sr^{-1}, and in space, 10^{-4} photon cm^{-2} s^{-1} sr^{-1}. These detectors must also discriminate a background flux of charged particles more than 1,000 times as intense as the γ radiation. The value of Ω (for 1°) is 10^{-3} sr, and ε is about 0·2. The only parameters that can change by orders of magnitude are area and time. The ultimate experiment is therefore a large-area detector in a satellite or on the Moon.

Three basic types of detectors have been used so far: scintillation-Čerenkov counters, nuclear emulsions, and spark chambers. Detailed descriptions of these detectors have been given in review articles by Greisen[58] and Kraushaar[59]. The scintillation-Čerenkov counter telescope was the principal detector used in the early days of γ-ray astronomy. A typical area was 100 cm² with an angular resolution of ±15°. These detectors reached their most sophisticated development in the satellite experiments of Explorer 11 and OSO 3 (Fig. 3). Although they offered high conversion efficiency and sufficient sensitive area, they had poor angular resolution and poor discrimination against background radiation. Nuclear emulsions offered high angular and energy resolution, low energy threshold (~20 MeV), and simplicity, but had the disadvantage of continuous sensitivity and long and tedious data reduction (in a sense that they contained too much information).

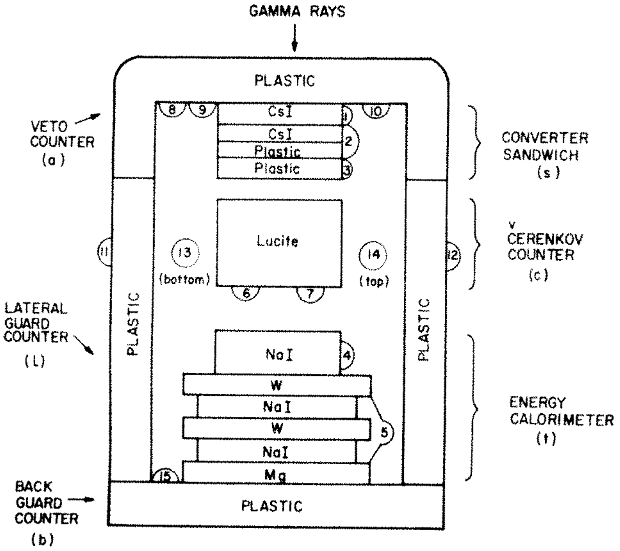


Fig. 3. Schematic diagram of the γ-ray detector flown aboard OSO 3 in 1967.

In the mid-1960s the spark chamber became the primary detector. It is essentially a device for making visible the electron–positron tracks by means of a spark discharge along the paths of the ionized particles. The tracks can be recorded by film, by a vidicon camera, by sonic pickups, or digitally by wire grids connected to a magnetic memory. The advantage of the spark chamber is that it is a visual device with selective sensitivity, so that unwanted background events are low or can be identified. The chamber has a relatively large sensitive area (10^2–10^4 cm²) and a large acceptance angle (±30°) with angular resolution of the order of ±1°, which make it an ideal survey instrument. The spark chamber has already provided γ-ray astronomy with a significant increase in sensitivity; the next major advance will occur in 1971 when the group at the Goddard Space Flight Center, led by C. Fichtel, will fly a large ($6\cdot5\times10^2$ cm²) digital spark chamber (Fig. 4) in the Small Astronomy Satellite (SAS-B). In 1972 a joint European group (University of Milan; Centre d'Etudes Nucléaires de Saclay, France; Max-Planck-Institut für Extraterrestrische Physik, Munich) will launch a vidicon spark chamber (132 cm²) in the ESRO satellite TD-1.

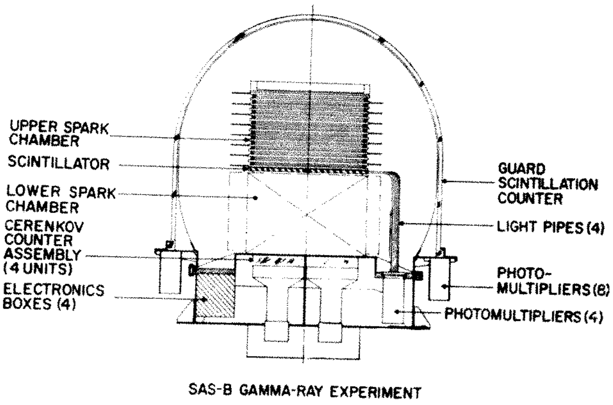


Fig. 4. Schematic diagram of the digital spark chamber to be flown on the Small Astronomy Satellite (SAS-B) in 1971 by the Goddard Space Flight Center.

Emulsion spark-chamber combinations are also being used, with the promise of greater angular resolution and lower threshold energy. In this detector the spark chamber is used to locate the γ-ray conversion point in the emulsion. Groups at Washington University (St Louis), University of Minnesota, and the Naval Research Laboratory are now using this technique.

Fig. 5 shows the minimum detectable flux at 100 MeV that could be measured by γ-ray detectors over the past ten years and the expected sensitivity of future detectors. Note that the minimum detectable flux has decreased by an order of magnitude every 2·3 years and should continue at that rate, reaching sensitivities of $10^{-6}-10^{-7}$ photon cm^{-2} s^{-1} within the next two years.

An attempt to increase significantly the area of detectors has brought about the development of the gas-Čerenkov detector by a joint Cornell University–Smithsonian Astrophysical Observatory–Naval Research Laboratory group. The single detector has an area of 5×10^4 cm² with an angular resolution of ±1°, an acceptance angle of ±3°, and (expected) excellent background rejection. The first balloon flight of the instrument is expected in 1970, and it should be able to measure sources as weak as 5×10^{-7} photon cm^{-2} s^{-1} in six hours. The detector is also being proposed for a large spacecraft mission. A smaller version of this detector ($1\cdot9\times10^3$ cm²) has been designed[60] for the energy region from 10 to 100 MeV.

Although the most sensitive experiments will be performed in satellites, much work can still be done by high-altitude balloons, particularly as balloons become larger and more reliable. For example, with a spark chamber it should be possible to confirm definitely the galactic γ-ray flux measurements of OSO 3. These experiments are now being performed by groups at the Goddard Space Flight Center, the Case Western Reserve University and the University of Southampton, and the results will be known soon. Balloon experiments at present compete with satellite experiments as regards sensitivity in the search for discrete sources. Because of atmospheric

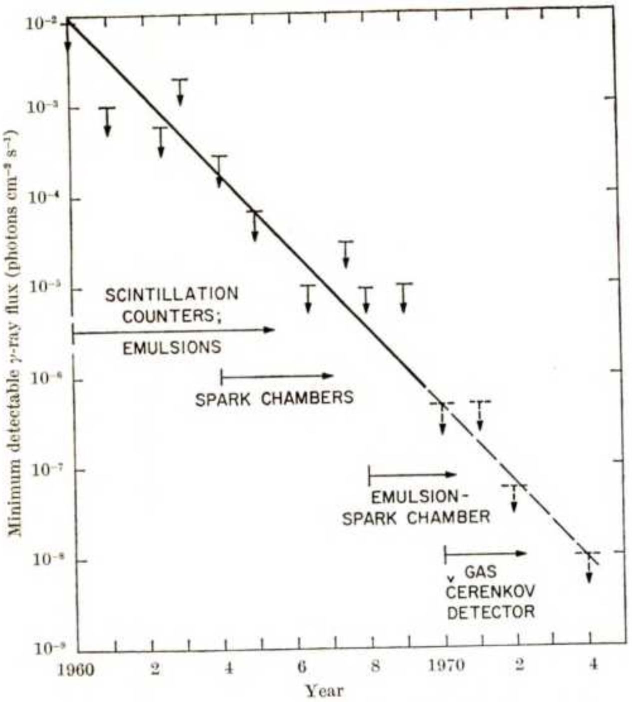


Fig. 5. Decrease in the minimum detectable γ-ray flux levels at 100 MeV over the years.

background radiation, the isotropic γ-ray flux will be best measured in satellite experiments. A continued balloon programme is also needed to develop and test new detectors.

I have mentioned that γ-ray astronomy above energies of 10^{11} eV is performed with ground-based detectors. A cosmic γ-ray of these energies interacts with air molecules and generates a cascade of high-energy electrons, positrons and secondary γ-rays. This cascade, called an air shower, is highly directional and proceeds with relativistic velocity in the direction of the initial γ-ray photon. The relativistic charged particles in the air shower generate a cone of Čerenkov light of about 2° total opening angle in the direction of the shower axis. The light, which is primarily in the blue and ultraviolet region of the visible spectrum, occurs as a burst lasting about 10^{-8} s, and covers a ground area of 10^8 cm². The detection of this light at the lowest possible γ-ray energies requires a dark site, a cloudless, moonless night, and a sensitive light collector. Unfortunately, high-energy cosmic ray particles, primarily protons, also generate air showers and bursts of Čerenkov light in the atmosphere, resulting in a uniform distribution of this light over the sky (approximately 10^{-4} of the total light of the night sky). Gamma rays can be distinguished at present only by increased numbers of light pulses coming from the direction of possible sources. A typical experiment therefore consists of positioning a mirror and phototube detector ahead of a suspected source and recording the rate of observed light pulses as the Earth's rotation carries the source across the field of view. Such a telescope can detect γ-rays over an area of 10^8 cm² with an angular resolution of about 1°. Jelley has written a review on this technique[61].

A typical detector used in this energy region consists of a cluster of searchlight mirrors, 1–1·5 m in diameter, mounted on a steerable frame. Such a detector, developed by the Atomic Energy Research Establishment, Harwell, and University College, Dublin, and now being used in Malta, is shown in Fig. 6. The Smithsonian Astrophysical Observatory has in operation, in Arizona, a fully steerable 10-m reflector consisting of a mosaic of 248 small hexagonal mirrors (Fig. 7).

Although no sources have been detected definitely in this energy region, the search continues, with longer exposure times and in an all-sky survey. Pulsars may be sources of 10^{16} eV particles and considerable effort, by all groups, is being devoted to a search for γ-rays from these objects.

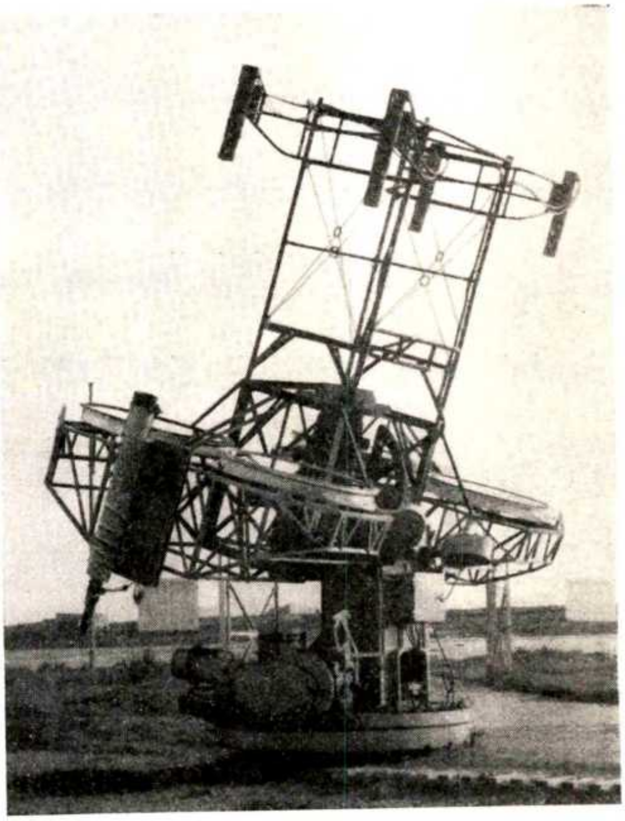


Fig. 6. The Čerenkov γ-ray installation operated jointly by the Atomic Energy Research Establishment, Harwell, and University College, Dublin. This installation consists of four 90 cm diameter f/2 mirrors and is sited at Qrendi, Malta.

It is very difficult to lower the threshold γ-ray energy by a significant amount; however, a considerable increase in sensitivity, perhaps a factor of 30, could be obtained if showers induced by γ-rays could be distinguished from proton-induced showers.

It is interesting to note that all major atmospheric Čerenkov light detectors are located in the northern hemisphere and almost no research has been carried out south of the equator.

Future Progress

Progress depends greatly on new experimental data. The origin of the galactic γ-ray flux remains a major mystery. Is it diffuse and generated by interactions of high-energy particles in the interstellar medium, or is it composed of discrete, unresolved sources ? Further theoretical work cannot proceed without more data. Another question is how many discrete sources, "γ-ray stars", exist ? Are they a transient phenomenon ? Are they galactic in origin ? A systematic all-sky survey is needed to answer these questions. Are pulsars γ-ray

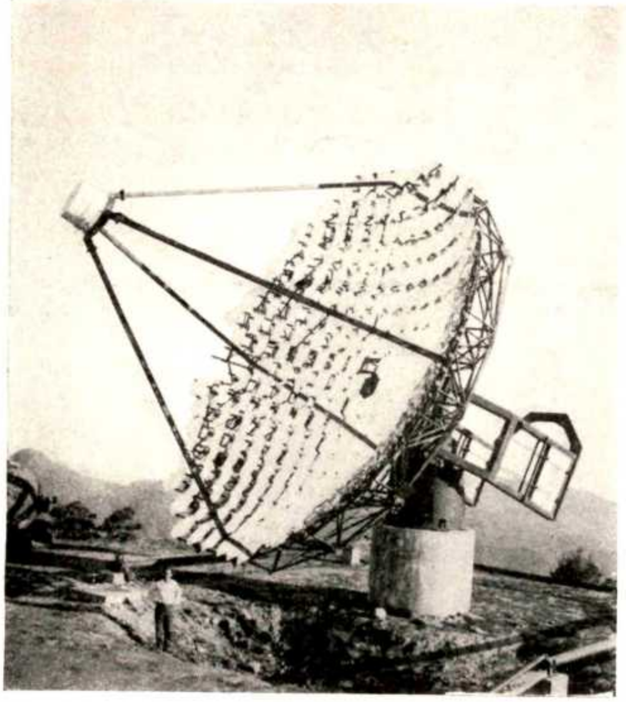


Fig. 7. The 10-m optical reflector at Mt Hopkins, Arizona, used by the Smithsonian Astrophysical Observatory to search for cosmic γ-rays at 10^{11} eV.

sources ? Detection of such radiation would provide very strong evidence that pulsars are the sources of cosmic rays. Of more cosmological significance is the origin of the diffuse γ radiation. Is it a remnant of an early stage of development of the universe ?

The experimental data necessary to answer these important questions may be slow in coming. The next major satellite detector, the digital spark chamber on the SAS-B satellite, will not be launched until 1971. Larger detectors in manned space stations will not be launched before 1974. Recent balloon experiments from Australia should yield more information on the γ-ray flux from the galactic centre, particularly its intensity and angular distribution; however, satellites are needed to investigate the galactic plane and the isotropic radiation.

New ideas in detector development are needed. For significant increases in sensitivity, $\sim 10^{-7}$ photon cm^{-2} s^{-1} at 100 MeV, there seems no alternative to the increase in the area-time product. The gas-Čerenkov detector or clusters of spark chambers may be a solution. With the use of large space stations or the Moon, there seems to be no serious limit to the sensitive area that can be obtained. Energy resolution must be increased to distinguish between mechanisms of γ-ray sources; this is particularly important in the 10–100 MeV region. Although difficult to achieve, higher angular resolution is also needed at all energies. A lunar-based detector, using the horizon as an occulting edge, could achieve resolutions of minutes of arc.

Questions about the distribution and density of high-energy particles, matter, and photons in the universe may be answered only by the future results of γ-ray astronomy. Compared with optical and radio astronomy, the field is relatively young and experiments are still difficult to perform. Exciting results are just now being produced, and the future should be even more promising.

[1] Morrison, P., *Nuovo Cim.*, **1**, 858 (1958).

[2] Clark, G. W., Garmire, G. P., and Kraushaar, W. L., *Astrophys. J. Lett.*, **153**, L203 (1968).

[3] Frye, jun., G. M., Staib, J. A., Zych, A. D., Hopper, V. D., Rawlinson, W. R., and Thomas, J. A., *Nature*, **223**, 1320 (1969).

[4] Morrison, P., in *Perspectives in Modern Physics* (edit. by Marshak, R. E.), 343 (Interscience Publ. Co., New York, 1966).

[5] Giacconi, R., Gursky, H., Paolini, F. R., and Rossi, B. B., *Phys. Rev. Lett.*, **9**, 439 (1962).

[6] Clark, G. W., Garmire, G. P., and Kraushaar, W. L., *Proc. IAU Symp. No. 37, Rome* (edit. by Gratton, L.) (in the press).

[7] Fichtel, C. E., Kniffen, D. A., and Ögelman, H. B., *Proc. IAU Symp. No. 37, Rome* (edit. by Gratton, L.) (in the press).

[8] Delvaille, J. P., Albats, P., Greisen, K. I., and Ögelman, H. B., *Canad. J. Phys.*, **46**, S425 (1968).

[9] Valdez, J. V., and Waddington, C. J., *Astrophys. J. Lett.*, **156**, L85 (1969).

[10] Sood, R. K., *Nature*, **222**, 650 (1969).

[11] Hutchinson, G. W., Pearce, A. J., Ramsden, D., and Wills, R. D., *Proc. IAU Symp. No. 37, Rome* (edit. by Gratton, L.) (in the press).

[12] Anand, K. C., Daniel, R. R., and Stephens, S. A., *Canad. J. Phys.*, **46**, S484 (1968).

[13] Kidd, J. M., *Nuovo Cim.*, **27**, 57 (1963).

[14] Duthie, J., Fowler, P. H., Kaddoura, A., Perkins, D. H., and Pinkau, K., *Nuovo Cim.*, **24**, 122 (1962).

[15] Abraham, F., Kidd, J., Koshiba, M., Levi Setti, R., Tsao, C. H., and Wolter, W., *Nuovo Cim.*, **28**, 221 (1963).

[16] Toyoda, Y., Suga, K., Murakami, K., Hasegawa, H., Shibata, S., Domingo, V., Escobar, I., Kamata, K., Bradt, H., Clark, G., and LaPointe, M., *Proc. Ninth Intern. Conf. Cosmic Rays, London*, **2**, 708 (1965).

[17] Gawin, **J**., Hibner, J., Wdowczyk, J., Zawadzki, A., and Maze, R., *Proc. Ninth Intern. Conf. Cosmic Rays, London*, **2**, 639 (1965).

[18] Catz, Ph., Maze, R., Gawin, J., Przasnycki, M., Wdowczyk, J., and Zawadzki, A., *Phys. Rev. Lett.*, **23**, 988 (1969).

[19] Steigman, G., *Nature*, **224**, 477 (1969).

[20] Hoyle, F., *Nature*, **224**, 477 (1969).

[21] Duthie, J. G., Cobb, R., and Stewart, J., *Phys. Rev. Lett.*, **17**, 263 (1966).

[22] Chudakov, A. E., Dadykin, V. L., Zatsepin, V. I., and Nesterova, N. M., *Cosmic Rays* (edit. by Skobel'tsyn, D. V.) (*Proc. (Trudy) P. N. Lebedev Phys. Inst.*); *Trans. Consultants Bureau*, **26**, 99 (1965).

[23] Fegan, D. J., McBreen, B., O'Mongain, E. P., Porter, N. A., and Slevin, P. J., *Canad. J. Phys.*, **46**, S433 (1968).

[24] Fazio, G. G., Helmken, H. F., Rieke, G. H., and Weekes, T. C., *Astrophys. J. Lett.*, **154**, L83 (1968).

[25] Tornabene, H. S., and Cusimano, F. J., *Canad. J. Phys.*, **46**, S81 (1968).

[26] Charman, W. N., Jelley, J. V., Orman, P. R., Drever, R. W. P., and McBreen, B., *Nature*, **220**, 565 (1968).

[27] O'Mongain, E. P., Porter, N. A., White, J., Fegan, D. J., Jennings, D. M., and Lawless, B. G., *Nature*, **219**, 1348 (1968).

[28] Porter, N. A., Jennings, D. M., and O'Mongain, E. P., *IAU Circ. No. 21*, (1969).

[29] Chatterjee, B. K., Murthy, G. T., Ramana Murthy, P. V., Sreekantan, B. V., and Tonwar, S. C., *Proc. Eleventh Intern. Conf. Cosmic Rays, Budapest* (in the press).

[30] Fazio, G. G., Hearn, D. R., Helmken, H. F., Rieke, G. H., and Weekes, T. C., *Proc. Eleventh Intern. Conf. Cosmic Rays, Budapest* (in the press).

[31] Charman, W. N., and Drever, R. W. P., *Nature*, **224**, 567 (1969).

[32] Fazio, G. G., Helmken, H. F., Rieke, G. H., and Weekes, T. C., *Proc. Eleventh Intern. Conf. Cosmic Rays, Budapest* (in the press).

[33] Gould, R. J., *Phys. Rev. Lett.*, **15**, 577 (1965).

[34] Rieke, G. H., and Weekes, T. C., *Astrophys. J.*, **155**, 429 (1969).

[35] Cocconi, G., *Proc. Intern. Conf. Cosmic Rays, Moscow*, **2**, 309 (1960).

[36] Gould, R. J., and Burbidge, G. R., *Handbuch der Physik*, **46/2**, 265 (1967).

[37] Fazio, G. G., Helmken, H. F., Rieke, G. H., and Weekes, T. C., *Proc. IAU Symp. No. 37* (edit. by Gratton, L.) (in the press).

[38] Stecker, F. W., *Nature*, **222**, 865 (1969).

[39] Felten, J. E., and Morrison, P., *Phys. Rev. Lett.*, **10**, 453 (1963).

[40] Felten, J. E., and Morrison, P., *Astrophys. J.*, **146**, 686 (1966).

[41] Cowsik, R., and Pal, Y., *Phys. Rev. Lett.*, **22**, 550 (1969).

[42] Shen, C. S., *Phys. Rev. Lett.*, **22**, 568 (1969).

[43] Shivanandan, K., Houck, J. R., and Harwit, M. O., *Phys. Rev. Lett.*, **21**, 1460 (1968).

[44] Ögelman, H., *Nature*, **221**, 753 (1969).

[45] Hayakawa, S., and Tanaka, Y., *Proc. IAU Symp. No. 37, Rome* (edit. by Gratton, L.) (in the press).

[46] Brecher, K., and Morrison, P., *Phys. Rev. Lett.*, **23**, 802 (1969).

[47] Stecker, F. W., *Nature*, **224**, 870 (1969).

[48] O'Connell, R. F., and Verma, S. D., *Phys. Rev. Lett.*, **22**, 1443 (1969).

[49] Longair, M. S., and Sunyaev, R. A., *Astrophys. J. Lett.*, **4**, 65 (1969).

[50] Gould, R. J., and Schréder, G., *Phys. Rev. Lett.*, **16**, 252 (1966).

[51] Jelley, J. V., *Phys. Rev. Lett.*, **16**, 479 (1966).

[52] Apparao, M. V. K., *Proc. Indian Acad. Sci.*, **65**A, 349 (1967).

[53] Ginzburg, V. L., and Syrovatskii, S. I., *The Origin of Cosmic Rays* (Pergamon Press, Macmillan Co., New York, 1964).

[54] Garmire, G., and Kraushaar, W., *Space Sci. Rev.*, **4**, 123 (1965).

[55] Hayakawa, S., and Matsuoka, M., *Prog. Theor. Phys. Suppl.*, **30**, 204 (1964).

[56] Fazio, G. G., *Ann. Rev. Astron. Astrophys.*, **5**, 481 (1967).

[57] Lüst, R., and Pinkau, K., in *Electromagnetic Radiation in Space* (edit. by Emming, J. G.), 231 (Reidel, Dordrecht, Holland, 1967).

[58] Greisen, K., in *Perspectives in Modern Physics* (edit. by Marshak, R. E.), 355 (Interscience Publ. Co., New York, 1966).

[59] Kraushaar, W. L., *Astronautics and Aeronautics*, **7**, 28 (1969).

[60] Helmken, H., and Hoffman, J., *Nuclear Instr. Meth.* (in the press).

[61] Jelley, J. V., in *Progress in Elementary Particle and Cosmic Ray Physics* (edit. by Wilson, J. G., and Wouthuysen, S. A.), **9**, 39 (North-Holland, Amsterdam, 1967).

Chromosomal and Nuclear Location of Mouse Satellite DNA in Individual Cells

by
K. W. JONES
MRC Epigenetics Research Group,
Institute of Animal Genetics,
University of Edinburgh

Single strands of mouse satellite DNA were annealed to nuclei and chromosomes in mouse cells. From the location of autoradiographic grains, it is suggested that there is a concentration of satellite DNA sequences close to the centromeres of the chromosomes.

THE DNA from many eukaryotes may be fractionated on CsCl gradients into a main component, usually referred to as the major DNA band, and minor components called satellites. With one or two exceptions[1,2] the precise location of these minor components is unknown and their biological significance is obscure in all but one case[3]. Satellite DNA from the mouse, one of the most extensively studied, and comprising 9–10 per cent of the total DNA[4], consists of short sequences of nucleotides repeated end to end to form molecules with a molecular weight of several million daltons[5]. The complementary strands have been shown to differ in base composition[6] and may be separated from each other by virtue of their difference in buoyant density on alkaline CsCl gradients[7]. Cell fractionation has shown that satellite sequences are enriched in isolated nucleoli[8] and in dense chromatin fractions[9]. Chromosome fractionation[10] has provided evidence of the distribution of sequences throughout the chromosome set.

Methods for specifically annealing nucleic acids to chromosomes and nuclei which allow the localization of the annealed or hybridized molecules by autoradiography have recently been developed[11], and I now describe the results of the application of these methods to the problem of the location of mouse satellite DNA. Purified single strands of mouse satellite DNA were annealed to mouse and to rabbit nuclei and chromosomes.

Primary embryonic mouse cell cultures from a local strain of inbred mice were grown in 250 ml. flasks (Falcon Plastics) in Eagle's minimal essential medium which contained 10 per cent calf serum and 100 units of penicillin and streptomycin per ml. Rabbit kidney cells were obtained as an established cell line from Flow Laboratories and were maintained in the same manner as the mouse cells. The cell cultures were maintained in an incubator at 37° C gassed with 5 per cent CO$_2$ in air.

Labelling and Isolation of DNA

Approximately 50 µCi of tritiated thymidine ('TRK 120', Amersham) with a specific activity of 20·2 Ci/mmole was added to each flask of mouse cells previously grown to half confluence. After 2 days of incubation, the medium was changed and fresh medium containing the same amount of tracer was added. Incubation was contained for a further 2 days. The cultures were then washed several times with Dulbecco saline, trypsinized away from the flasks and harvested by pelleting in a bench centrifuge. The pellets were resuspended in a very small volume of saline and then lysed by addition of $2 \times$ SSC (SSC = 0·15 M NaCl–0·015 M sodium citrate) in 1 per cent in sodium lauryl sulphate and containing 200 µg/ml. protease (Sigma) predigested at 37° C for 20 min. The lysate was incubated for 2 h at 37° C, cooled to room temperature, mixed with CsCl (1·26 g/ml.), overlayered with paraffin oil and centrifuged at 42,000 r.p.m. in an MSE centrifuge rotor No. 2410 for 40 h at 20° C. The tubes were pierced and 0·5 g fractions were collected. The absorbance at 260 nm of the fractions was determined in a 'Unicam *SP* 800' spectrophotometer. Samples were spotted on Whatman No. 1 filter disks, which were washed twice with 5 per cent trichloroacetic acid, followed by ice cold ethanol and ether (three washes each). Radioactivity was determined by scintillation counting in toluene based scintillation fluid using a Nuclear Chicago 'Unilux' counter. The coincident peaks of absorbance at 260 nm and radioactivity were then pooled and the DNA was recovered by pelleting, after suitable dilution, in an MSE 2418 rotor at 30,000 r.p.m. at 4° C for 18 h. The pellets were resuspended in $0{\cdot}1 \times$ SSC. 4 ml. of the dissolved pellet containing 100 µg of DNA was then mixed with 5·02 g of CsCl overlayered with paraffin oil and were centrifuged for 40 h at 42,000 r.p.m. 0·2 g

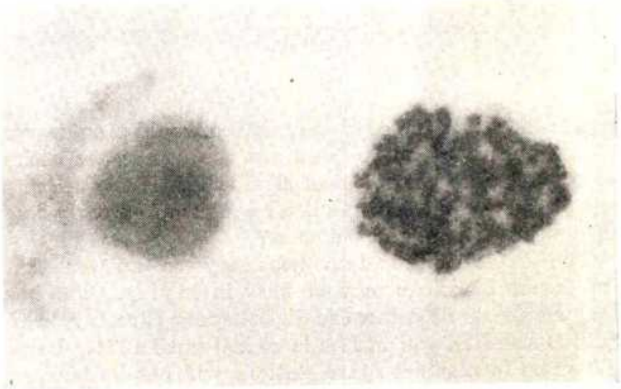


Fig. 1. Rabbit interphase and metaphase nuclei after annealing to mouse satellite. Note absence of grains.

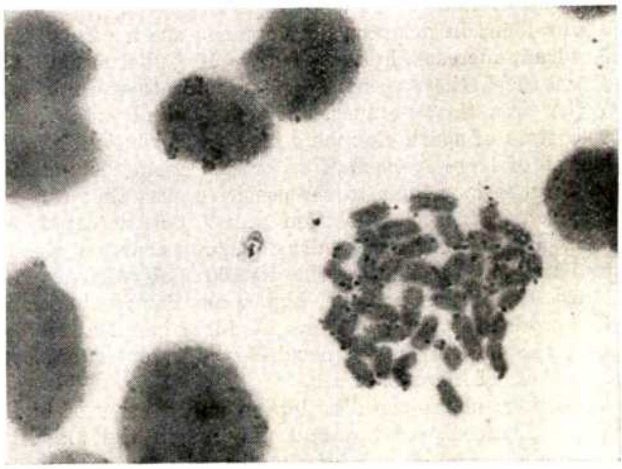


Fig. 2. Mouse interphase and metaphase nuclei after annealing to mouse satellite. Note absence of autoradiographic background and grains above both kinds of nuclei with localization in groups over dense chromatin at interphase and over chromosomes at metaphase. Focused at level of grains.

fractions were collected and their absorbance at 260 nm was determined as before. The light satellite fractions were collected and pooled. The pooled material was recycled on CsCl gradients and was then fractionated on alkaline CsCl gradients[12]. The peak fractions of the light strand of the satellite DNA were pooled and the buoyant density of a small sample of this material was determined in a Spinco model *E* analytical ultracentrifuge, using a marker of denatured *Pseudomonas aeruginosa* DNA (buoyant density, 1·736 g/cm^3). A value of 1·700 was obtained and the DNA was observed to migrate as a single symmetrical band. An aliquot of this material was taken for the determination of its specific radioactivity which was estimated to be approximately $2{\cdot}2 \times 10^6$ d.p.m./μg by scintillation counting in a toluene based scintillator using a counter with an efficiency (unchecked) of about 10 per cent. The remaining single stranded material was then diluted with 0·1 × SSC brought to *p*H 7·4 and recovered by pelleting at 30,000 r.p.m. as previously described.

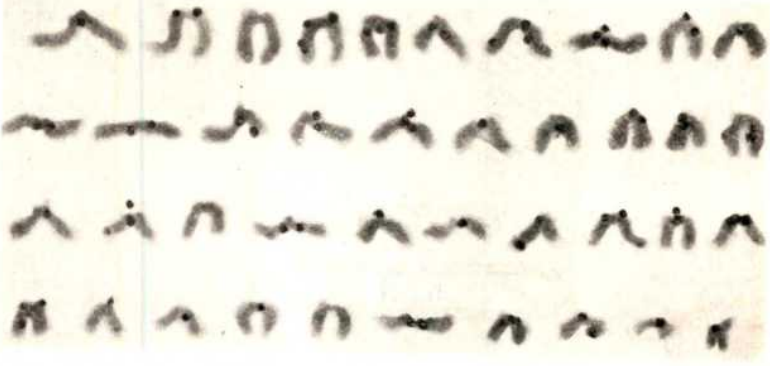


Fig. 4. Chromosomes from Fig. 3 arranged roughly in order of size, to show relative distribution of grains.

Chromosome preparations were obtained from cell cultures after treatment with colcemid (0·4 μg/ml. for 6 h). The cells were trypsinized away from culture flasks, pelleted gently, resuspended in hypotonic saline for 15 min, repelleted and fixed in ice cold ethanol–acetic acid (3 : 1). The fixed cells were resuspended in 45 per cent acetic acid and drops of the cell suspension were air dried onto coverglasses, and then stored at 4° C *in vacuo* until required.

Annealing in Rabbit and Mouse Cells.

Before annealing, the cell preparations were either dipped into 0·1 × SSC at 100° C and then quenched in 0·1 × SSC at 0° C, or alternatively treated with 0·07 M NaOH for 5 min at room temperature[14] to denature the DNA *in situ*. Denatured preparations were then dehydrated through 70 per cent, 90 per cent and absolute alcohol, and then dried *in vacuo*. The dry preparations were inverted over a 1 μl. drop of single stranded satellite DNA at a concentration of 0·6 μg/μl. on a siliconized slide, sealed to the slide with rubber solution and then incubated at 60° C for 2 h. The coverslips with cells were then detached and washed three times in 2 × SSC at room temperature to remove excess unbound radioactive DNA and then further incubated in a stirred waterbath containing 2 × SSC at 55° C for a further 2 h, to remove non-specifically trapped radioactive DNA. The preparations were rinsed twice again in 2 × SSC at room temperature and were then dehydrated in the usual way. Dehydrated preparations were mounted on slides and were dipped in Ilford 'L4' emulsion diluted 1 : 1 with water, and stored in lightproof boxes at 4° C. After 10 days exposure the autoradiographs were developed in Kodak 'D19b' developer, stained in 0·02 per cent toluidine blue at *p*H 6·0, and were photographed on Ilford '*R*40' plates using a Zeiss 'Ultraphot II' photomicroscope equipped with an interference filter.

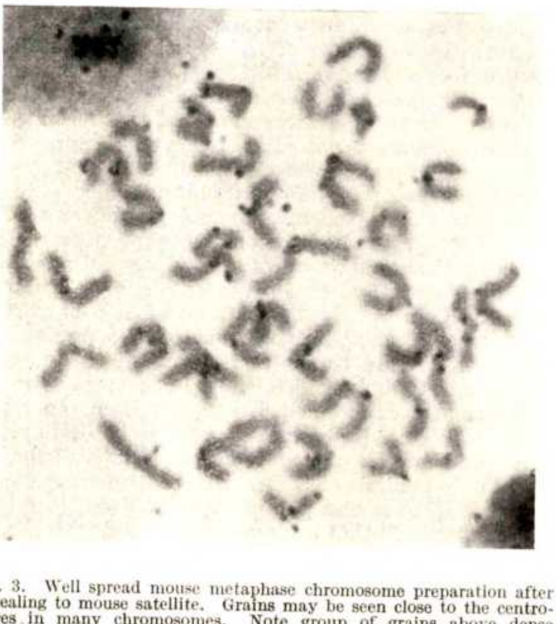


Fig. 3. Well spread mouse metaphase chromosome preparation after annealing to mouse satellite. Grains may be seen close to the centromeres in many chromosomes. Note group of grains above dense chromatin in nucleus at upper left.

The rabbit cells showed no significant grains located over any cellular structure after annealing with mouse light satellite radioactive DNA, and further autoradiographic exposure, beyond that used in the experimental group, failed to reveal any significant label. This control was therefore taken to be negative, indicating no tendency for the radioactive DNA to become non-specifically trapped by heterologous cell structures or by non-cellular materials in the preparations. An example of these preparations is shown in Fig. 1. They are compared with mouse cells in Figs. 2–4.

Autoradiographic grains were located over both interphase nuclei and chromosomes in the mouse cells. The cytoplasmic and non-cellular backgrounds were similar to that observed in rabbit cells. Grains were located over most of the chromosomes in any given group (Figs. 2 and 3), and there were few background grains. To see if the grains were polarized in favour of the centromeric regions, each chromosome was divided into three regions, proximal (*A*), median (*B*) and distal (*C*), with respect to the centromere, and grains were counted over each region. The counts on five well spread metaphase preparations were averaged and are shown as a histogram in Fig. 5. It is clear that there is a marked skewing of the grain distribution in favour of the centromeric regions. The pattern of grains over the centric regions of daughter chromosomes was often strikingly symmetrical, indicating that the annealing is to homologous sites and it is very likely that annealing occurs at an available site. That some chromosomes were unlabelled is potentially significant. The unlabelled chromosomes were therefore recorded into size classes designated small, medium and large (Table 1). The numbers of labelled and unlabelled chromosomes in five well spread metaphase plates are recorded in Fig. 5. Unlabelled chromosomes do not seem to belong exclusively to a particular size class, but most of the unlabelled chromosomes found were classified as medium sized. This is probably not significant, however, because medium sized chromosomes predominate in the mouse. Because the number of grains per chromosome is low, a class of chromosome without label would be expected by chance and from trivial causes such as self

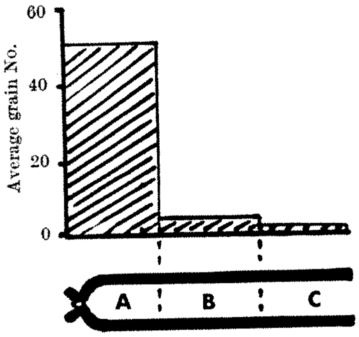


Fig. 5. Histogram based on average grain count from five well spread metaphase plates of mouse chromosomes showing distribution of grains relative to the centromere.

absorption of radioactivity; further experiments may, however, reveal truly unlabelled chromosomes.

In interphase nuclei, grains were largely associated with the dense chromatin and nucleoli. In nuclei without dense regions the grains were more scattered and this shows that the binding of radioactive DNA occurs to both diffuse and concentrated arrangements of the available specific annealing sites and is not merely an artefact of binding to lumps of chromatin. The absence of binding to rabbit nuclei, which present ostensibly similar targets for non-specific binding, confirms this. Differences in the intensity of labelling were noted in nuclei of similar size and conformation lying in the same regions of the preparation. It is not known whether these appearances reflect real differences in the availability of annealing sites or are the results of some more fortuitous aspect of the technique. In occasional nuclei, prematurely condensed individual chromosomes were present within the mass of uncondensed chromatin, and it was sometimes possible to see the polarized distribution of the associated label similar to that noted in chromosomes at metaphase.

It has previously been shown[11] that it is possible to anneal RNA molecules to complex structures such as chromosomes and nuclei. Molecular hybrids thus formed have been re-isolated and characterized by biophysical methods. There is clear evidence of the specificity of the molecular interactions and similar results have been obtained independently[13–15]. The results of the experiments reported here show clearly that DNA annealing to cytological preparations is possible and they constitute the first demonstration of the location of a chemically defined class of DNA on mammalian chromosomes. Specificity similar to that obtained using RNA[11] is found as can be seen from the failure of heterologous nuclear structures to bind radioactivity. The species specificity of mouse satellite has been previously shown *in vitro* using the DNA from a wide variety of different organisms[16]. In this experiment advantage has been taken of the facility of obtaining pure single strands of satellite DNA in order to promote annealing to the specimen by overcoming the rapid self-annealing which would otherwise be expected. It has been shown[17] that the $C_0t_{1/2}$ of mouse satellite DNA is about 10^{-3} and it is the second fastest renaturing DNA that has yet been isolated. In the conditions of our experiments the efficiency of annealing may be calculated as follows: specific activity of the input single stranded DNA is approximately 2×10^6 d.p.m./µg; assuming 10 per cent autoradiographic efficiency[18] 2×10^5 grains/µg/min or 0·2 grains/pg/min can be expected; if it is assumed that there are 7 pg DNA/nucleus, then each nucleus has 0·7 pg/satellite, which represents 0·35 pg of DNA available for annealing. With 100 per cent efficiency of annealing, $0{\cdot}2 \times 0{\cdot}35$ grains/nucleus/min or 0·07 grains can therefore be expected; with 10 days exposure, 100 per cent annealing would produce approximately 1,000 grains/nucleus. A maximum of about eighty grains per nucleus was in fact observed: this represents an efficiency of about 8 per cent.

Table 1. NUMBERS OF LABELLED AND UNLABELLED CHROMOSOMES IN FIVE METAPHASE PLATES INDICATING RELATIVE SIZE DISTRIBUTION OF UNLABELLED CHROMOSOMES

	Chromosomes				
	Unlabelled				Labelled
Cell No.	Large	Medium	Small	Total	Total
1	0	4	1	5	35
2	0	4	0	4	36
3	1	1	1	3	37
4	1	3	0	4	36
5	1	1	1	3	37
Total	3	13	3	19	181

Location of Satellite Sequences

The location of the bound radioactivity is not general, but is restricted to definite regions in both nuclei and chromosomes. This localized binding is similar in homologous daughter chromosomes in many instances, as can be seen from the matched pattern of grains, and reflects underlying homology of chromosomal structure. The discontinuity in labelling pattern is not necessarily related to the concentration of complementary satellite sequences, but could equally well reflect local differences in accessibility to the incoming radioactive molecules. Chromatin fractionation[8] indicates, however, that nucleoli are enriched three to four-fold in satellite sequences compared with DNA from whole nuclei and provides independent evidence of discontinuity in satellite distribution. This has been confirmed in our work which reveals a preferential localization of grains over nucleoli and dense chromatin. It is therefore logical to interpret the chromosomal pattern on similar lines and to conclude that a concentration of satellite DNA sequences exists close to the centromeres in most, if not all, of the chromosomes of the mouse. A similar distribution of reiterated DNA sequences has been found in *Drosophila melanogaster* in studies of the hybridization pattern of c-RNA with salivary chromosomes (K. W. J. and F. W. Robertson, in preparation). The distribution of satellite sequences among most or all of the chromosomes in the mouse has been previously inferred from analyses of gradient fractionated chromosome preparations[10].

Significance of DNA Annealing

One of the potentially significant aspects of this approach is that it can discover the location of defined DNA sequences on the chromosomes and relate this to their functional distribution at interphase. Thus it seems clear, from the evidence of the enriched satellite DNA content of nucleoli and of centric regions of chromosomes, that these become associated in interphase. The respective functions of centromeres and satellite DNA in this phenomenon are not clear, but a mechanism which obviously coordinates the physical, and perhaps the functional, aspects of different chromosomes may rely to some extent on the chemical homology of the associated satellite DNA. Non-homologous association particularly of telocentric chromosomes occurs widely in vertebrates and can be extensive and persistent in certain circumstances[19]. Centromeric association of non-homologous chromosomes in the rainbow trout (*Salmo irredeus*) has been shown to lead to the appearance of tissue specific chromosomal polymorphism[20]. The concentration of satellite sequences at nucleoli has also been reported in human and vole (*Microtus agrestis*) cells[8] implying a general tendency for aggregative or associative behaviour of these unique kinds of DNA. The centric concentration of satellite DNA is also potentially significant in relation to the suggestion[21] that it may be connected with chromosome pairing.

It is obvious that satellite DNA is concentrated close to regions which are usually referred to as heterochromatin. The term heterochromatin is used, however, in reference to a wide assortment of chromosomal phenomena whose common denominator is that they are present as characteristically staining regions. For this reason, it would not yet be wise to identify satellite DNA too closely with heterochromatin in all its aspects. Nevertheless, properties of satellite DNA which may serve to explain some aspects of certain kinds of heterochromatin require mention. For example, the recent report[22] that mouse satellite DNA replication is differentially repressed by sulphur mustard may impart new significance to the classical finding that these chemicals preferentially produce aberrations in heterochromatin[23]. This is especially relevant in view of the finding[24] that DNA synthesis in late *S* is localized to the proximal chromosomal regions in mouse—that is, those nearest the centromeres. Much of the heterochromatin is also localized in these regions[25].

The encouraging precision of the technique of DNA annealing to cytological preparations should also allow the identification of chromosomes and nuclei of mouse in situations in which these are intimately mixed with those of another species. This is interesting in relation to studies on interspecific cell hybrids and heterokaryons. The application of the method to other species combinations will become possible as more DNA sequences with similar properties to the mouse satellite become available. The technique should also find applications in studies of mixed cell populations such as in connexion with tissue immunology and radiation chimaeras.

I thank Professor Waddington for his support and for reading this manuscript; also Dr Max Birnstiel and P. M. B. Walker for helpful discussions. I am indebted to Mr J. Speirs for the buoyant density analysis, and to Mrs K. Hall, Miss B. Geddes and Miss M. Smith for expert technical assistance. This work was supported by the Science Research Council and the Muscular Dystrophy Research Group of Great Britain.

Received January 5, 1970.

[1] Chun, E. H. L., Vaughan, M. H., and Rich, A., *J. Mol. Biol.*, **7**, 130 (1963).

[2] Granick, S., and Gibor, A., in *Progress in Nucleic Acid Research and Molecular Biology*, **6**, 143 (edit. by Davidson, J. N., and Cohn, W. E.), 143 (Academic Press, New York, 1967).

[3] Birnstiel, M., Spiers, J., Purdom, I., Jones, K. W., and Loening, U. E., *Nature*, **219**, 454 (1969).

[4] Kit, S., *J. Mol. Biol.*, **3**, 711 (1961).

[5] Waring, M., and Britten, R. J., *Science*, **154**, 791 (1966).

[6] Chun, E. H. L., and Littlefield, J. W., *J. Mol. Biol.*, **7**, 245 (1963).

[7] Flamm, W. G., McCallum, M., and Walker, P. M. B., *Proc. US Nat. Acad. Sci.*, **57**, 1729 (1967).

[8] Schildkraut, K. L., and Maio, J. J., *Biochim. Biophys. Acta*, **161**, 76 (1968).

[9] Yasmineh, W. G., and Yunis, J. J., *Biochem. Biophys. Res. Commun.*, **35**, 779 (1969).

[10] Maio, J. J., and Schildkraut, K. L., *J. Mol. Biol.*, **24**, 29 (1967).

[11] John, H. A., Birnstiel, M. L., and Jones, K. W., *Nature*, **223**, 582 (1969).

[12] Flamm, W. G., Birnstiel, M. L., and Walker, P. M. B., in *Subcellular Components* (edit. by Birnie, G. D.), 125 (Butterworth, London, 1969).

[13] Gall, J. G., *Genetics* (in the press).

[14] Gall, J. G., and Pardue, M. L., *Proc. US Nat. Acad. Sci.*, **63**, 378 (1969).

[15] Buongioro-Nardelli, M., and Amaldi, F., *Nature*, **225**, 946 (1970).

[16] Flamm, W. G., Walker, P. M. B., and McCallum, M., *J. Mol. Biol.*, **40**, 423 (1969).

[17] Britten, K. J., and Kohne, D. E., *Science*, **161**, 529 (1968).

[18] Perry, R. P., in *Methods in Cell Physiology* (edit. by Prescott, D. M.), **1**, 324 (Academic Press, New York, 1964).

[19] Ohno, S., Stenius, C., Faisst, E., and Zenzes, M. T., *Cytogenetics*, **4**, 117 (1965).

[20] Ohno, S., Christian, L. C., and Stenius, C., *Exp. Cell Res.*, **32**, 590 (1963).

[21] Walker, P. M. B., Flamm, W. G., and McLaren, A., in *Handbook of Molecular Cytology* (edit. by Lima-de-Faria) (North-Holland, Amsterdam, in the press).

[22] Flamm, W. G., Bernheim, N. J., and Spalding, J., *Biochim. Biophys. Acta*, **195**, 273 (1969).

[23] Kihlman, B. A., *Actions of Chemicals on Dividing Cells* (Prentice-Hall, Englewood Cliffs, 1966).

[24] Evans, H. J., *Exp. Cell Res.*, **35**, 381 (1964).

[25] Ohno, S., Kaplan, W. D., and Kinosita, R., *Exp. Cell Res.*, **13**, 358 (1957).

Variations in the DNA from Two Rodent Families (Cricetidae and Muridae)

by
W. HENNIG*
P. M. B. WALKER
MRC Research Group on the Mammalian Genome,
Department of Zoology,
University of Edinburgh,
Edinburgh 9

Even within a closely related family, the banding profiles in CsCl and reassociation rates of the DNA exhibit considerable differences.

THE DNA of higher organisms has been shown to contain easily distinguishable fractions by following the rate of reassociation after denaturation[1–4]. Because the reassociation follows second order kinetics it must be dependent on the concentration of those single-stranded molecules which are able to reform stable duplex molecules. Consistent with this is the finding of a linear relationship between the rate of reassociation and the complexity or size of the genome over a wide range of organisms[2,3]. Higher organisms, however, contain a proportion of nuclear DNA which reassociates more rapidly than expected, indicating that such fractions have multiple nucleotide sequences with some degree of similarity. In certain higher organisms some of these sequences are distinguished by their uniformity, and, if their base compositions are sufficiently different from that of the bulk DNA, they can be isolated by centrifugation in CsCl to give so-called "satellite" DNA[5]. Mouse "satellite" DNA is one species which has been extensively studied[6–10].

Some of the difficulties raised by the evolution and function of these related sequences have been discussed elsewhere[11] together with preliminary comparisons of the DNA from different organisms[12]. We report here studies on sixteen different species, subspecies and geographically separated populations within two rodent families, the Cricetidae and the Muridae. *Microtus*, *Clethrionomys* and *Arvicola* all belong to the tribe Microtini within the family Cricetidae, while *Rattus*, *Mus*, *Acomys* and *Apodemus* are members of the sub-family Murinae of the family Muridae. They represent therefore both of the great families comprising the super-family Muroidea, sub-order Myomorpha. From reassociation kinetics and CsCl centrifugation of the DNA, we conclude that the proportion of these sequences varies even within this closely related group of animals.

Differences in Banding Properties in CsCl

The banding properties in CsCl after centrifuging to equilibrium in an analytical centrifuge show that the average buoyant density—and therefore the average content of C+G—is nearly the same for the bulk DNA in all species investigated, but some exhibit a minor component distinct from the main DNA band, while in others a similar component is visible within it (Fig. 1). The latter are not likely to be artefacts because centrifuging in alkaline CsCl, which denatures the DNA, reveals a banding profile which is very like that found in the neutral conditions (Fig. 2). We have also fractionated some of these DNA samples on a preparative neutral CsCl gradient, before banding the separated fractions in the analytical centrifuge (Fig. 3). The minor component is present and enriched in the fraction corresponding to its average base composition. We conclude from this that, like guinea-pig DNA[14,15], several other rodent DNAs have minor components analogous to the "satellite" components found in the mouse. From the banding pattern in alkaline CsCl (Fig. 2) it also seems—in confirmation of earlier studies on *Apodemus sylvaticus*[11]—that strand separation, which is so characteristic of mouse[7], guinea-pig[14,15] and human[16] minor components, is not a general property of such fractions.

---

* Present address: Max Planck Institut für Biologie, Abt. Beermann, Tübingen.

The proportion of the DNA found in these minor components seems to vary between about 2 and 10 per cent, but fractions like that from human DNA[16], which is less than 2 per cent of the total, would not be detected in our experiments. The banding position in CsCl of the minor component, indicative of its average C+G content, varies considerably between the species investigated. Even the satellites of the closely related *Apodemus flavicollis*, *sylvaticus* and *agrarius* species display (Fig. 4) small differences in density of the order of 0·001 g/cm³, and differences in relative amounts. These three species, together with *A. mystacinus* (Fig. 1), thus show a remarkable variation within a single genus.

The most important factor affecting measurements of the relative proportion of these components is molecular weight, because there is evidence from studies on the mouse[10] that these sequences are covalently linked to main band DNA. Band sedimentation studies indicate that the molecular weight of our DNA preparation is about 3×10^7 daltons. Mechanical shearing of the DNA

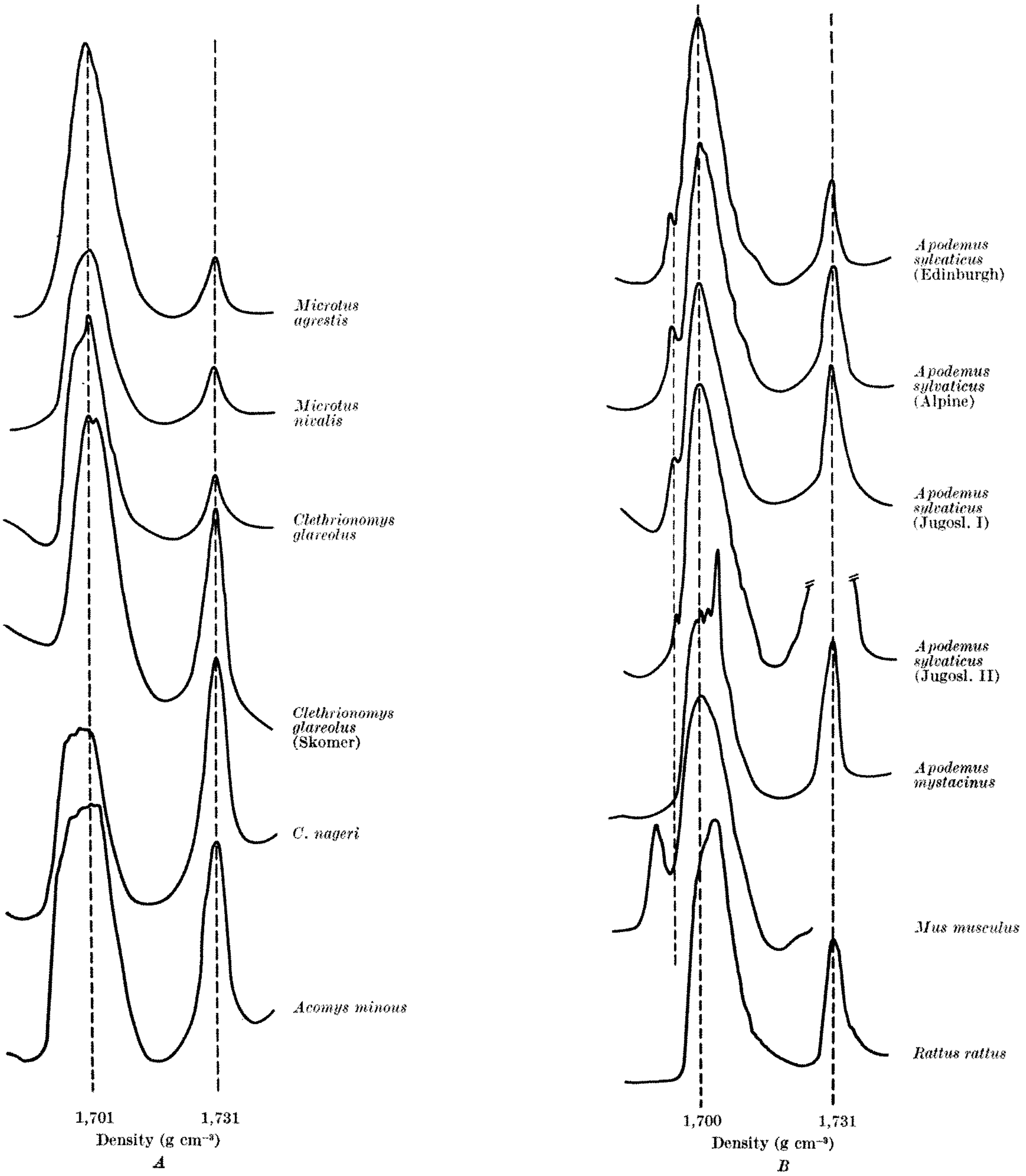


Fig. 1. Analytical centrifugation of different rodent DNAs in neutral CsCl (Harshaw, optical grade). Approximately 3 μg of DNA from liver nuclei and 1–3 μg DNA from *Micrococcus lysodeikticus* (density 1·731 g cm⁻³) were dissolved in 0·01 M Tris (*p*H 8) and mixed with CsCl to an initial density of 1·715 to 1·720 g cm⁻³. Centrifugation was carried out at 44,700 r.p.m. and 25° C in an MSE analytical centrifuge for 22 to 28 h using bromine and chlorine gas filters and Kodak film. Liver nuclei were prepared by homogenization and centrifugation through nearly isopycnic sucrose[13].

(at about 4×10^6 daltons) before centrifugation does not affect the proportion of minor components recovered.

Kinetics of Reassociation

In the mouse and the guinea-pig, minor satellite fractions have been isolated and their rates of reassociation determined[10,15]. These are very rapid and indicate that those sequences which are responsible for the reformation of stable duplexes must recur, in the mouse for example, once every 150–300 nucleotides. On the other hand, both the melting behaviour of the reassociated DNA[6,7] and the more precise technique of pyrimidine tract analysis[17] indicate that the 150–300 nucleotide sequences are similar but not identical (E. Southern and A. Carr-Brown, manuscript in preparation). For mouse and guinea-pig DNA, therefore, the fast reassociating fractions are homogeneous in base composition, and after CsCl centrifugation occur as narrow bands either superimposed on or separable from the main band DNA.

It is convenient[18] to distinguish between these "fast" reassociating fractions and those which reassociate at an intermediate rate: that is, intermediate between the fast fraction and a principal component of the DNA which reassociates at the much slower rate predicted[2,3] for sequences having only a single copy in each genome. These intermediate and slowly reassociating DNAs occur in the main band which contains sequences with a wide range of different base compositions. Intermediate fractions have been found in all the higher organisms so far studied[2]. They have rates of reassociation indicative of many different "families of repeated sequences". Each family can be defined by a degree of repetitivity, bearing in mind again that repetitivity denotes similarity but not necessarily identity. One of the difficulties in measuring the size of families is that the degree of similarity, or goodness of fit, between complementary chains is highly dependent on the conditions during reassociation of the polynucleotide chains[18]. Relaxed conditions, such as low temperature and high Na$^+$ concentration in the incubation mixture, will allow less specific interactions to be measured and the family

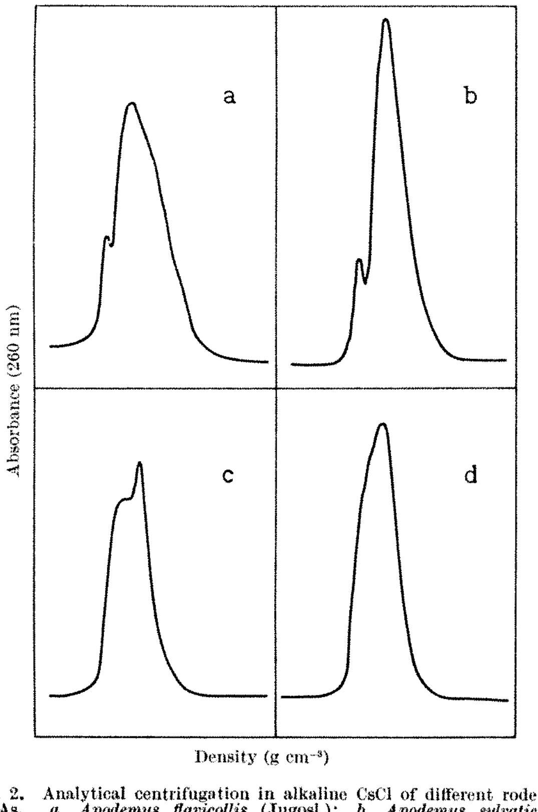


Fig. 2. Analytical centrifugation in alkaline CsCl of different rodent DNAs. *a*, *Apodemus flavicollis* (Jugosl.); *b*, *Apodemus sylvaticus* (Alpine); *c*, *Rattus rattus* (Jugosl.); *d*, *Acomys minous*. 3 μg of DNA was dissolved in 0·58 ml. of 0·01 M Tris (pH 8), denatured with 0·1 ml. 0·2 M NaOH and adjusted with 0·93 g CsCl to an initial density of 1·740 to 1·750 g cm^{-3}. Centrifugation was carried out as in Fig. 1.

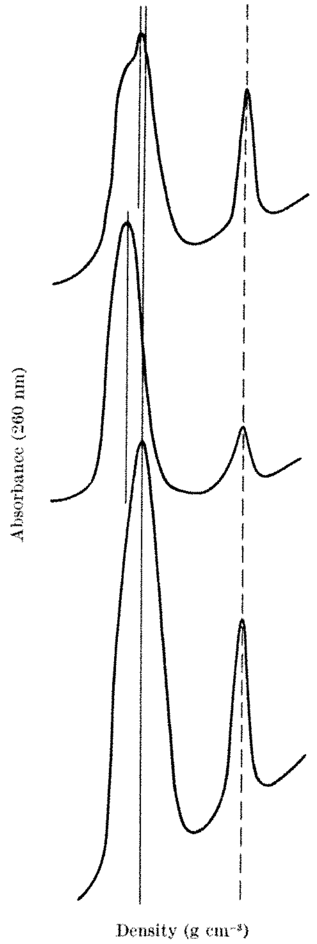


Fig. 3. Analytical centrifugation of DNA from liver nuclei of *Arvicola amphibius* (female) after prefractionation on a preparative ultracentrifuge. *a*, Total DNA; *b*, light fraction of the preparative run; *c*, heavy fraction of the preparative run. The distributions in *c* are non-Gaussian and therefore indicate further heterogeneity in the heavy fraction. Centrifugation was carried out as in Fig. 1.

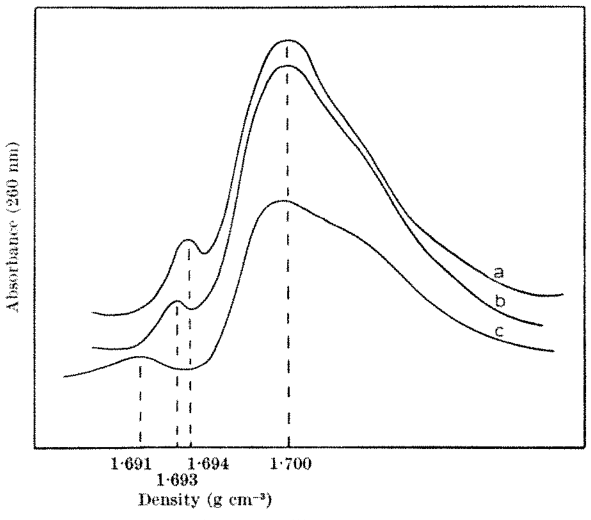


Fig. 4. Comparison of the satellites of different *Apodemus* species. Whole liver DNA was centrifuged as described in Fig. 1 and the traces aligned as shown. The densities were obtained with reference to the marker DNA peak not shown at this magnification. *a*, *Apodemus sylvaticus* (Edinburgh); *b*, *Apodemus flavicollis* (Hereford); *c*, *Apodemus agrarius* (Bohemia).

size will be larger. Nevertheless, just as the fast minor components vary in size in different species, so does the size of the intermediate fraction. Britten has reported[2] a variation from 20 to 80 per cent in the intermediate fraction, using standardized conditions of reassociation.

For our comparisons of reassociation rates between different rodent DNAs we have measured ultraviolet hypochromicity in relatively stringent conditions; the reassociation temperature was 60° C in 0·12 M Na^+ which defines a T_m for the native DNA of about 85° C (ref. 19). Second order rate plots similar to those of Wetmur and Davidson[3] have been used to compare the kinetics of reassociation. The reciprocal of the fraction unreassociated is plotted against time, and in simple DNAs such as those from T4 or bacteria this gives a straight line. For higher organisms, a number of more or less straight lines of different slopes are found (Fig. 5). Extrapolating to zero time gives a crude estimate of the proportion of each component in test systems with mixtures of T4 and *E. coli* DNA (unpublished data of M. L. Birnstiel and W. H.). Calculated in this way, the proportion of rodent DNA sequences reassociating at an intermediate rate varies between 4 and 13 per cent. The proportion of fast reassociating sequences has an even greater variation, between 0 and 12 per cent: in *Microtus agrestis* and *Clethrionomys glareolus* fast fractions cannot be detected by the method, which, like CsCl centrifugation, does not immediately reveal the presence of small minor components.

The Biological Significance of Repetitive DNA

The evidence for any biological function for fast reassociating fractions like the mouse satellite is small, although mouse satellite is probably not transcribed[10]. The hypothesis that highly repetitive DNA has a "chromosomal housekeeping" function[12] is supported by recent findings that the DNA which remains in chromosomes after salt extraction[9], and also condensed chromatin (ref. 20 and personal communication from B. J. McCarthy), are enriched in satellite DNA. In addition, similar proportions of satellite sequences have been found in isolated chromosomes of different size classes[9]. More recently, Smith[8] has shown that mouse satellite is the first DNA fraction to be replicated after polyoma infection, and Jones[8a] has used the technique of *in situ* hybridization to indicate that a high proportion of satellite sequences are localized near the centromeres of each mouse chromosome. These findings should not be interpreted to indicate that heterochromatin, which is only definable by cytological and genetic observations, is exclusively composed of highly repetitive DNA. Indeed, our experiments contradict this because *Microtus agrestis*, in which about 20 per cent of the genome is contained in the giant heterochromatic sex chromosomes[21], has no large satellite (Fig. 1) nor any fast reassociating component (Fig. 5). No other correlation between karyotype and the occurrence of multiple sequences is evident from our data (Table 1). Nor is there any precise information on the DNA content

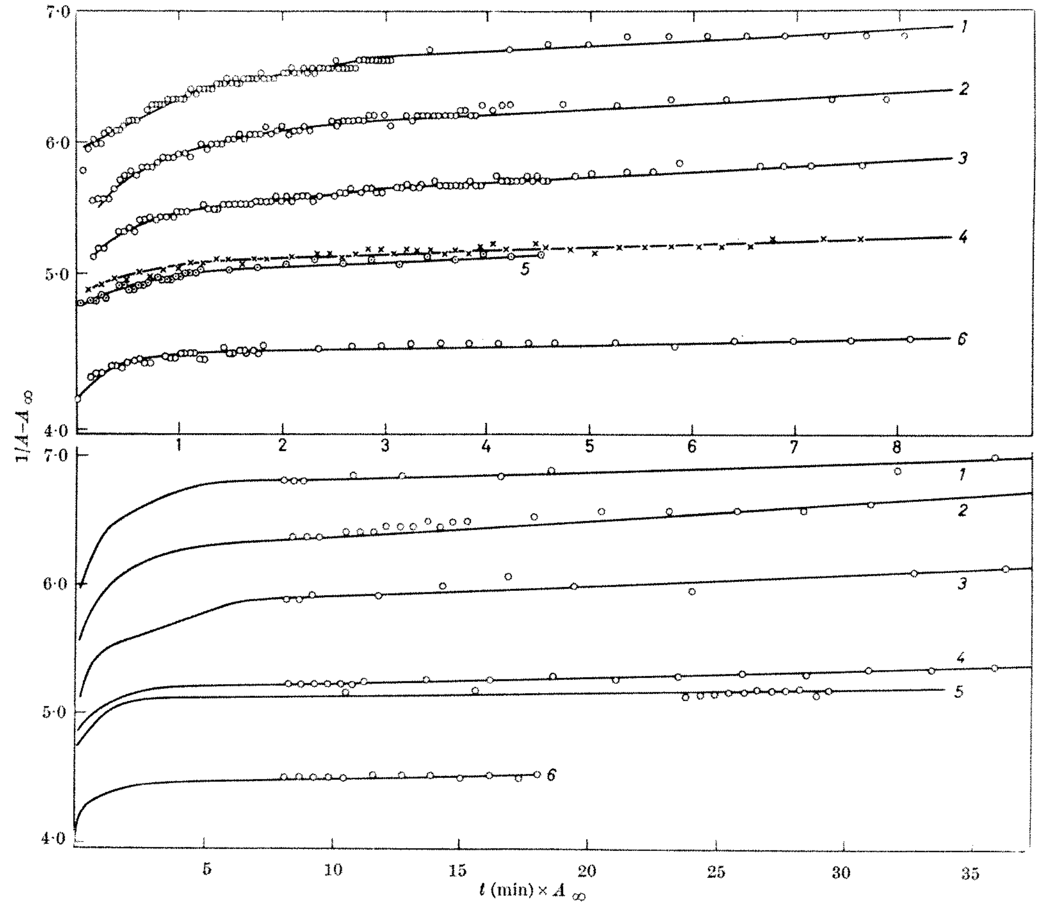


Fig. 5. Renaturation behaviour of some rodent DNAs. 1, *Microtus arvalis* (Orcadensis); 2, *Apodemus sylvaticus*; 3, *Apodemus flavicollis*; 4, *Microtus agrestis*; 5, *Clethrionomys glareolus*; 6, *Apodemus mystacinus*. Sonicated DNA (average size 500–1,000 nucleotides according to molecular weight determinations by boundary centrifugation) at a concentration of 10 to 20 μg/ml. was denatured at 100° C in 0·08 M phosphate buffer (pH 6·8) and quickly injected into a 1 cm or a 5 cm cuvette of a Zeiss spectrophotometer 'PMQ II'. The cuvettes were kept at 60° C as measured by a platinum resistance thermometer and bridge circuit (Rosemount Engineering). The renaturation temperature reached 60° C after 10 to 20 s. The changes in absorbance were then recorded at (initially) 3 s intervals with a 'Unilog' digital print-out (Joyce, Loebl). Usually the reaction was followed for about 48 h and the initial hyperchromicity was checked by an additional melting of the duplexes at the end of the run. The data were plotted according to Wetmur and Davidson[3]. A_∞, Absorbance (260 nm) of the native DNA; A, absorbance at time t. To facilitate comparison ($t \times A_\infty$) is plotted on the abscissa. The lower half of the figure consists of the data at longer times of renaturation, together (< 8) with the curves from the upper figure after an appropriate scale correction.

of these species, which could allow a comparison between the proportion of multiple sequences and the genome size.

A much canvassed possibility is that repetitive DNA is associated with the presence of multiple copies of genes as suggested by the chromosome models of Callan[22], Beermann[23] and Whitehouse[24]. It is well established that the DNA sequences specifying ribosomal[25] and 5S RNA[26] are present in multiple copies. Recently[27] a tissue specific RNA has been localized in the *Drosophila hydei* genome and correlated with distinct functions in sperm histogenesis. The conditions of this experiment were such that the DNA complementary to this RNA must have been highly reiterated. This provides direct evidence that these repeated sequences are not fortuitously similar but are functionally related.

Table 1

Species	2 N	Per cent of the genome "Fast"	"Interm."	Total Repetitive
Microtus arvalis (orcadensis)	46	6	8	14
Microtus agrestis	50	—	7	7
Microtus nivalis	56	5	5	10
Clethrionomys glareolus britannicus	56	—	11	11
C. glareolus skomerensis	56	7	8	15
C. nageri	56 ?	7	8	15
Arvicola amphibius	36	5	5	10
Rattus rattus	42	10	4	15
Mus musculus	40	12	8	20
Acomys minous	?	5	7	13
Apodemus mystacinus	?	5	5	10
Apodemus flavicollis	48	7	13	20
Apodemus sylvaticus (Edinburgh)	48	11	9	19
Apodemus sylvaticus (Yug. I)	48	11	9	19
Apodemus sylvaticus (Yug. II)	48	11	9	19
Apodemus sylvaticus (Alpine)	48	9	9	18

Chromosome numbers (2 N) are given in the first column. Percentages of the genome are obtained by extrapolating the different parts of the reaction curves to zero time and calculating the corresponding $A\infty$ (see text for an empirical justification). The column "fast" refers to the initial rapid phase of the reaction curve and "intermediate" to all other components except the final slow reaction which occurs at values greater than 10 for $t \times A\infty$ in Fig. 5. In *Microtus agrestis* it is not possible to distinguish between "fast" and "intermediate" within the small amount of repetitive DNA and in one *Clethrionomys* nearly all the repetitive DNA renatures at an intermediate rate. The amounts of the "fast" component will not necessarily be the same as that in satellite DNAs defined by CsCl centrifugation.

On the other hand, about 80 per cent of the genome of the rodents studied in this work reassociates at the rate characteristic of sequences having only a single copy on each genome. Such measurements, however, cannot exclude the presence, of, for example, two copies of each gene, but they appear to exclude a chromosomal model based on the general large-scale multiplication of all genes. The hypothesis of "masters" and "slaves" could be rescued if it were confined to cistrons specifying proteins and if these were confined to the intermediate fraction of DNA. The number of such cistrons would be relatively small, however, because the rate of reassociation of the intermediate fraction is consistent with the presence of about 10,000 copies of each cistron, on average, while the size of the fraction (20 per cent intermediate DNA) limits the number of such copied cistrons to about 100. Even if each of these families of copied cistrons specified several related proteins, it would still seem to be essential to involve the unique sequences in a protein-specifying role. It should also be remembered that the quality of reassociated DNA from the intermediate fractions indicates that the "copies" are far from perfect.

Further, even such a homogeneous repetitive fraction as the mouse satellite discussed earlier is not composed of identical copies (E. Southern and A. Carr-Brown, manuscript in preparation), and except perhaps for the cistrons transcribing 5S RNA[28,29], and possibly the ribosomal DNA in *Xenopus*[25], there is no evidence that other repeated DNA sequences are identical.

If repetitive DNA in higher organisms is not largely associated with multiple gene copies, can it be explained in other ways ? Any explanation has to account for the wide variations in the amount of DNA which reassociates at fast and intermediate rates (Table 1): it seems as if the genomes of these rodents are in a state of continuous and large scale fluctuation.

Earlier[12], one of us (P. M. B. W.) considered the possibility that there are frequent base substitutions at non-critical positions in the DNA. Thus interchange between synonymous codons might be selectively more nearly neutral—even more than substitutions resulting in conservative[31] amino-acid replacements. A random selection of available synonyms may destroy the ability of two cistrons coding for similar proteins to reassociate and form stable duplexes[18], so that if all "single-copy" DNA specified proteins, the size of the intermediate fraction may be altered in different species by varying degrees of bias in the selection of the available synonyms. Even if "single-copy" DNA is largely untranslated, this explanation may still hold, because it is likely that base substitutions could also occur in non-protein specifying regions of the DNA without damaging their function.

The gross differences which we have revealed in one rodent family, and which seem to involve up to 500 million base pairs (10 per cent of the genome), must surely reflect the operation of significant molecular mechanisms in higher organisms. They need to be accounted for in any theory of genome organization or, indeed, any theory of satellite DNA function. Moreover, we need to know to what extent these differences are caused by the evolutionary amplification of DNA sequences—which has certainly occurred—and how much by the rapid substitution of bases at less critical but nevertheless not neutral positions in the genome.

Rattus rattus, *Apodemus mystacinus* and some *A. sylvaticus* were collected by Mr T. Day in Yugoslavia with the help of the Weir Fund of the University of Edinburgh. *A. flavicollis* was collected by Mr H. Jones from Herefordshire. Mr T. Warwick collected or arranged for the collection of the *Microtus* spp., some of the *A. sylvaticus* and *Arvicola amphibius*. *Clethrionomys* spp. were obtained from the laboratory populations of Dr J. Godfrey, who also collected the alpine individuals of *A. sylvaticus*. *Acomys minous* was a gift from the London Zoo. We thank those who have assisted and also acknowledge the support of a research grant from the Medical Research Council and by the Max-Planck-Gesellschaft and the Deutsche Forschungsgemeinschaft for W. H.

Received January 5, 1970.

[1] Waring, M., and Britten, R. J., *Science*, **154**, 791 (1966).

[2] Britten, R. J., and Kohne, D. E., *Science*, **161**, 529 (1968).

[3] Wetmur, J. G., and Davidson, N., *J. Mol. Biol.*, **31**, 349 (1968).

[4] Walker, P. M. B., and McLaren, A., *Nature*, **208**, 1175 (1965).

[5] Kit, S., *J. Mol. Biol.*, **3**, 711 (1961).

[6] Bond, H. E., Flamm, W. G., Burr, H. E., and Bond, S. B., *J. Mol. Biol.*, **27**, 289 (1967).

[7] Flamm, W. G., McCallum, M., and Walker, P. M. B., *Proc. US Nat. Acad. Sci.*, **57**, 1729 (1967).

[8] Smith, B. J., *J. Mol. Biol.*, **47**, 101 (1970).

[8a] Jones, K. W., *Nature*, **225**, 912 (1970).

[9] Maio, J. J., and Schildkraut, C. L., *J. Mol. Biol.*, **40**, 203 (1969).

[10] Flamm, W. G., Walker, P. M. B., and McCallum, M., *J. Mol. Biol.*, **40**, 423 (1969).

[11] Walker, P. M. B., Flamm, W. G., and McLaren, A., in *Handbook of Molecular Cytology*, 52 (edit. by Lima-de-Faria) (North-Holland, Amsterdam, 1969).

[12] Walker, P. M. B., *Nature*, **219**, 228 (1968).

[13] Desjardins, R., Smetana, K., Grogan, D., Higashi, K., and Busch, H., *Cancer Res.*, **26**, 97 (1966).

[14] Corneo, G., Ginelli, E., Soave, C., and Bernardi, G., *Biochemistry*, **7**, 4373 (1968).

[15] Flamm, W. G., Walker, P. M. B., and McCallum, M., *J. Mol. Biol.*, **42**, 441 (1969).

[16] Corneo, G., Ginelli, E., and Polli, E., *J. Mol. Biol.*, **33**, 331 (1968).

[17] Burton, K., and Peterson, G. B., *Biochem. J.*, **75**, 17 (1960).

[18] Walker, P. M. B., *Prog. Nucleic Acid Res. and Mol. Biol.*, **9**, 301 (1969).

[19] Marmur, J., and Doty, P., *J. Mol. Biol.*, **5**, 109 (1962).

[20] Yasmineh, W. G., and Yunis, J. J., *Biochem. Biophys. Res. Commun.*, **35**, 779 (1969).

[21] Schmid, W., Smith, D. W., and Theiler, K., *Archiv. der Julius Klaus-Stiftung*, **XL**, 35 (1965).

[22] Callan, H. G., *J. Cell Sci.*, **2**, 1 (1967).

[23] Beermann, W., in *Heritage from Mendel* (edit. by Brink, R. A., and Styles, E. D.) (Univ. Wisconsin, Madison, 1967).

[24] Whitehouse, H. L. K., *J. Cell Sci.*, **2**, 9 (1967).

[25] Birnstiel, M. L., Wallace, H., Sirlin, J. L., and Fischberg, M., *Nat. Cancer Inst. Monog.*, **23**, 431 (1966).

[26] Brown, D. D., and Weber, C. S., *J. Mol. Biol.*, **34**, 661, 681 (1968).

[27] Hennig, W., *J. Mol. Biol.*, **38**, 227 (1968).

[28] Forget, B. G., and Weissman, S. M., *J. Biol. Chem.*, **244**, 3148 (1969).

[29] Brownlee, G. G., Sanger, F., and Barrell, B. G., *J. Mol. Biol.*, **34**, 379 (1968).

[30] Birnstiel, M. L., Grunstein, M., Speirs, J., and Hennig, W., *Nature*, **223**, 1265 (1969).

[31] Smith, E. L. *Harvey Lectures*, Ser. 62, 231 (1967).

Position of the Initiator and Peptidyl Sites in the *E. coli* Ribosome

by
ERNST KUECHLER*
ALEXANDER RICH
Department of Biology,
Massachusetts Institute of Technology,
Cambridge, Massachusetts

If an initiation complex is formed with *E. coli* ribosomes, viral messenger RNA and formylmethionyl-tRNA and the unprotected messenger RNA is then digested with ribonuclease, the ribosome is still able to synthesize a pentapeptide fragment. The point of entry for messenger RNA on the ribosome therefore seems to be four codons removed from the initiator site. The same distance was found for the peptidyl site.

THE function of the ribosome is more fully understood than the details of its structure. There are two subunits with a combined diameter in the electron microscope of approximately 200 Å. It is usually believed that the addition of amino-acids takes place in the interior of the ribosome, but there is very little information about the site of this process. The fact[1] that proteolytic enzymes acting on the growing polypeptide chains in reticulocyte ribosomes are unable to digest the thirty to thirty-five amino-acids proximal to the transfer RNA suggests that a portion of the nascent chain is buried within the ribosome. Experiments[2,3] involving both synthetic and natural messenger RNAs have shown that the fragment of messenger RNA bound to the ribosome is also resistant to ribonuclease digestion.

We have extended this kind of study by binding *Escherichia coli* ribosomes to the initiator site of R17 viral RNA in the presence of labelled formylmethionyl-transfer RNA[4], subjecting this initiation complex to extensive ribonuclease treatment and then allowing it to incorporate amino-acids. The product was found to be the N-terminal pentapeptide fragment of the coat protein, which was attached to the ribosomal structure through transfer RNA. We interpret these experiments to indicate that the initiator site is four codon units away from the position where ribonuclease can act at the ribosomal site of entry for messenger RNA. A similar interpretation is also suggested by experiments in which the sequence was determined of viral RNA segments protected by ribosomes attached to initiator sites[5,6]; and further by an experiment in which an amber codon in the viral RNA is brought into the ribosome before ribonuclease digestion. When the amber is four codon units removed from the peptidyl site, ribonuclease digestion of the messenger RNA does not affect its normal function in bringing about termination and release of the peptide chain. These experiments also show that ribonuclease digested ribosomes are able to carry out the normal steps of polypeptide chain elongation and termination.

The single stranded messenger RNA extracted from the R17 bacteriophage virus[7] was added to purified *E. coli* ribosomes in the presence of ^{35}S-labelled formylmethionine transfer RNA, the initiator of protein synthesis[4], to give the initiation complex shown in Fig. 1*a*. The complex was exposed to pancreatic ribonuclease (10 μg/ml.) and incubated for 1 or 2 h at 0° C, resulting in an almost complete digestion of the R17 messenger RNA and considerable digestion of the ribosomal RNA. In these conditions, however, the formylmethionyl-tRNA remains intact (E. K. and A. R., manuscript in preparation). The nuclease digested ribosomes were retrieved by centrifugation through sucrose and placed in a complete system for amino-acid incorporation. The oligopeptide obtained was characterized by thin layer electrophoresis.

Another variant of this experiment was carried out by using a mutant of R17 virus, amB$_2$ (ref. 8), which has an amber codon in the seventh position of the coat protein gene. When this RNA is used in an amino-acid incorporating system a free hexapeptide is produced, fMet-Ala-Ser-Asn-Phe-Thr, corresponding to the N-terminus of the *in vitro* synthesized R17 virus coat protein[9]. The initiation complex is again formed with the amB$_2$ RNA using ^{35}S-labelled formylmethionyl-tRNA and ribosomes (Fig. 1*b*). A limited addition of amino-acids was carried out using an *in vitro* incorporation system deficient in asparagine, the fourth amino-acid[10]. This incorporation results in the formation of tripeptide which is still attached to the tRNA in the ribosome. In this complex the amber codon is four codons away from the peptidyl site which is occupied by the tRNA containing the tripeptide. When ribonuclease digestion was carried out and the ribosomes used in an *in vitro* system in the presence of all amino-acids, the hexapeptide fragment of the coat protein was released into the supernatant.

Oligopeptides were identified using thin layer electrophoresis at *p*H 4·8 (ref. 11), which gave a good separation of all of the peptides (Fig. 2). The mobility of the N-terminal oligopeptides is plotted relative to the mobility of formylmethionine as a function of molecular weight. In the absence of side effects, mobility should be inversely related to molecular weight: the observed points in fact fall very close to the solid line in Fig. 2. For electrophoresis, *p*H 4·8 was chosen because it is far enough from the *pK* value of the carboxyl groups to minimize individual differences between carboxy terminal amino-acids.

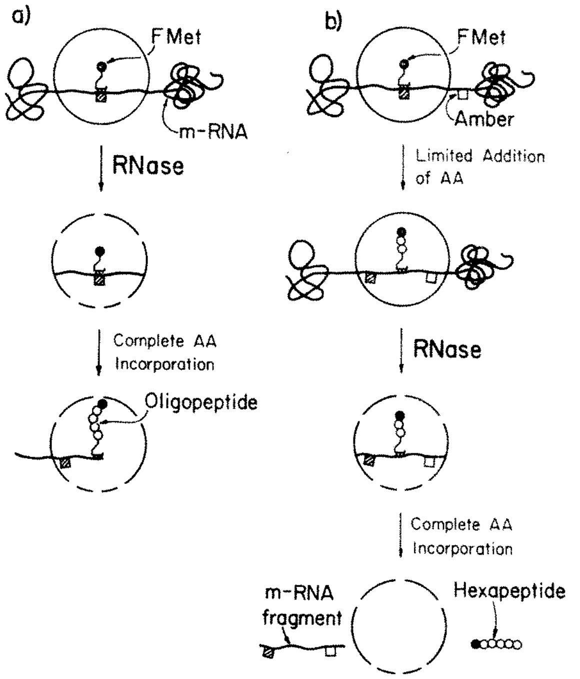


Fig. 1. Schematic diagram of the experiments: (*a*) Formation of the initiation complex with wild type R17 RNA as messenger, followed by ribonuclease digestion and incorporation in a complete *in vitro* protein synthesizing system. The cross-hatched square represents the initiation triplets; the hook is tRNA and the large circle is a ribosome. (*b*) Formation of the initiation complex with amB$_2$ RNA. The open square represents the amber triplet. A limited addition of two amino-acids is followed by ribonuclease digestion and complete amino-acid incorporation results in released hexapeptide.

---

* Present address: Institut für Biochemie, University of Vienna, Vienna 9.

The ribosome is able to carry out normal peptide synthesis after treatment with ribonuclease. The N-terminal peptides of the coat protein, fMet-Ala and fMet-Ala-Ser, are formed sequentially in an incorporation system which is deprived of asparagine[10], the fourth amino-acid in the polypeptide chain (Fig. 3). After ribonuclease treatment of the initiation complex, ribosomes were incubated at 15° C in the presence of alanyl and seryl-RNA plus supernatant. At various times, aliquots were analysed by thin layer electrophoresis. There is a rapid disappearance of formylmethionine with a corresponding sharp rise in the amount of radioactive fMet-Ala which reaches a peak approximately 10 s after the onset of incubation and then falls slowly. Concurrently there is a gradual rise in the radioactivity of the tripeptide fMet-Ala-Ser which continues for the entire 5 min incubation period. These kinetic curves would be expected in a system in which fMet-Ala is an intermediate in the synthesis of the fMet-Ala-Ser tripeptide. The rapid appearance of the dipeptide fMet-Ala results from a fast initial transpeptidation step; the somewhat slower appearance of the tripeptide fMet-Ala-Ser may reflect a limiting amount of a supernatant factor[12]—tripeptide formation is known to require all the factors for polypeptide chain elongation[13,14]. Similar results are obtained with ribosomes which have not been subjected to ribonuclease treatment, so that functions essential for protein synthesis are unimpaired and it implies that the degraded ribosomes are capable of synthesizing longer peptides.

Identification of Peptide Sequences

The initiation complex (Fig. 1) containing ^{35}S-labelled formylmethionyl-tRNA was isolated and then incubated with a complete amino-acid mixture and supernatant. After various times, aliquots of the incubation mixture were precipitated with 5 per cent trichloroacetic acid (TCA) and the precipitate was hydrolysed with alkali to release the nascent peptides from the tRNA. At zero time of incubation it can be seen that, where no ribonuclease treatment took place, all of the radioactivity is associated with formylmethionine. At 5 and 10 min of incubation there are a series of radioactive products which migrate to positions both above and below the position of the marker compounds of radioactive N-terminal penta and hexapeptides of the coat protein. The more slowly migrating oligopeptides present after both 5 and 10 min of incubation include nona and decapeptides; no larger peptides are found because of the method of isolation. Following incubation with alkali to release the oligopeptides bound to tRNA, peptides containing more than ten amino-acids were precipitated with 5 per cent TCA, so accounting for the fairly sharp discontinuity in the longer peptides. In the absence of R17 RNA in the incubation mixture, there is no appreciable accumulation of peptides, which suggests that there is only a very small background of endogenous messenger RNA present in the ribosome preparation.

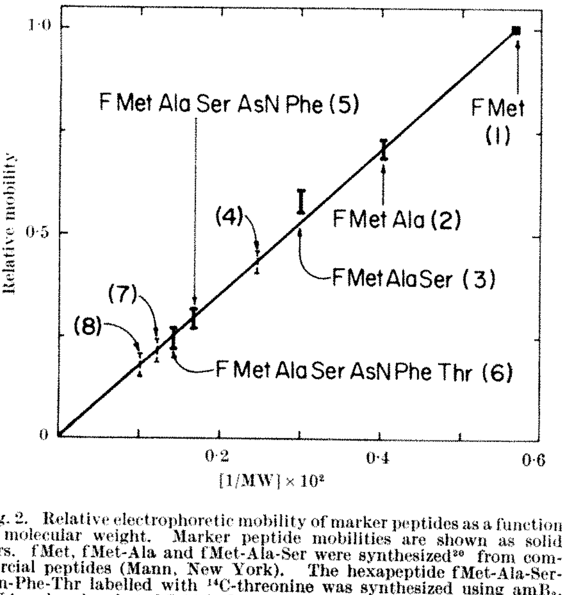


Fig. 2. Relative electrophoretic mobility of marker peptides as a function of molecular weight. Marker peptide mobilities are shown as solid bars. fMet, fMet-Ala and fMet-Ala-Ser were synthesized[20] from commercial peptides (Mann, New York). The hexapeptide fMet-Ala-Ser-Asn-Phe-Thr labelled with ^{14}C-threonine was synthesized using amB$_2$, RNA and preincubated S-30 (ref. 10). The pentapeptide fMet-Ala-Ser-Asn-Phe labelled with ^{14}C-phenylalanine was obtained from the hexapeptide containing ^{14}C-phenylalanine by incubation with 50 μg/ml. of chymotrypsin (Worthington) in 0·1 M Tris (*p*H 8) and 0·005 M CaCl$_2$ at 37° C for 1 h. The dashed bars indicate the positions of radioactive peptides formed *in vitro* cell free synthesis. Their electrophoretic mobilities characterize them as the N-terminal tetra, hepta and octapeptide of the R17 coat protein. The length of the bars characterizes the range of relative electrophoretic mobilities obtained. Electrophoresis was carried out at 4° C on 20 × 20 cm cellulose thin layer glass plates (Merck, Germany) at *p*H 4·8 (12·5 ml. acetic acid, 14 ml. pyridine per l. buffer) using 38 V/cm (ref. 11). Electrophoresis times were either 105 min or 210 min. To correct for the endosmotic effect, ^{14}C-uridine was used as a marker for the origin.

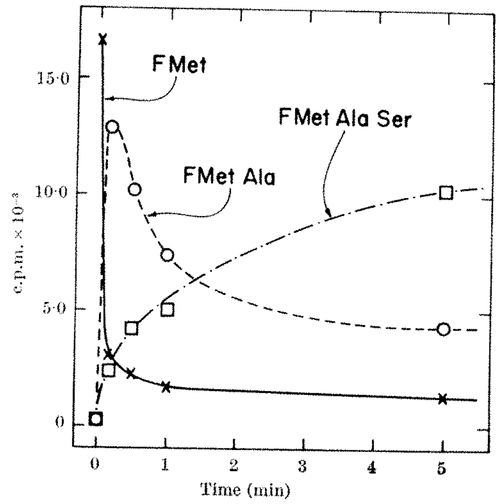


Fig. 3. Kinetics of the sequential formation of the N-terminal peptides fMet-Ala and fMet-Ala-Ser on ribonuclease treated ribosomes. *E. coli* Q13 ribosomes containing about 70 per cent native subunits were used throughout[19]. The ribosomes were preincubated for 30 min at 37° C in the presence of 3 mM ATP, 0·2 mM GTP and 5 mM PEP; 20 μg of PEP kinase and 100 μg of N^5-formyltetrahydrofolic acid were added per ml. The ribosomes were purified by centrifugation through 6 ml. 1 M sucrose containing 0·03 M NH$_4$Cl, 0·05 M Tris (*p*H 7·6), 0·01 M magnesium acetate, 0·001 M EDTA, 0·001 M Cleland's reagent for 14 h at 60,000 r.p.m. in a Spinco 65 rotor. ^{35}S-fMet-*t*RNA was freed from aminoacyl-tRNA by treatment with 10 mM CuSO$_4$ at *p*H 5·5 (refs. 21, 22). The mixture for the formation of the initiation complex (2 ml.) contained per ml.: 2·7 mg *E. coli* ribosomes, 0·75 mg R17 RNA, 2·3 μmoles GTP, 5 μmoles magnesium acetate, 1·8 × 10^6 c.p.m. ^{35}S-fMet-tRNA (2 × 10^5 c.p.m. per $A_{260\text{ nm}}$ unit), 50 μmoles KCl, 10 μmoles NH$_4$Cl, 100 μmoles Tris (*p*H 7·2) and 6 μmoles mercaptoethanol. After 10 min at 37° C and subsequent cooling to 0° C the Mg^{2+} concentration was raised to 10 mM and the solution was incubated for 1 min at 37° C. 10 μg/ml. of pancreatic ribonuclease was added at 0° C and the digestion continued for 1 h at 0° C. All subsequent operations were carried out at 0–4° C. The solution was layered on 6 ml. of 1 M sucrose-containing NH$_4^+$-Tris-Mg^{2+} buffer; 0·03 M NH$_4$Cl, 0·05 M Tris (*p*H 7·6), 0·012 M magnesium acetate and 0·001 M Cleland's reagent. It was then centrifuged for 5 h at 60,000 r.p.m. at 2° C in a Spinco 65 rotor. The pellet was washed and resuspended in a 1·6 ml. NH$_4^+$-Tris-Mg^{2+} buffer. Part of this suspension (0·3 ml.) was used to make a 1 ml. final solution. The final solution for limited amino-acid addition contained per ml.: 800 μg tRNA ("stripped" in 0·5 M Tris, *p*H 9, at 30° for 45 min and purified on 'Sephadex *G*-25'), 3 μmoles ATP, 0·2 μmoles GTP, 5 μmoles PEP, 20 μg PEP kinase, 40 nmoles each of alanine and serine, S-100 (purified by ribonuclease treatment and phosphocellulose chromatography)[10] containing 1 mg protein, 30 μmoles NH$_4$Cl, 50 μmoles Tris (*p*H 7·6), 12 μmoles magnesium acetate and 1 μmole Cleland's reagent. The mixture was preincubated for 10 min at 37° and cooled to 0° before the addition of the ribosome suspension. Incubations were carried out at 15° C and stopped by freezing in solid CO$_2$–alcohol. TCA was added to 5 per cent and the sample was centrifuged at 1,000*g* for 5 min. The precipitate was treated with 0·1 M NaOH for 1 h at 0° C. TCA was again added to 5 per cent and the sample was centrifuged. The supernatant was extracted with cresol–ethyl acetate and the product purified according to the technique of Capecchi[11]. The eluate from the 'Dowex 50' column (0·5 × 2·5 cm) was lyophilized and redissolved in 25 μl. H$_2$O. 10 μg each of synthetic fMet, fMet-Ala and fMet-Ala-Ser were added. Electrophoresis was for 105 min as described in Fig. 2. The peptides were detected by spraying with platinic iodide reagent[11] and by autoradiography. Spots were removed from the plate and collected in scintillation vials; 0·5 ml. H$_2$O and 20 ml. of Bray's solution were added and the samples were counted in a liquid scintillation counter.

Fig. 4*b* shows the peptide fragments synthesized by ribosomes which had been subjected to 1 h of ribonuclease action. No products are formed at 0 min incubation, but after both 5 and 10 min of incubation there are peptides smaller than the hexapeptide. Most of the radioactivity released by alkali is found in a very intense spot which has the same electrophoretic mobility as the synthetic pentapeptide fMet-Ala-Ser-Asn-Phe of the viral coat protein. Smaller amounts of the intermediates fMet-Ala, fMet-Ala-Ser and fMet-Ala-Ser-Asn are also found. The pentapeptide product is absent when R17 RNA is not added to the incubation mixture. If the initiation complex is formed using RNA from the mutant amB$_2$ virus and treated analogously, the results are identical to those with wild type R17 RNA. In the electrophoretogram of Fig. 4 there is only a small separation between the penta and hexapeptide. Experiments using paper electrophoresis for longer time periods demonstrated that the oligopeptide product had the same electrophoretic mobility as the tritiated pentapeptide marker fMet-Ala-Ser-Asn-^{3}H-Phe and was clearly separated from the hexapeptide marker. The identity of the product in Fig. 4*b* with the synthetic pentapeptides was confirmed by co-electrophoresis after acid deformylation (E. K. and A. R., manuscript in preparation). Fig. 4*c* illustrates the experiment carried out with ribosomes subjected to 2 h of digestion with ribonuclease. After 10 min incubation the pentapeptide is still the chief product. Corresponding supernatants from the experiments in Fig. 4 were also analysed by thin layer electrophoresis and autoradiography. There was some release of the N-terminal di and tripeptide, which occurred equally in the ribonuclease and non-ribonuclease treated ribosomes, but no appreciable release of pentapeptide. Apparently when this product is formed on the ribonuclease digested messenger RNA it is securely held to the ribosome and released only by incubation with alkali which hydrolyses RNA.

In the amB$_2$ virus, the amber is the seventh codon in the coat protein gene. The amB$_2$ RNA was used as outlined in Fig. 1*b*. After limited incorporation of amino-acids in an asparagine-deprived system, the major product is the tripeptide fMet-Ala-Ser (Fig. 5*a*), which is not found in the absence of added amB$_2$ RNA. Following ribonuclease digestion, the ribosomes were incubated with a complete mixture of amino-acids and the radioactive peptides found in the supernatant and in the TCA precipitate were separated. The supernatant product (Fig. 5*b*) consists exclusively of the N-terminal hexapeptide of the coat protein, as indicated by co-electrophoresis with synthetic fMet-Ala-Ser-Asn-Phe-Thr. It is absent when no RNA from the amber mutant bacteriophage is added. The TCA precipitated ribosomes were treated with alkali to release nascent polypeptide chains, and autoradiography of the products (Fig. 5*c*) reveals a small amount of hexapeptide bound to the ribosome; quantitative analysis showed that 94 per cent of the hexapeptide is released. Thus, by contrast with the experiment of Fig. 4 in which the pentapeptide remains bound to the ribosome, the addition of three amino-acids before ribonuclease treatment resulted in the protection of the amber codon against ribonuclease and release of hexapeptide into the supernatant on *in vitro* incubation.

We have also used wild type R17 RNA in the experiment in which a tripeptidyl-tRNA-ribosome complex is formed before ribonuclease digestion. In this case the precipitate contained largely heptapeptide (Fig. 5*d*) which was identified by its mobility (Fig. 2). A quantitative analysis showed that 80 per cent of the heptapeptide was in the precipitate. This is in accord with the first experiment where formylmethionine was on the initiator site (the position occupied by fMet-tRNA, in the presence of GTP and initiation factors), indicating that four additional amino-acids can be added following ribonuclease digestion. But the tripeptidyl-tRNA occupies the peptidyl site on the ribosome, so that the initiator site seems to be identical to the peptidyl site on the ribosome within the resolution of this experiment—the length of one codon.

Significance of the Protected Region

The ribosome is able to carry out many of its normal functions following extensive exposure to ribonuclease. In experiments with polyuridylic acid directed polyphenylalanine synthesis, it has been shown that *E. coli* ribosomes are able to form polyphenylalanine even after

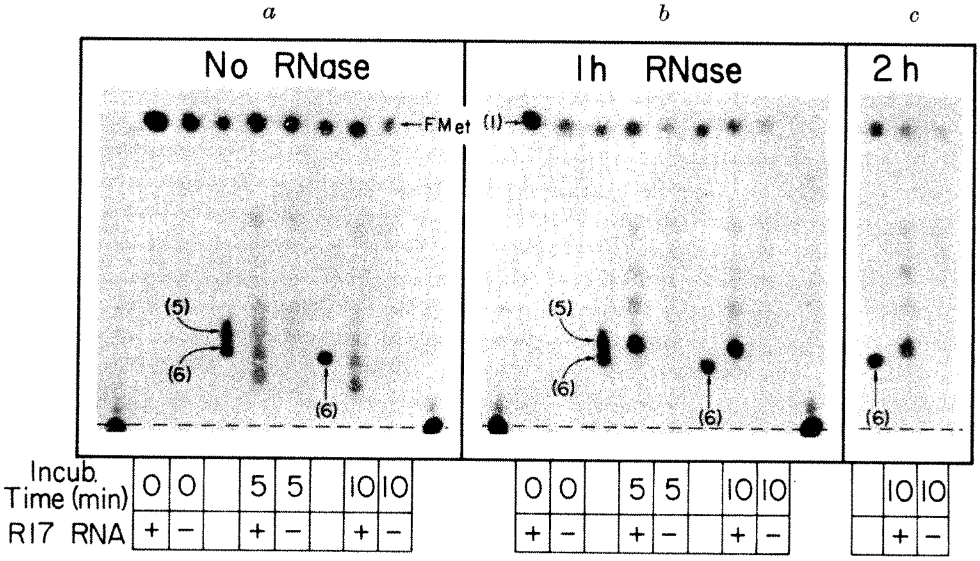


Fig. 4. Oligopeptide synthesis using the untreated and ribonuclease treated initiation complex containing ^{35}S fMet-tRNA, ribosomes and wild type R17 RNA. The initiation complex (2 ml.) was formed as described in Fig. 3; $3 \cdot 4 \times 10^6$ c.p.m. of ^{35}S-fMet-tRNA was used ($3 \cdot 7 \times 10^5$ c.p.m. per $A_{260\ \mathrm{nm}}$ unit) per ml. After the incubation and adjustment of the Mg^{2+} concentration to 10 mM, the mixture was split into three equal parts: *a* was not treated further; *b* and *c* were incubated with 10 μg/ml. of pancreatic ribonuclease at 0° C for 1 h and 2 h, respectively. Centrifugation and resuspension of the ribosomes were as described above. The *in vitro* protein synthesizing system was the same as in Fig. 3, except for the following components: 800 μg tRNA was precharged with all twenty amino-acids; 100 μg N^5-formyltetrahydrofolic acid and 100 nmoles of all amino-acids were present per ml. of incubation mixture. In addition a crude *E. coli* B S-100 was used at a concentration of 1·5 mg protein/ml. After incubation for 0, 5 and 10 min at 15° C, the reaction products were isolated from the TCA precipitate as described above. The electrophoresis was run for 2 h. The numbers (5) and (6) refer respectively to synthetic fMet-Ala-Ser-Asn-^{14}C-Phe and fMet-Ala-Ser-Asn-Phe-^{14}C-Thr. Radioactive uridine was added as a marker to show the position of the origin corrected for endosmotic migration (the dashed line at the bottom of the autoradiograph which passes between two uridine markers at either side). The plates were exposed to X-ray film for 6 days.

the 50S ribosomal subunit has been digested with ribonuclease to the point where there is no remaining intact 23S ribosomal RNA[15]. In those experiments it was necessary to increase the concentration of divalent ions above that used in a normal incubation. Now it has been shown that optimum incorporation of amino-acids occurs with a divalent ion concentration of 12 mM Mg^{2+}, by contrast with the optimum of 7–10 mM Mg^{2+} which is normally used with natural messenger RNA[9,10,16]. Our experiments show that the digested ribosomes (whose characterization will be described elsewhere) not only elongate polypetide chains but also release them when directed by an amber codon.

The R17 bacteriophage RNA codes for three proteins: maturation protein, coat and RNA synthetase protein. We are considering primarily the coat protein. Most of the binding of formylmethionyl-tRNA is on the coat protein initiation site if the viral RNA is intact[17]. In addition, however, we are using Capecchi's procedure of assaying the supernatant and the ribosome bound peptides[11,16] which involves an extraction of uncharged peptides into a cresol–ethyl acetate phase followed by chromatography on 'Dowex 50' and yields only N-blocked peptides which do not contain basic amino-acids. The N-terminal peptides of both the maturation and synthetase protein contain arginine and lysine[17]. Our analytical procedure would thus preferentially select for coat protein fragments and, because the amber mutation is in the coat protein, the substantial release of hexapeptide in the experiment described in Fig. 5 shows that we are primarily analysing peptides directed by the coat protein gene.

In experiments with synthetic oligonucleotide messenger RNA, it has been shown that ribosomes are capable of reading to the end of a complete codon in the oligonucleotide[18]. If a codon is incomplete—does not contain three nucleotides—the ribosome does not add another amino-acid. In assessing the results of the experiment which yielded the pentapeptide fMet-Ala-Ser-Asn-Phe, we therefore conclude that the pancreatic ribonuclease cleaves at least beyond the fifteenth nucleotide of the coat protein gene. The ribonuclease could have cleaved through the codon which specifies threonine in the sixth position, but it is unlikely that the ribonuclease left any appreciable amount of the threonine codon intact because no hexapeptide was detected attached to the ribosome (Fig. 4). The presence of the N-terminal heptapeptide (Fig. 5*d*) reinforces this interpretation. These results are in agreement with experiments in which the portion of the messenger RNA protected by the ribosome was isolated and sequenced and which indicated that ribonuclease cleavage occurs immediately after the fifteenth residue in the polynucleotide chain[5]. Similar results have also been obtained with Qβ virus[6]. The results of these and the amino-acid incorporation experiments therefore indicate that the initiator site is removed from the ribosomal entrance for messenger RNA by four codons, or a total of fifteen nucleotides if we include the initiator codon. Formation of a heptapeptide when the peptidyl site is occupied by tripeptidyl-tRNA suggests that that site is similarly removed some fifteen nucleotides from the ribosomal point of entry for messenger RNA, although the same result would be obtained if the peptidyl site were sixteen or seventeen nucleotides removed.

If we assume that the ribonuclease is able to act at the periphery of the ribosome and that the messenger RNA has its bases stacked with a separation of 3·4 Å per nucleotide, a distance of approximately 50 Å from the initiator or peptidyl sites to the periphery is suggested. But the polynucleotide chain can also be extended to an internucleotide spacing near 7 Å per base, so that the maximum distance could be as great as 100 Å, or approximately half the ribosomal diameter. It is of interest that proteolytic digestion of the nascent polypeptide chain on the ribosome[1] also indicated that the site for amino-acid addition is a similar distance from the point at which the proteolytic enzymes can act. Thus it is likely that the enzymatic functions of the ribosome occur deep within the ribosomal structure.

This research was supported by grants from the US National Institutes of Health and the National Science Foundation. We thank Dr M. Capecchi for supplying us

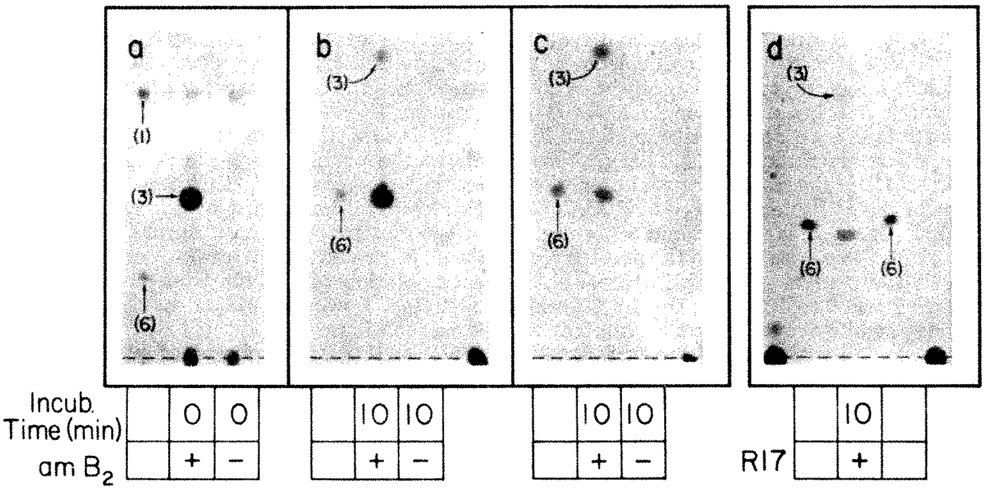


Fig. 5. Experiments using RNA from the virus mutant amB_2. The tripeptidyl-tRNA–ribosome–mRNA complex was formed in a purified *in vitro* system (2 ml.) by limited addition of alanine and serine as described in Fig. 3 except that 0·75 mg amB_2 RNA, 2·7 mg *E. coli* ribosomes and $1·9 \times 10^6$ c.p.m. ^{35}S-fMet-tRNA ($3·3 \times 10^5$ c.p.m. per $A_{260 \text{ nm}}$ unit) were added per ml. The incubation was carried out at 7 mM Mg^{2+} for 10 min at 37° C. After increasing the Mg^{2+} concentration to 10 mM, the mixture was again incubated for 1 min at 37° C and rapidly chilled. Ribonuclease digestion was carried out at 0° for 1 h at 10 μg/ml. Following centrifugation and resuspension of the ribosomes, a complete amino-acid incorporation system identical to that in Fig. 4 was used. The oligopeptides were isolated from the 5 per cent TCA precipitate after hydrolysis at 0° C in 0·1 M NaOH[10]. *a*, The tripeptide fMet-Ala-Ser isolated from the TCA precipitate before amino-acid incorporation in the complete *in vitro* system; *b*, hexapeptide released into the supernatant; *c*, the peptides which remain bound to tRNA. Electrophoresis was for 105 min in *a* and 210 min in *b* and *c*. The plates were exposed to X-ray film for 4 days. *d*, Heptapeptide precipitated with the ribosomes when wild type R17 RNA was used instead of amB_2 RNA. The *in vitro* system (2 ml.) was as described except that $0·53 \times 10^6$ c.p.m. ^{35}S-fMet-tRNA ($0·8 \times 10^6$ c.p.m. per $A_{260 \text{ nm}}$ unit) was added per ml. There is no incorporation in the absence of R17 RNA. The electrophoresis was run for 3 h. The plate was exposed to X-ray film for 6 days. The position of N-terminal marker peptides is indicated by the numbers in parentheses. The dashed line at the bottom is the corrected origin which is marked by radioactive uridine.

with the R17 bacteriophage strains. We also thank Dr Harvey Lodish for discussions.

Received December 16, 1969.

[1] Malkin, L. I., and Rich, A., *J. Mol. Biol.*, **26**, 329 (1967).

[2] Takanami, M., and Zubay, G., *Proc. US Nat. Acad. Sci.*, **51**, 834 (1964).

[3] Takanami, M., Yan, Y., and Jukes, T. H., *J. Mol. Biol.*, **12**, 761 (1965).

[4] Adams, J. M., and Capecchi, M. R., *Proc. US Nat. Acad. Sci.*, **55**, 147 (1966).

[5] Steitz, J. A., *Nature*, **224**, 957 (1969).

[6] Hindley, J., and Staples, D. H., *Nature*, **224**, 964 (1969).

[7] Gesteland, R. F., and Boedtker, H., *J. Mol. Biol.*, **8**, 496 (1964).

[8] Gussin, G. N., *J. Mol. Biol.*, **21**, 435 (1966).

[9] Zinder, N. D., Engelhardt, D. L., and Webster, R. E., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 251 (1966).

[10] Bretscher, M., *J. Mol. Biol.*, **34**, 131 (1968).

[11] Capecchi, M. R., *Biochem. Biophys. Res. Commun.*, **28**, 773 (1967).

[12] Lucas-Lenard, J., and Lipmann, F., *Proc. US Nat. Acad. Sci.*, **55**, 1562 (1966).

[13] Haenni, A. L., and Lucas-Lenard, J., *Proc. US Nat. Acad. Sci.*, **61**, 1363 (1968).

[14] Erbe, R. W., Nau, M. M., and Leder, P., *J. Mol. Biol.*, **39**, 441 (1969).

[15] Cahn, F., Schachter, E. M., and Rich, A., *Biochim. Biophys. Acta* (in the press).

[16] Capecchi, M. R., *Proc. US Nat. Acad. Sci.*, **58**, 1144 (1967).

[17] Lodish, H., *Nature*, **220**, 345 (1968).

[18] Thach, R. E., Dewey, K. F., and Mykolajewycz, N., *Proc. US Nat. Acad. Sci.*, **57**, 1103 (1967).

[19] Friedman, H., Lu, P., and Rich, A., *Nature*, **223**, 909 (1969).

[20] Sheehan, J. C., and Yang, D. H., *J. Amer. Chem. Soc.*, **80**, 1154 (1958).

[21] Schofield, P., and Zamecnik, P. C., *Biochim. Biophys. Acta*, **155**, 410 (1968).

[22] Sanger, F., Bretscher, M. S., and Hocquard, E. J., *J. Mol. Biol.*, **8**, 38 (1964).

Origin and Dynamical Behaviour of Thermal Protons in the Venusian Ionosphere

by

P. M. BANKS
Department of Applied Physics and
Information Science, and
Institute for Pure and Applied Physical Sciences,

W. I. AXFORD
Department of Physics,
Department of Applied Physics and
Information Science, and
Institute of Pure and Applied Physical Sciences,
University of California, San Diego,
La Jolla, California 92037

An important source of thermal H^+ and/or D^+ has been overlooked in the ionosphere of Venus which supports the choice of H^+ and/or D^+ as the dominant light ion at high altitudes.

DIRECT measurements of the thermal plasma environment of Venus were made in late 1967 by radio occultation methods using Mariner V (refs. 1–3). The data have led to the following conclusions regarding the ionosphere of Venus.

(1) The main ionospheric region in the sunlit hemisphere appears as a well defined layer of CO_2^+. Models based on photoionization of CO_2, followed by dissociative recombination of CO_2^+, are in substantial agreement with the density profiles deduced experimentally[4,5]. The peak CO^+ density for a solar zenith angle $\Psi = 40°$ is approximately 6×10^5 ions cm^{-3} at an altitude of 140 km (we assume a mean planetary radius of 6,053 km). Above the ionization peak CO_2^+ decreases approximately as $\exp(-z/H_0)$ where z is the altitude and $H_0 = 2kT/M(CO_2)g$ is the scale height. Here k is Boltzmann's constant, $M(CO_2)$ is the mass of a CO_2 molecule, g is the acceleration of gravity, and T is the neutral gas temperature. Near the peak of the layer $T \simeq 300$ to 400 K, giving a CO_2^+ scale height of about 15 km, while above 200 km a temperature of about 700 K is expected with $H_0 \simeq 30$ km (ref. 6).

(2) At an altitude of about 500 km in the daytime ionosphere a very sudden transition occurs[2] from the relatively cool ($\lesssim 0 \cdot 1$ eV/particle) ionospheric plasma of moderate density ($\sim 10^4$ cm^{-3}) to interplanetary conditions where energetic protons (~ 1 KeV/particle) predominate with densities of 1 to 10 cm^{-3}. This transition boundary, or plasmapause, is thought[7,8] to arise from a shielding of the Venus ionosphere by compression of the interplanetary magnetic field against the highly conducting planetary plasma. Essentially, as the solar wind flows past Venus the imbedded interplanetary magnetic field is unable to diffuse rapidly through the Venusian ionosphere and, as a result, electromagnetic forces are established which deflect the solar wind around the planet in such a way that no intermixing of the solar wind and ionospheric plasma takes place. From this point of view the region of magnetic field enhancement forms a protective boundary layer between the planetary environment and the vastly different conditions of the solar wind. The existence of such a boundary depends, of course, on there being a balance between the ram pressure of the solar wind, the compressed magnetic field pressure, and the pressure of the planetary ionosphere. In the case of Venus the planetary ionosphere is thought to arise from photoionization of the neutral atmosphere, with ionospheric energy densities of the order of 10^3 eV cm^{-3} being required to balance the external solar wind pressure in the subsolar regions.

(3) Below the Venusian dayside plasmapause, but above the peak ionospheric density, there seems to be a transition from CO_2^+ to some unidentified light ion. Unfortunately, it is just in this region that the formation of caustics prevents an accurate analysis of the electron density profile from radio occultation studies. The extrapolation of the electron density profile from lower altitudes, however, indicates that CO_2^+ cannot be the dominant ion.

(4) In the initial phases of planetary occultation the night time ionosphere was probed by Mariner V (ref. 2). The results seem consistent with a main ionospheric layer of CO_2^+ having a peak density of 10^4 cm^{-3} at an altitude of about 170 km. Due to the long rotation period of Venus and in the absence of any known local ionization processes, it has been suggested[2,9] that the night time ionosphere results from He^+–CO_2 charge transfer. If so, it is generally assumed that lateral transport of He^+ from the daytime ionosphere is adequate to maintain CO_2^+ in the dark regions. No detailed calculations have yet been made of this effect, however, and in the face of rapid He^+ neutralization in CO_2 and N_2, it may prove difficult to supply an adequate flux of He^+ to overcome the rapid rate of CO_2^+ dissociative recombination[10].

These general features are illustrated in Fig. 1. At the bow shockfront[11] the originally supersonic solar wind is reduced to subsonic speeds as it flows around the planet.

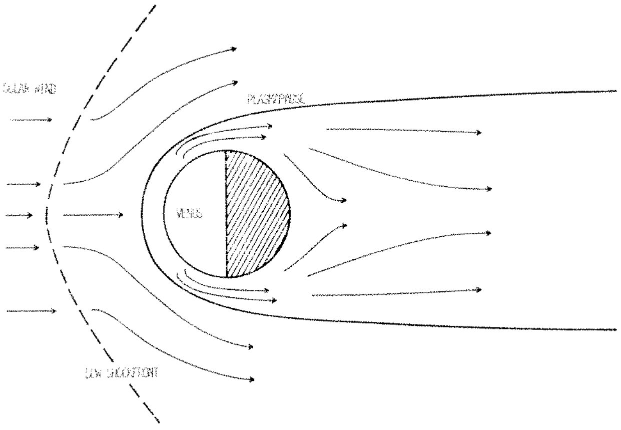


Fig. 1. Plasma environment of Venus. The solar wind passes through the bow shockfront and around the planet after being deflected by the compressed interplanetary magnetic field. The magnetic field boundary layer protects the high ionosphere from direct contact with the solar wind. Within the daytime ionosphere H^+ or D^+ created through CO_2^+–H/D charge exchange flows upwards and rearwards forming a thermal plasma tail behind the planet.

Penetration of the solar plasma into the planetary atmosphere is limited by the formation of a compressed magnetic field boundary layer across which transport of solar wind or ionospheric plasma cannot occur. The shape of the plasmapause, discussed later, is determined by a balance between the normal component of solar wind pressure and the ionospheric pressure. The arrows in the region outside the plasmapause indicate the solar wind flow, while the interior arrows show a proposed flow pattern of ionospheric plasma into the tail region which will be discussed in this article.

Although many aspects of the main ionospheric layer of Venus can be discussed in terms of the CO_2^+ photoionization model, there are some unexplained features of considerable importance. Among these must be included the following.

(1) The Stanford University occultation experiment, operating at 49·8 and 423·3 MHz, detected an extended region of enhanced thermal plasma lying behind Venus in the antisolar direction[2]. A mean ion density of 300 cm^{-3} was deduced extending 10,000 km behind the planet. Although it was suggested[2] that this planetary tail was composed of light ions, the particles have yet to be identified and their origin remains unknown.

(2) On the nightside of Venus at altitudes between 750 km and 1,450 km the presence of a light ion was inferred from the measured altitude variation of electron density[2]. The source of this cool plasma is not known, but probably it does not arise from nightside ionization processes.

(3) In the dayside ionosphere the composition transition from CO_2^+ to a light ion requires the presence of H^+, D^+, H_2^+ or He^+. Of these possibilities it is difficult to argue that H_2^+ and He^+ are dominant ion species because of their rapid neutralization reactions with CO_2 and N_2. In fact, He^+ is lost so rapidly that to maintain the night time ionosphere alone requires either a very large density of He in the Venusian atmosphere[9] or a photoionization rate much greater than appropriate for terrestrial helium. With regard to H^+ or D^+ the low rate of photoionization ($\sim 3\times 10^{-7}$ s^{-1}) has precluded serious consideration of H^+ or D^+ as important ionic components. (The problem concerned with the existence of H, D, or H_2 in the Venusian atmosphere has been discussed at length elsewhere[12]. For the purposes of this article it is not necessary to differentiate between H and D because their ionization energies are nearly the same.)

It is evident that the presence of light ions is required in the outer regions of the Venusian ionosphere to explain both the formation of the dayside plasmapause and the existence of a tail of ionization behind the planet. Of the various light ions available (H^+, D^+, H_2^+, He^+) neither H_2^+ nor He^+ is likely to be the main ion species at high altitudes, while the small photoionization rate of H^+ (or D^+) seems to remove this ion from consideration. We wish to point out, however, that an important source of thermal H^+ and/or D^+ has been overlooked in the Venusian ionosphere which supports the choice of H^+ and/or D^+ as the dominant light ion at high altitudes.

We suggest that the charge exchange reaction

$$CO_2^+ + \binom{H}{D} \xrightarrow{k_f} CO_2 + \binom{H^+}{D^+} + 0{\cdot}17 \text{ eV} \qquad (1)$$

proceeds rapidly in the forward (f) direction and provides a copious supply of protons (or deuterium ions) in the Venusian ionosphere. Although the forward reaction rate k_f for reaction (1) does not seem to have been measured, the exothermic (0·17 eV) nature of the exchange, together with the large number of final energy states available in CO_2, imply that reaction (1) is a rapid process at thermal energies. Thus, as in the terrestrial ionosphere where H^+ is produced principally through O^+-H charge exchange rather than photoionization, we propose that photoionization of H is relatively unimportant in the Venusian ionosphere but that, through reaction (1), H^+ and/or D^+ are the chief ionic constituents of the daytime ionosphere above the CO_2^+ layer.

At low altitudes where diffusion proceeds slowly, the steady state H^+ and /or D^+ density is determined through a balance of ion production and loss. Although reaction (1) has been identified as a source of light ions, the reverse reaction, k_r, also occurs. But the 0·17 eV energy defect of the reaction, together with the large CO_2 statistical weight factor, implies that $k_f/k_r > 250$. Because radiative recombination of H^+ or D^+ is generally a slow process, the equilibrium ion density can be expressed as

$$\frac{n(H^+/D^+)}{n(CO_2^+)} = \frac{k_f n(H/D)}{k_r n(CO_2)} > 250\ \frac{n(H/D)}{n(CO_2)} \qquad (2)$$

indicating an increase in the H^+/D^+ density relative to CO_2^+ in the regions of chemical equilibrium.

With the identification of reaction (1) as the ionization source for H^+ and/or D^+ in the Venusian ionosphere, it is possible to consider in more detail a reasonable model for the spatial distribution of plasma about Venus. As described previously, the compressed interplanetary magnetic field imposes a pressure boundary condition upon the Venusian upper ionosphere such that in the steady state the plasmapause must form at an altitude where the approximate relation

$$N_{sw}\, M V^2 \cos^2\xi = nk(T_e + T_i) \qquad (3)$$

is satisfied. Here N_{sw} is the solar wind proton number density, V is the solar wind speed (both measured outside the bow shockfront), M is the proton mass, ξ is the angle between the plasmapause normal and the solar wind direction, n is the ionospheric plasma density, and T_e, T_i are the ionospheric electron and ion temperatures. Using typical reported parameters[1,2,11] we find that the plasmapause should form at an altitude where $n \simeq 10^4$ cm^{-3} for $\Psi = 40°$.

Taking into account lateral plasma flow, the equations of continuity and momentum for the ith ion species in a multi-ionic constituent plasma can be written for steady state conditions as

$$\frac{1}{r^2}\frac{\partial}{\partial r}(n_i U_r r^2) + \frac{1}{r\sin\Psi}\frac{\partial}{\partial\Psi}(n_i \sin\Psi\, U_\Psi) = p_i - l_i \qquad (4)$$

$$U_r = -\frac{kT_i/m_i}{\sum\limits_k \nu_{ik}}\left\{\frac{1}{n_i}\frac{\partial n_i}{\partial r} + \frac{T_e/T_i}{n_e}\frac{\partial n_e}{\partial r} + \frac{1}{H_i}\right\} \qquad (5)$$

$$U_\Psi = -\frac{kT_i/m_i}{r\sum\limits_k \nu_{ik}}\left\{\frac{1}{n_i}\frac{\partial n_i}{\partial\Psi} + \frac{T_e/T_i}{n_e}\frac{\partial n_e}{\partial\Psi}\right\} \qquad (6)$$

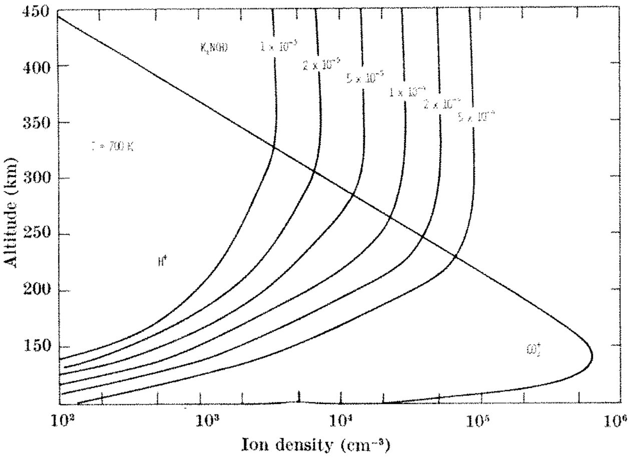


Fig. 2. Daytime ion composition profiles for diffusive equilibrium without lateral transport charge exchange reaction (1) with various ionization rates. A previous model neutral atmosphere of McElroy and Strobel[9] was used with parameters pertaining to CO_2^+ being taken from McElroy[5]. The introduction of D^+ for H^+ does not alter the results appreciably.

neglecting thermal gradients. Here r is a radial coordinate, U is the transport speed (r, Ψ components), p_i and l_i are the production and loss rates, ν_{ik} is the collision frequency between ith and kth type particles, $n_e = \Sigma n_i$, and $H_i = kT_i/m_i g$.

The boundary conditions to be used in conjunction with equations (4) to (6) determine the actual shape of the different ion density profiles. At the plasmapause boundary, the altitude of which is implicit in equation (3), the normal component of the plasma velocity is zero while the lateral component is unrestricted. Neglecting lateral transport in the subsolar wind regions, it follows that the approximate location of the plasmasphere can be found by computing solutions to the coupled ion density equations for CO_2^+ and H^+ and/or D^+ in diffusive equilibrium and searching for the altitude where equation (3) is satisfied.

Using the reaction rates and parameters given previously[5,9], profiles of ion composition have been computed for the daytime Venusian ionosphere for $\Psi \simeq \xi = 40°$ neglecting the effect of lateral transport. The results, shown in Fig. 2 for H^+, indicate the importance of reaction (1) even for relatively small reaction rates. Because the H or D densities vary slowly in the altitude range 200 to 500 km, the effective rate coefficient for ionization production, $k_f n(H)$, is indicated as a parameter in Fig. 2 to remove uncertainties connected with the neutral atomic hydrogen or deuterium density. In fact, reaction rates as fast as $k_f \simeq 2 \times 10^{-9}$ cm^{-3} s^{-1} may be typical for reaction (1), while H and/or D densities as great as 10^5 cm^{-3} may be present in the high atmosphere of Venus[12]. The substitution for D^+ in place of H^+ in reaction (1) and equations (4) to (6) does not alter significantly the results obtained for H^+; the factor of two difference in scale height is not important over the small interval between the CO_2^+ peak and the plasmapause. Further, there is no reason to expect a strong dependence on nuclear mass for the cross-section of reaction (1) even though a small relative velocity effect will be present in k_f.

From this discussion and Fig. 2, it is seen that the existence of H or D in the Venusian atmosphere, in the presence of CO_2^+, provides a sufficient source of ionization to explain the relatively high altitude of the plasmapause (consideration of CO_2^+ alone would lead to a much lower altitude) and the relatively slow variation of electron density with altitude characteristic of light ions. Furthermore, for $\Psi < 60°$ little variation is expected in the CO_2^+ density profile due to the optical depth factor, and from (1) and (3) it follows that the altitude of the plasmapause must increase with Ψ on the dayside of the planet.

Complete calculations of the ion density about Venus, including lateral flow of ionization, are in progress. For $\Psi \gtrsim 70°$ lateral flow is responsible for removing H^+ (or D^+) from the dayside and carrying it into the regions behind the planet from whence it is probably lost to interplanetary space. The chief contribution to the lateral flow arises at 400–500 km altitude where the ion-neutral diffusion coefficient is very large, that is, small lateral pressure gradients lead to large lateral plasma flows. When viewed from behind the planet, the upper ionosphere at the terminator should appear as a ring-shaped ionization source of H^+ and/or D^+ ions with flow into the dark regions as indicated in Fig. 1. The maximum rate of vertical efflux of H^+ and/or D^+ through the atmosphere has been calculated as $\sim 10^8$ cm^{-2} s^{-1}. If this efflux is uniform over the daylit hemisphere, a total flux of 2×10^{26} ions s^{-1} would be involved. If such a large flux were to exist, only about one hour would be required to fill a tail 10,000 km long to a mean density of 300 ions s^{-1}. But this estimate neglects both H^+ loss processes and the fact that only weak lateral flows can exist in the subsolar regions due to the smallness of the lateral pressure gradient. Estimates based on more realistic, but still arbitrary, conditions indicate that the actual time required to replenish the tail with ionization is of the order of several days.

Although it would be convenient to assume that the H^+ or D^+ contained in the plasma tail is in hydrostatic equilibrium, this is undoubtedly not the case. We envisage that a fountain effect takes place in the daytime Venusian ionosphere with the electric field established through $CO_2^+ - e$ charge separation (brought about by gravity and chemical processes) acting to drive H^+ (or D^+) outwards towards the plasmapause and, through lateral pressure gradients, rearwards into the tail region. While the original source of ionization lies in the photoionization of CO_2, reaction (1) provides a process leading to the eventual loss of H^+ and/or D^+ from the planetary atmosphere.

The actual speed of lateral flow of the plasma into the tail remains uncertain. In the absence of a strongly diverging geometry or frictional effects, a subsonic flow would be appropriate. Photoelectrons from the daytime upper atmosphere, however, may also escape into the tail region and lead to an enhancement of the plasma flow energy. In this case, average ion energies of the order of 10 eV per ion might be reached at great distances from the planet.

With regard to the night time ionosphere below 500 km, H^+ and/or D^+ from the dayside can clearly contribute to the observed electron density. It is noted, however, that H^+ and/or D^+ do not react readily with CO_2 to form CO_2^+ and hence probably cannot explain the night time CO_2^+ layer. As mentioned before, either lateral transport of He^+ or some low intensity ionization source (photons or energetic particles) may be involved.

This work was supported in part by NASA and by the Advanced Research Projects Agency of the Department of Defense, and was monitored by the US Army Research Office, Durham.

Received September 22, 1969.

[1] Snyder, C. W., *Science*, **158**, 1665 (1967).
[2] Mariner Stanford Group, *Science*, **158**, 1678 (1967).
[3] Kliore, A., Levy, G. S., Cain, D. L., Fjeldbo, G., and Rasool, S. I., *Science*, **158**, 1683 (1967).
[4] McElroy, M. B., *J. Atmos. Sci.*, **25**, 574 (1968).
[5] McElroy, M. B., *J. Geophys. Res.*, **74**, 29 (1969).
[6] McElroy, M. B., *J. Geophys. Res.*, **73**, 1513 (1968).
[7] Dessler, A. J., in *The Atmospheres of Venus and Mars* (edit. by Brandt, J. C., and McElroy, M. B.), 241 (Gordon and Breach Pub. Co., New York, 1968).
[8] Johnson, F. S., *J. Atmos. Sci.*, **25**, 658 (1968).
[9] McElroy, M. B., and Strobel, D. F., *J. Geophys. Res.*, **74**, 1118 (1969).
[10] Donahue, T. M., *J. Atmos. Sci.*, **25**, 568 (1968).
[11] Bridge, H. S., Lazarus, A. J., Snyder, C. W., Smith, E. J., Davis, L., Coleman, P. J., and Jones, D. E., *Science*, **158**, 1669 (1967).
[12] McElroy, M. B., and Hunten, D. M., *J. Geophys. Res.*, **74**, 1720 (1969).

LETTERS TO THE EDITOR

PHYSICAL SCIENCES

How Significant are the Sharp Peaks in the Frequency Distribution of QSO Redshifts ?

RECENT work by Plagemann, Feldman and Gribbin[1] seems to show that quasar redshifts are not intrinsic. I take no issue here with the more specific result of the Cambridge group, that there is no significant evidence for periodicity of peaks in the distribution of z (redshift). But, using the same observational material, which was taken from a list by Burbidge and Burbidge[2], I find that the well known peak at $z=0{\cdot}060$ cannot be due to chance, although there is some uncertainty of the significance level. On the other hand, the conclusion of Burbidge[3] that the peak at $z=1{\cdot}95$ is highly significant is not confirmed. It can be shown that the difference between our result and that of Burbidge is due to the interpretation of the statistics.

Before discussing the redshifts, we first consider the random distribution of points on a line. Let a line AB be divided in N equal parts (cells). We distribute s points randomly over the line so that the probability ($p=1/N$) for a point to fall in any cell is the same for all cells and points. In an actual experiment, the frequency of points will vary from cell to cell.

The probability $w(n)$ that any cell shall contain exactly n points is then given by Bernoulli's formula

$$w(n) = \frac{s\,!}{s\,!\,(s-n)\,!}\,p^n \cdot (1-p)^{s-n} \qquad (1)$$

The Poisson approximation to equation (1) is good enough for our purpose. Hence

$$w(n) = \frac{\mu^n}{n\,!}\,\mathrm{e}^{-\mu} \qquad (2)$$

where $\mu=s/N$ is the average number of points per cell.

The expected number of cells containing exactly n points is found by multiplying $w(n)$ with the total number of cells.

$$E(n) = N \cdot w(n) = N \cdot \frac{\mu^n}{n\,!}\,\mathrm{e}^{-\mu} \qquad (3)$$

The gross distribution of redshifts with $0{\cdot}02<z<2{\cdot}18$ and nine equal intervals $\Delta z=0{\cdot}24$ is shown in Fig. 1. The lack of uniformity is evident and a complete explanation is likely to involve a number of factors with observational selection as a prominent contributor. Here I make no attempt to understand Fig. 1, but only investigate the reality of the peaks in the z distribution. The lack of uniformity implies that the *a priori* probability of z is not uniform and that the analysis leading to equation (3) would be inappropriate. Fortunately this difficulty can be overcome by choosing a smooth function x of z, having a uniform gross distribution. The distribution in x, then, can be compared with the random distribution given by equation (3). No peaks will have been either destroyed or created in the smooth transformation from z to x, so the question of the significance of the peaks in the distribution of z may be answered by investigating the distribution of x. Fig. 2 shows the gross distribution of the function x that was finally decided on. The uniformity is satisfactory, and it can be shown that the variation in the gross cell frequency is of the order normally found in random distributions.

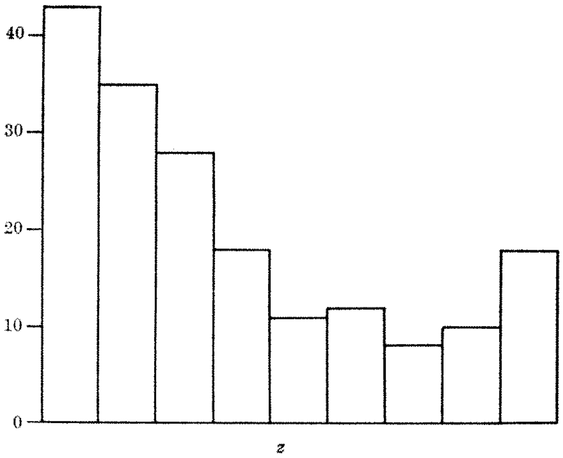


Fig. 1. Gross frequency distribution of redshifts for $0{\cdot}02<z<2{\cdot}18$ with $\Delta z=0{\cdot}24$.

Although the exact formula for x as a function of z is of no further consequence, it may be mentioned that $x(z)$ is the polynomial approximation to the number of QSOs with redshifts smaller than z, fitting exactly at $z=0$, 2/3, 4/3 and 2. The polynomial in fact is

$$x(z) = 24z^3 - 109z^2 + 208z$$

184 redshifts with $0{\cdot}024<z<2{\cdot}192$ corresponding to $5<x<185$ were analysed for the presence of peaks. The cell size chosen was $\Delta x=0{\cdot}33$, corresponding to a total number of cells $N=540$. The total number of QSOs is $s=184$ and the mean number per cell $\mu=0{\cdot}341$. The second column of Table 1 gives the observed number of cells containing exactly n QSOs. The frequency as calcu-

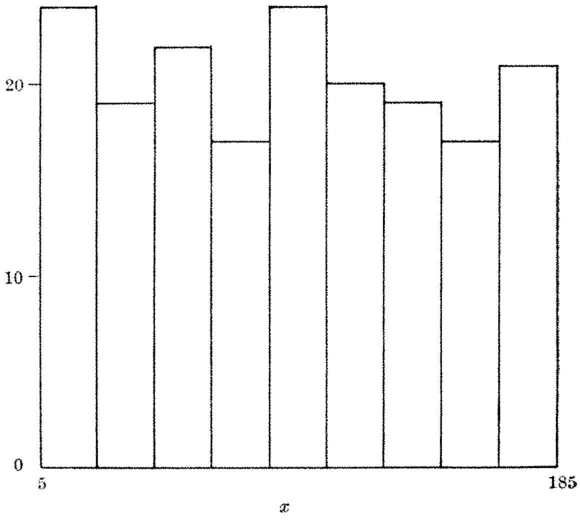


Fig. 2. Gross frequency distribution of x for $5<x<185$ with $\Delta x=20$.

lated from equation (3) is given in the third column and assumes a random distribution. The agreement is satisfactory and the differences not significant for $n=0$, 1, 2 and 3. For $n=4$ the residual is somewhat large but not likely to be significant. The single cell containing five QSOs demands attention, however. Because the expected theoretical frequency for $n=5$ and higher is only 0·01, it follows that a single count at $n=5$ or higher cannot be expected more frequently than once in a hundred experiments, on the average. This, in other words, establishes the group at $n=5$ as significant at the 1 per cent level. The peak occurs at $x=12{\cdot}3$ and the corresponding redshift is 0·060, which was already found significant by the Burbidges. No significant evidence is found for any of the other peaks which have been pronounced significant before. For instance, we find that the well known group at $z=1{\cdot}95$ causes a count at $n=4$, but the expected frequency 0·22 is too high to conclude significance.

Table 1. FREQUENCY OF CELLS CONTAINING n QSOs

n	Observed number of cells	Equation (3)
0	390	384·1
1	126	130·8
2	17	22·3
3	5	2·5
4	1	0·22
5	1	0·010

The test apparently consists of the comparison of observed with theoretical frequencies, the sensitivity increasing with diminishing theoretical frequency. I chose $\Delta x=0{\cdot}33$, but a reduction of Δx, for the purpose of increasing the sensitivity, is not practical, for $\Delta x=0{\cdot}33$ corresponds to $\Delta z=0{\cdot}002$ at $z=0{\cdot}060$, which is of the order of the accuracy of a determination of z. The theoretical frequencies at $n=5$ and larger, which are critical for the significance level, are rather sensitive to the value of μ used in equation (3). μ would have to be raised from 0·341, as used, to 0·40 to find the group at $z=0{\cdot}060$ only significant at the 5 per cent level. A corresponding error in μ seems out of the question, however.

The standard technique for testing frequency distributions for significant differences is the chi-square test. It is remarkable that this test when applied to our data shows no evidence for a significant departure from a random distribution. This is because the chi-square test cannot be applied unless the expected frequencies are larger than five. To apply the chi-square test, it would be necessary to pool the frequencies at $n=3$ and larger, with a consequent serious reduction in sensitivity at the low frequency end of the distribution[4].

In an alternative experiment, I tried the so-called "interval distribution", but found no evidence for nonrandomness. In the interval distribution, however, any non-random peak is pooled with a considerable number of random counts. The consequent high "noise level" makes this method particularly insensitive as a significance test for peaks.

None of the five objects with $z=0{\cdot}060$ seems to show any common characteristic other than redshift.

I thank Dr E. M. Standish for help. This work was partly supported by the US National Science Foundation.

A. J. WESSELINK

Yale University Observatory,
New Haven, Connecticut.

Received February 5, 1970.

---

[1] Plagemann, S. H., Feldman, P. A., and Gribbin, J. R., *Nature*, **224**, 875 (1969).

[2] Burbidge, G. R., and Burbidge, E. M., *Nature*, **222**, 21 (1969).

[3] Burbidge, G. R., *Astrophys. J. Lett.*, **154**, 41 (1968).

[4] Jeffreys, H., *Theory of Probability*, 88 (Clarendon Press, Oxford, 1939).

Resonance Fluorescence of the CN Free Radical

COMETARY emission spectra exhibit features of such diatomic radicals as CN, C_2, OH, CO^+, N_2^+ (ref. 1) which almost certainly arise from photon or corpuscular irradiation of polyatomic species in the comet head[2]. The emission spectra are almost certainly excited by resonance fluorescent excitation by solar photons[3]. Little laboratory work has been done on the kinetics of excitation processes of cometary spectra. In this report we record some recent work on the fluorescent excitation of CN in a static system.

Free CN was produced by gas-phase photolysis of ICN at 10^{-2} torr pressure using a 1·6 kW high pressure Xe lamp which emits a continuous spectrum between 2000 Å and 10,000 Å. The absorption spectrum of ICN exhibits a photodissociation continuum between 2000 Å and 3000 Å. The dissociation products are then I and vibrationally unexcited $\mathrm{CN}(X^2\Sigma)$ (ref. 5). In our experiments the Xe lamp not only photolysed ICN but also excited it to the $B^2\Sigma$ state from which fluorescence of the bands of the violet $B^2\Sigma$–$X^2\Sigma$ system were observed with a photoelectric scanning 0·25 m monochromator. The fluorescence signal was not strong, and, because of the operation of the Franck–Condon principle in excitation and radiation, consisted mostly of bands of the $\Delta v=0$ sequence near 3883 Å. Bands of the $\Delta v=\pm 1, \pm 2, \pm 3\ldots$ sequences were very weak. Fig. 1 is a scan of the $\Delta v=0$ sequence. The instrumental band pass was 3·3 Å and the strongest feature is the (0,0) band. The integrated signal from this sequence was used in the kinetic studies described later. Mechanisms related to the fluorescent excitation of CN are photodissociation, $\mathrm{ICN} + h\nu \rightarrow \mathrm{I} + \mathrm{CN}(X^2\Sigma, v''=0)$; fluorescent excitation, $\mathrm{CN}(X^2\Sigma) + h\nu \rightarrow \mathrm{CN}(B^2\Sigma, v'=0,1,2\ldots)$; radiation, $\mathrm{CN}(B^2\Sigma) \rightarrow \mathrm{CN}(X^2\Sigma)$; quenching, $\mathrm{CN}(B^2\Sigma)+\mathrm{ICN} \rightarrow \mathrm{CN}(X^2\Sigma)+\mathrm{ICN}$; recombination, $\mathrm{CN}+\mathrm{CN}+\mathrm{ICN} \rightarrow \mathrm{C_2N_2}+\mathrm{ICN}$; CN diffusion and loss to walls.

Recombination was not an important process at the experimental pressures. On the other hand, diffusion and quenching were major radical sinks. In the steady state the fluorescent intensity as a result of this would be

$$I_{fl} = \frac{K(E^2/D)[\mathrm{ICN}]}{1+K'[\mathrm{CN}]} \tag{1}$$

where K is a physical and geometrical proportionality constant, K' is the quenching constant, D is the diffusion coefficient, E is the lamp flux and ICN and CN are species concentrations.

Typical plots of I_{fl} against E^2 and [ICN] are displayed in Fig. 2 which confirm the phenomenological application

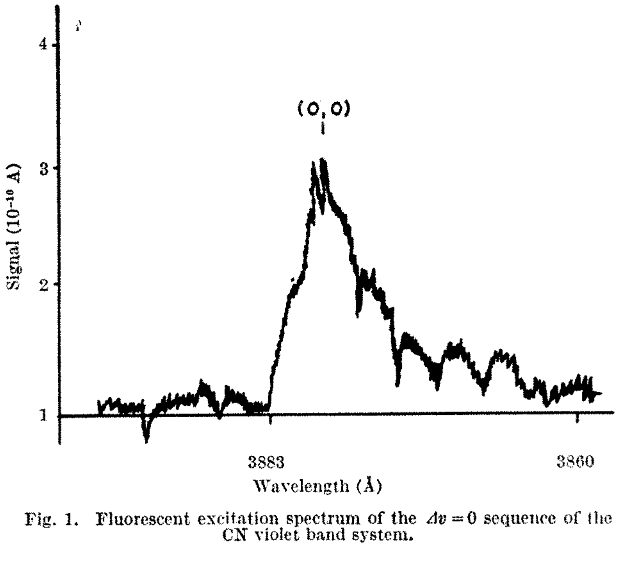


Fig. 1. Fluorescent excitation spectrum of the $\Delta v=0$ sequence of the CN violet band system.

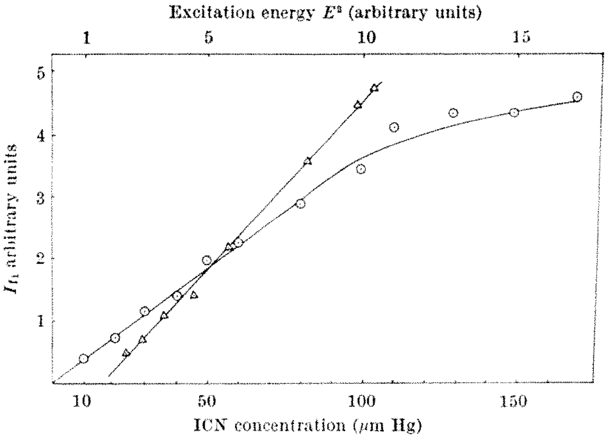


Fig. 2. Plots of intensity of the $\Delta v = 0$ sequence against [ICN] and E^2. ○, I_{f_1} versus [ICN]; △, I_{f_1} versus E^2.

of equation (1). The I_{f_1} versus [ICN] is of a characteristic Stern–Vollmer shape and from it a quenching cross-section of 90 (Å)^2 is derived, which attests to the efficiency of the quenching process.

This work was supported by research grants from the National Research Council of Canada. One of us (J. A. M.) was the recipient of National Research Council of Canada scholarships.

J. A. Myer*
R. W. Nicholls

Centre for Research in Experimental Space Science,
York University,
Toronto.

Received January 8, 1970.

* Present address: Avco Everett Research Laboratory, Everett, Massachusetts 02149.

[1] Wurm, K., *The Moon, Meteorites and Comets* (edit. by Kuiper, G. P.) (Univ. Chicago Press, 1963).
[2] Whipple, F. L., *Astrophys. J.*, **111**, 375 (1950).
[3] Swings, P., *Astrophys. J.*, **94**, 320 (1941).
[4] Myer, J. A., thesis, York Univ. (1968).
[5] Basco, N., Nicholas, J. E., Norrish, R. G. W., and Vickers, W. H., *Proc. Roy. Soc.*, A, **272**, 147 (1963).

Transient Lunar Phenomena and Electrostatic Glow Discharges

Transient lunar phenomena (TLPs) are evanescent localized glows and obscurations on the Moon; Aristarchus, Alphonsus and Schroeter's Valley are well known areas of activity[1]. The emitted light is usually described as reddish or pinkish, sometimes with a "sparkling" or "flowing" appearance[2,3]. The coloration may extend for a distance of 10 miles or more on the lunar surface, with brighter spots 2–3 miles across, and is commonly associated with veiling of the surface features. The average duration of an event is some twenty minutes, but it may persist intermittently for a few hours. No permanent alteration has been detected at the site of any TLP. These phenomena are regarded as distinct from the possible low-level general luminescence most easily detected during total lunar eclipse[1].

Middlehurst[4] has shown that TLPs are most frequent around perigee, and suggests that trapped gases are released by the accompanying tidal stress. So far only Kozyrev has succeeded in obtaining spectra, in which he identifies emission bands of molecular hydrogen, carbon and nitrogen[5–7]. He proposed that solar radiations produce ionization and excite fluorescence—but TLPs have also been reported on the dark side of the Moon[2,4]. Other suggestions include proton luminescence, thermoluminescence, shock-wave heating and emission from glowing lava or fire-fountains; but all have serious shortcomings as explanations of the phenomenon under consideration[8].

I have suggested that one mechanism for the formation of lunar craters was fluidization during degassing at an early stage in the evolution of the solar system[9]. A slight continuous evolution of gas is expected to persist to the present time, some accumulating in fissures and cavities until released by the tidal mechanism. In my model experiments I noted that tapping the apparatus could temporarily reactivate a few of the smaller craterlets. Kozyrev's spectra, and the absence of carbonates and hydrates in the returned lunar samples, suggest that the escaping gases are those retained by the Jovian planets—hydrogen, noble gases, methane and ammonia—rather than carbon dioxide and water vapour.

The release of trapped gases, and their rapid expansion, must lead to disturbance of the fine dust covering the lunar surface. Now it has long been known[10] that movement and dispersion of finely divided material result in separation of charge and the generation of considerable electrostatic potentials. Faraday[11], and later Rudge[12] and others, showed that a positive charge was left on the plate of an electroscope if sand was blown from it. I have found charges of the same sign to be produced by the dispersion of powdered samples of basalt, olivine, ilmenite, limonite, ash-flow deposits and chondritic meteorites.

Detailed studies have proved that both positively and negatively charged particles are present in a dust cloud, but generation in heterogeneous systems (especially when conducting surfaces are present) leaves a net excess of charge of one sign on the cloud. Dissimilar particles in heterogeneous clouds are often oppositely charged, as, for example, the classical red lead and sulphur mixture for the development of Lichtenberg figures. Particles moving in nominally homogeneous systems can also become strongly charged, but the overall net charge of the cloud remains close to zero[13,14].

It is apparent that separation of the particulate components of a heterogeneous cloud, for example, by elutriation or gravitational settling, could produce oppositely charged regions within it. Regional charge separation may occur even in homogeneous clouds if there is a correlation between the size of a particle (including aerosol material) and the sign of its preferred charge.

The generation of considerable electrostatic potentials in fluidized beds has been reported[15,16]. On a larger scale, many industrial accidents have been traced to the movement of dust-laden air generating potential differences sufficient to promote an incendiary spark[17]. Natural dust storms cause violent disturbances of the terrestrial electric field[18]. Lightning displays within the ash cloud from volcanic eruptions[19,20] (including nuées ardentes[21]) have been described by observers from Pliny[22] onwards.

Situations giving rise to spark discharges occur at pressures around atmospheric, but at reduced pressures charge equalization is facilitated by glow discharge[23]. Experiments indicate this mechanism to be most important in the pressure range 10–0·1 torr (refs. 24 and 25). Studies of the light accompanying the generation of frictional electricity *in vacuo* were begun by Hauksbee[26] in 1704 and continued by others[19], but little has been added since.

I therefore propose that the obscuration associated with TLPs is due to fine dust raised by lunar degassing. In certain areas, sporadic escape of accumulated gas occurs through channels opened by tidal stress. Movement and separation of the heterogeneous particles result in separation of charge and, occasionally, sufficient potential difference is built up to promote glow discharge through the transient gas phase[27]. The predominance of hydrogen in the discharge gives the reddish tint.

Triboelectric charging and glow discharge synthesis and polymerization could have been especially significant in the early stages of planetary evolution, independent of the mechanism for the production of cratered surfaces. It also seems worthwhile to look for electric fields and glow discharges generated by the dust storms believed to occur on Mars.

A. A. MILLS

Departments of Geology and Astronomy,
University of Leicester.

Received January 16; revised February 17, 1970.

[1] Kopal, Z., *An Introduction to the Study of the Moon* (Reidel, 1966).
[2] Herschel, W., *Phil. Trans. Roy. Soc.*, **77**, 229 (1787).
[3] Greenacre, J. C., *Ann. NY Acad. Sci.*, **123**, 811 (1965).
[4] Middlehurst, B. M., and Moore, P. A., *Science*, **155**, 449 (1967).
[5] Kozyrev, N. A., in *Physics and Astronomy of the Moon* (edit. by Kopal, Z.) (Academic Press, 1961).
[6] Kozyrev, N. A., *Nature*, **198**, 979 (1963).
[7] *Nature*, **222**, 404 (1969).
[8] Cameron, W. S., and Gilheany, J. J., *Icarus*, **7**, 29 (1967).
[9] Mills, A. A., *Nature*, **224**, 863 (1969).
[10] Bennet, A., *Phil. Trans. Roy. Soc.*, **77**, 26 (1787).
[11] Faraday, M., *Experimental Researches in Electricity*, **2**, para 2138 (London, 1844).
[12] Rudge, W. A. D., *Proc. Roy. Soc.*, A**90**, 256 (1914).
[13] Kunkel, W. B., *J. Appl. Phys.*, **21**, 820 (1950).
[14] Harper, W. R., *Contact and Frictional Electrification* (Oxford Univ. Press, 1967).
[15] Ciborowski, J., and Wlodarski, A., *Chem. Eng. Sci.*, **17**, 23 (1962).
[16] Končar-Djurdjević, S., and Vuković, D., *Nature*, **193**, 58 (1962).
[17] Dale, R. A., *Electrostatic Hazards in Coal Mining, Safety in Mines Res. Est., No. 118* (1955).
[18] Latham, J., *Quart. J. Roy. Met. Soc.*, **90**, 91 (1964).
[19] Priestley, J., *History of Electricity* (London, 1775).
[20] Anderson, R., *et al.*, *Science*, **148**, 1179 (1965).
[21] Perret, F. A., *Carnegie Inst. Washington, Publ. 458*, 99 (1937).
[22] Pliny (the Younger), *Second Letter to Tacitus* (*c.* 106).
[23] Llewellyn Jones, F., *The Glow Discharge* (Methuen, 1966).
[24] Peterson, J. W., *J. Appl. Phys.*, **25**, 907 (1954).
[25] Foord, T. R., *J. Sci. Ins., Series 2*, **2**, 411 (1969).
[26] Hauksbee, F., *Physico-Mechanical Experiments on Various Subjects* (London, 1709).
[27] Sartory, P. K., *J. Brit. Astron. Assoc.*, **77**, 430 (1967).

Dielectric and Anelastic Effects in Inorganic Glasses

THE dielectric properties of inorganic glasses in alternating fields are usually described in terms of the complex dielectric constant

$$\varepsilon^* = \varepsilon' - i\varepsilon'',$$

where ε' is the real part and ε'' is the imaginary part or dielectric loss. The values of ε' and ε'' are usually obtained from measurements of the capacitance and resistance of a disk shaped specimen fitted with metal electrodes. If the value of C_p/C_o (where C_p is the equivalent parallel capacitance and C_o is the capacitance with a vacuum between the electrodes) is plotted against log ω (where ω is the angular frequency), then, in the audiofrequency range and with blocking electrodes (for example, gold or platinum), a curve of the form shown in Fig. 1 is usually obtained with glasses containing appreciable amounts of alkali-metal ions.

The high values for the capacitance at low frequencies are the result of electrode polarization effects[1,2]. At higher frequencies, the curve resembles that which would be expected for a Debye relaxation process and in this region C_p/C_o is taken to represent ε', the real part of the complex dielectric constant. In the absence of electrode polarization effects, it is assumed that at zero frequency, ε' will correspond to the static dielectric constant ε_s.

The dielectric losses in the audiofrequency region are generally considered to be due to (*a*) "conduction" losses and (*b*) "dipole relaxation" losses[3]. To separate the latter from the "conduction" losses, the d.c. conductivity is subtracted from the a.c. conductivity when calculating the value of ε'' corresponding to the "dipole relaxation" process[4,5] so that

$$\varepsilon''(\omega) = \frac{G_{ac} - G_{dc}}{\omega C_o}$$

where G_{ac} is the equivalent parallel a.c. conductance and G_{dc} is the d.c. conductance. (To be strictly accurate, the "conduction" losses with blocking electrodes would perhaps be better regarded as a form of dielectric loss associated with space-charge polarization at the electrodes and arising from the movement of charge carriers through the bulk of the specimen.) When plotted against log ω, the values of ε'' for the "dipole relaxation" loss show a dispersion similar to, but broader than, that which would be expected from a simple Debye model (Fig. 1).

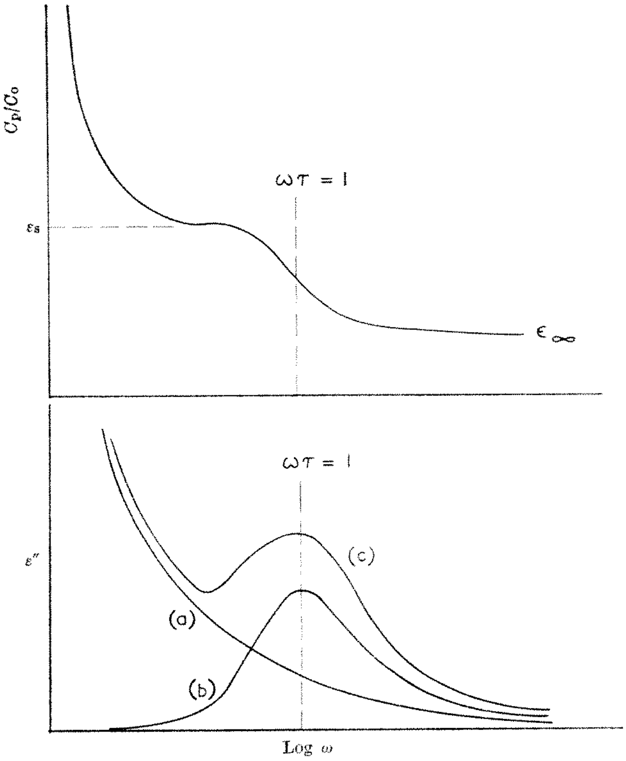


Fig. 1. Dielectric properties of silicate glasses containing alkali-metal ions. (*a*) "Conduction" loss; (*b*) "dipole relaxation" loss; (*c*) total dielectric loss.

Although the dielectric properties of glasses have been studied extensively, little progress has been made in the identification of the nature of the "dipole relaxation" losses. These were attributed by Stevels[3] to the movement of alkali-metal ions through small distances and over low potential energy barriers. This would lead to a polarization of the glass which would follow the external a.c. field with a relaxation time determined by the time needed for the ions to acquire sufficient energy to pass over the potential barriers. Taylor[6], however, showed that the value of the activation energy for this relaxation process was close to that for the d.c. conductivity. Although this supported the view that the movement of alkali-metal ions was responsible both for the d.c. conductivity and the relaxation effect, it meant that it was more difficult to distinguish between the two processes. Charles[7] suggested that around each non-bridging oxygen there was a number of equivalent sites available for an alkali-metal ion. Polarization could then occur through movement of an alkali-metal ion from one site to another. This could be a direct movement or could take place indirectly as part of the conduction process. The dielectric behaviour could then be interpreted using Fröhlich's model[8] in which charges in a dielectric may each occupy one of two equilibrium positions. There are, however, two difficulties with this theory. The first is that it is difficult to explain why, for example, the partial replacement of Na_2O by CaO in a sodium silicate glass does not lead to a reduction in the value of ε_s[6,9]. The second is that when we take the expressions derived by

Charles for τ the relaxation time, σ the conductivity and $(\varepsilon_s - \varepsilon_\infty)\varepsilon_0$, we find that, if we neglect a correction factor for a distribution of jump frequencies,

$$\frac{\tau\sigma}{(\varepsilon_s - \varepsilon_\infty)\varepsilon_0} = \frac{1}{6}\left(\frac{r}{b}\right)^2$$

where r is the average "jump distance" for conductivity, b is the effective jump distance in the polarization process, ε_∞ is the high-frequency dielectric constant and ε_0 is the permittivity of free space. Experimentally, with silicate glasses, the factor on the left-hand side of this equation does not differ greatly from 1·3 even for glasses of very different alkali-metal ion content[9], so we have the relationship $b \simeq 0{\cdot}4\ r$. This requirement is not easily correlated with physically plausible structures for glasses of varying alkali-metal oxide content.

These difficulties with existing theories and the close inter-relationship between the conduction and relaxation processes suggest the possibility of a general reinterpretation of the low-frequency dielectric behaviour of inorganic glasses in terms of a Maxwell relaxation process.

For a homogeneous conducting dielectric Maxwell[10] showed that the relaxation time for the conduction process is given by

$$\tau_M = \frac{\varepsilon_s\ \varepsilon_0}{\sigma}$$

where σ is the conductivity. This relationship is derived from the Poisson equation $\nabla . \nabla\Psi = -\rho/\varepsilon_s\ \varepsilon_0$ and the continuity equation

$$\frac{\mathrm{d}\rho}{\mathrm{d}t} = \sigma\nabla\ .\nabla\Psi$$

where Ψ is the potential and ρ is the charge density.

It follows that if we have a disturbance in potential in a dielectric through, for example, the rotation of a dipole or the displacement of a bound charge, then there will be changes in the charge density in the surrounding medium which will die away exponentially with a relaxation time τ_M.

Table 1. LOW-FREQUENCY DIELECTRIC PROPERTIES OF SOME INORGANIC GLASSES

Glass composition (mole per cent)	Temperature (°C)	ε_s	$1/\sigma$ (Ω cm)	$\tau_M = \varepsilon_s\ \varepsilon_0/\sigma$ (s)	τ_{obs} (s)	Ref.
18 Na_2O, 10 CaO, 72 SiO_2	121 159	27·5 25·7	$6{\cdot}3\times10^8$ $7{\cdot}1\times10^7$	$1{\cdot}5\times10^{-3}$ $1{\cdot}6\times10^{-4}$	$1{\cdot}2\times10^{-3}$ $1{\cdot}0\times10^{-4}$	6
43·6 Fe_2O_3, 11·4 FeO, 45·0 P_2O_5	22·8 76·2	18·5 17·4	$1{\cdot}2\times10^{10}$ $5{\cdot}6\times10^8$	$2{\cdot}0\times10^{-2}$ $8{\cdot}6\times10^{-4}$	$1{\cdot}6\times10^{-2}$ $5{\cdot}7\times10^{-4}$	11
Vitreous silica containing 0·04 p.p.m. sodium and approx. 0·1 to 1·0 per cent water	655	7·3	$2{\cdot}5\times10^8$	$1{\cdot}6\times10^{-4}$	$1{\cdot}1\times10^{-4}$	12
17·5 Na_2O, 9·6 CaO, 0·5 MnO, 0·4 ZnO, 1·4 Al_2O_3, 70·6 SiO_2	150	28	$8{\cdot}5\times10^7$	$2{\cdot}1\times10^{-4}$	$1{\cdot}7\times10^{-4}$	2

If we examine the experimental results we find that for many inorganic glasses the observed relaxation time (τ_{obs}) for the "dipole relaxation" loss does not differ greatly from $\tau_M = \varepsilon_s\ \varepsilon_0/\sigma$. Some values for glasses of very different structure are given in Table 1. This approximate agreement suggests that in these systems the relaxation time is determined by a flow of charge and a Maxwell relaxation mechanism. It follows that the entity in the glass structure responsible for polarization may, in the absence of electrical interactions with the surrounding medium, be readily displaced or rotated.

In the case of the silicate glasses containing appreciable amounts of alkali-metal ion, it is suggested that the primary polarization process is a displacement of non-bridging oxygen ions. At low frequencies there is a simultaneous movement and redistribution of the surrounding alkali-metal ion charge carriers. At higher frequencies, however, when $\omega > 1/\tau_M$ appreciable displacement of the non-bridging oxygens is prevented by the retarding force due to the slow movement of the charge carriers. This interpretation is also supported by the relationship between the static dielectric constant and the non-bridging oxygen content which is apparent from the work of Taylor[6] and Barton[9].

In the iron-phosphate glass studied by Hansen and Splann[11] the charge carriers are electrons migrating between Fe^{3+} and Fe^{2+} ions. Again, there is the same approximate agreement between τ_{obs} and $\varepsilon_s\ \varepsilon_0/\sigma$, so that it would seem likely that, in this case too, the high value for the dielectric constant at low frequencies is also due to the displacement of non-bridging oxygens. In the case of vitreous silica, Owen and Douglas[12] showed that ε_s depended on the water content of the silica so that the low frequency polarization can probably be ascribed to the movement of OH groups. At a temperature of 655° C, however, the silica has an appreciable conductivity and the relaxation time is determined by the conductivity.

For silicate glasses containing alkali-metal ions, Owen[13,14] suggested that the polarization mechanism might be similar to that underlying the Debye–Falkenhagen effect in electrolyte solutions. The mechanism suggested here does in fact show similarities, but there are important differences; the non-bridging oxygens in the glasses are here considered to be non-diffusible and, although they can be regarded as being surrounded by an atmosphere of charge carriers, the conduction mechanism in the glass is very different from that in an aqueous electrolyte solution.

When we consider the corresponding anelastic effects it would appear that two types of relaxation process involving the migration of alkali-metal ion charge carriers might be expected. The first would correspond to the Maxwell relaxation process described here and would involve the displacement of non-bridging oxygens or other polar groups and a local flow of charge carriers. The relaxation time and activation energy would again be determined by the conductivity. This process can be considered to be responsible for the low-temperature loss peak observed in internal friction experiments using torsion pendulum techniques. With glasses containing alkali-metal oxides, these peaks have previously been ascribed to "stress-induced migration" of alkali-metal ions. With the iron phosphate glass, a similar mechanical loss peak was observed with longitudinal oscillations and frequencies of 50 and 150 kHz and attributed to the stress-induced movement of electrons[15]. In this case, too, it would seem likely that the primary process may have been the displacement of non-bridging oxygens with a relaxation time and activation energy determined by the electronic conductivity.

The second type of mechanical relaxation process involving the migration of alkali-metal ions would involve their diffusion through the bulk of the specimen and, with appropriate stress systems, changes in charge concentration at or near the surfaces. A loss process of this type would be expected in flexural vibration experiments and would show similarities with the space-charge polarization effects observed in dielectric experiments. The losses in this case would be due to stress-induced migration, but the relaxation time (as with the space-charge polarization effects) would be determined by the conductivity and geometry of the specimen.

The interpretation of dielectric effects in aluminosilicate glasses and their significance in the study of glass structure and phase-separation processes will be discussed in a future publication.

D. K. HALE

National Physical Laboratory,
Division of Inorganic and Metallic Structure,
Teddington,
Middlesex.

Received December 19, 1969.

[1] Isard, J. O., *J. Sci. Instrum.*, **40**, 403 (1963).
[2] Volger, J., Stevels, J. M., and van Amerongen, C., *Philips Res. Reports*, **8**, 452 (1953).
[3] Stevels, J. M., *The Electrical Properties of Glass, Handbuch der Physik*, **20**, p. 350 (Springer-Verlag, Berlin, 1957).
[4] McCrum, N. G., Read, B. E., and Williams, G., *Anelastic and Dielectric Effects in Polymeric Solids*, p. 211 (John Wiley and Sons, 1967).
[5] Taylor, H. E., *J. Soc. Glass Technol.*, **41**, 350T (1957).
[6] Taylor, H. E., *J. Soc. Glass Technol.*, **43**, 124T (1959).
[7] Charles, R. J., *J. Appl. Phys.*, **32**, 1115 (1961).
[8] Fröhlich, H., *Theory of Dielectrics* (Oxford University Press, 1949).
[9] Barton, J. L., *Verres et Refr.*, **20**, 328 (1966).
[10] Maxwell, J. C., *Electricity and Magnetism.*, third ed., **1**, 450 (Clarendon Press, Oxford, 1892).
[11] Hansen, K. W., and Splann, Mary T., *J. Electrochem. Soc.*, **113**, 895 (1966).
[12] Owen, A. E., and Douglas, R. W., *J. Soc. Glass Technol.*, **43**, 159T (1959).
[13] Owen, A. E., *Progress in Ceramic Science* (edit. by J. E. Burke), **3**, p. 77 (Pergamon Press, 1963).
[14] Owen, A. E., thesis, Univ. Sheffield (1959).
[15] Miller, R. A., and Hansen, K. W., *J. Electrochem. Soc.*, **116**, 254 (1969).

Charge Moving with the Speed of Light

I HAVE recently shown[1] that Maxwell's equations admit solutions for charge moving in a straight line with the speed of light c. The accompanying electromagnetic field F_{ik} is that of a plane-fronted wave, that is, one whose front is plane and whose amplitude varies over the front. The source is a null four-current, J^i, satisfying

$$g_{ik}\, J^i\, J^k = 0 \qquad (1)$$

where g_{ik} is the metric tensor of flat space-time. The charge is localized in a world-tube about the z-axis of coordinates; all appropriate conditions are satisfied at the boundary of the tube, and the F_{ik} are everywhere continuous. Although charge is radiated away from the source, conservation of charge is of course automatically ensured by Maxwell's equations. The solutions have precise analogues in Einstein–Maxwell theory[2].

These solutions are perhaps not entirely conclusive because the streams of charge originate at infinity. I now wish to point out that solutions exist for null currents emitted from a point.

It is convenient to use the metric of flat space-time in null polar coordinates

$$ds^2 = -r^2(d\theta^2 + \sin^2\theta d\varphi^2) + 2\, du\, dr + du^2 \qquad (2)$$

where $u = t - r$ is the retarded time, and where we number the coordinates

$$x^1 = r,\ x^2 = \theta,\ x^3 = \varphi,\ x^4 = u$$

We choose unrationalized Gaussian units but with the unit of time chosen so that $c = 1$, and take the vector potential as

$$A_i = A\ \delta_i^4$$

where A is a function of θ, φ and u only; then the only non-zero components of F_{ik} are

$$F_{42} = -F_{24} = A_\theta,\ F_{43} = -F_{34} = A_\varphi$$

the subscripts θ and φ meaning differentiation with respect to θ and φ. The cartesian components of F_{ik} are proportional to r^{-1}, as expected for a wave field.

The electromagnetic energy tensor is

$$E^{ik} = r^{-2}\, (A_\theta{}^2 + A_\varphi{}^2 \operatorname{cosec}^2\theta)\ \delta^i_1\ \delta^k_1 \qquad (3)$$

and the current, obtained from

$$4\pi J^i = (-g)^{-\frac{1}{2}}\ \frac{\partial}{\partial x^k}\ [(-g)^{\frac{1}{2}}\ F^{ik}]$$

(g being the determinant of g_{ik}) is

$$J^i = -(4\pi r^2)^{-1}\ \nabla^2 A\ \ \delta^i_1 \qquad (4)$$

where

$$\nabla^2 A = A_{\theta\theta} + A_\theta \cot\theta + A_{\varphi\varphi} \operatorname{cosec}^2\theta$$

is the laplacian of A on the unit sphere. J^i satisfies (1) and is therefore a null current. Provided that, at each world-point, charge of only one sign is present[1], J^i represents charge moving radially away from (or towards) the origin with speed c. We could write (3) as

$$E^{ik} = v^i\, v^k \qquad (5)$$

where

$$v^i = r^{-1}\, (A_\theta{}^2 + A_\varphi{}^2 \operatorname{cosec}^2\theta)^{\frac{1}{2}}\ \delta^i_1$$

is a null vector parallel to J^i. From (5) it follows that the charge is accompanied by the field energy, also travelling radially with the speed of light. Both charge and energy automatically satisfy the usual conservation laws. The ponderomotive face, $F_{ik}\ J^k$ vanishes, which means that the charge moves freely, and no external force is necessary to maintain the current.

In regions free of current we have from (4)

$$\nabla^2 A = 0$$

and it is known that this equation has no solution continuous over the entire sphere (other than A = constant). Hence for fields of this type the current must be non-zero for some range of θ, φ.

It appeared in ref. 1 that a pulse of charge of one sign emitted at infinity and moving in a straight line with speed c is always accompanied by infinite field energy. This result now seems to have been due to the artificiality of the model used, because from equation (3) the integrated field energy of a pulse of spherical null current of finite duration u_1 to u_2, namely,

$$\int_0^{2\pi}\int_0^{\pi}\int_{u_1}^{u_2} (A_\theta{}^2 \sin\theta + A_\varphi{}^2 \operatorname{cosec}\theta)\ du\ d\theta d\varphi$$

is finite for suitable functions A. This removes a possible objection to null currents.

Charged particles moving with speed c have not yet been identified in cloud chambers, and the idea that charge, like energy, might be radiated through empty space is perhaps somewhat revolutionary. Nevertheless, it is clearly permitted by Maxwell's equations, and these have hitherto proved a sound guide to experience within their terms of reference. It is therefore suggested that some experiments be carried out to look for the phenomenon. The theory does not tell us how to generate a null current, but possibly one might arise when a suitable pulse of light is generated at a charged source.

W. B. BONNOR

Department of Mathematics,
Queen Elizabeth College,
Campden Hill Road,
London W8.

Received January 9, 1970.

[1] Bonnor, W. B., *Intern. J. Theor. Phys.*, **2**, 373 (1969).
[2] Bonnor, W. B., *Intern. J. Theor. Phys.* (in the press).

Electron Spin Resonance of Hydrogen Atoms in a Free-burning Flame at Atmospheric Pressure

STUDIES of the kinetic behaviour of hydrogen atoms in atmospheric flames[1] usually involve detection of hydrogen atoms by indirect methods, but direct detection actually in the flame itself has not been reported. We report here the observation of hydrogen atoms in free-burning flames at atmospheric pressure by electron spin resonance.

Previous measurements[2] of hydrogen atoms in gas-phase combustion systems by electron spin resonance (ESR) have been made at low pressures (about 10 torr or less). With one exception[3], the reaction has been initiated and sustained by passing the gas mixture through a heated tube (about 850 K) located either immediately before or inside the sample cavity of the ESR spectrometer. Westenberg and Fristrom[3] used a pin-hole probe to sample the gas from a flame burning at 0·1 atmosphere pressure and detected hydrogen atoms by ESR in the low pressure (0·1 torr) region downstream of the pin-hole. Similarly, ESR studies[4,5] of other gas-phase reactions of hydrogen atoms, produced by passing molecular hydrogen through a radiofrequency or microwave discharge, have been carried out at relatively low total pressures.

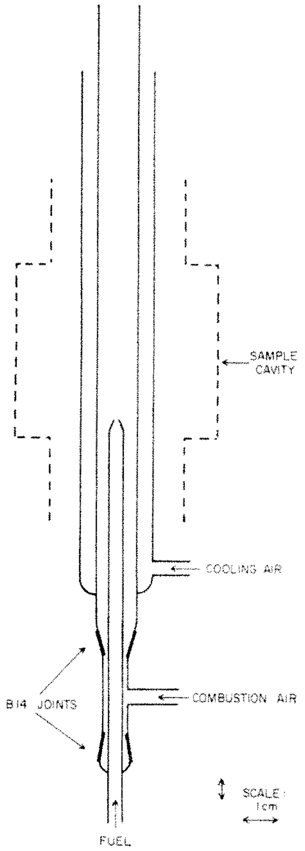


Fig. 1. Cross-section of burner.

It is usually assumed that the spectral lines of the hydrogen atom would be so severely pressure-broadened that they could not be detected at high pressures. But it has been shown[6] that when hydrogen atoms are produced by thermal dissociation of molecular hydrogen on a heated tungsten filament, there is virtually no increase in the line width at total pressures up to 500 torr. The main source of broadening in a flame at atmospheric pressure may therefore be interaction with other paramagnetic species, in particular with molecular oxygen. The effect of the temperature of the flame (about 2,100 K) on the Boltzmann distribution between the magnetic levels will cause about a seven-fold decrease in the intensity of the spectral lines compared with that at room temperature. But the hydrogen atom concentration in the reaction zone of a hydrogen/air flame is 10^{16}–10^{17} atoms/cm^3 (ref. 1), which is well above the limit of detectability by ESR for hydrogen atoms in the gas phase.

A simple burner (Fig. 1) was made to fit in a wide-access sample cavity of a Varian spectrometer. A stable flame, a few centimetres high, could be maintained on the central tube over a wide range of fuel/air ratios. (Hydrogen flow rates through the central tube were in the range 100–600 cm^3/min, and the air flow rates between 20 and 2,000 cm^3/min.)

With this burner, hydrogen atoms were easily detected in a hydrogen/air diffusion flame burning at atmospheric pressure. For a fuel-rich flame, the width of individual hyperfine lines was about 0·13–0·15 oersted, which is not more than twice the width observed (with similar operating conditions of the spectrometer) for hydrogen atoms in a low pressure flow system. For an oxygen-rich flame, however, the line width was about 2·5 oersted. The marked increase in line width occurred in a very narrow range of fuel/air ratios close to the stoichiometric value. A rough estimate of the relative intensities of the spectral lines showed that there was no abrupt change in the concentration of hydrogen atoms accompanying the change in line width. These observations suggest that the main cause of line broadening was indeed interaction with molecular oxygen.

The system is not ideally suited to measuring the absolute concentration of hydrogen atoms in the flame, but the total concentration of hydrogen atoms in a small flame (0·4 cm diameter × 0·8 cm high) was found to be about $0 \cdot 4 \times 10^{15}$ atoms/cm^3. In view of the assumptions that have to be made in the calculation, this value agrees reasonably well with that of 2×10^{15} atoms/cm^3, estimated from existing values[1] for the concentration profiles in a premixed flat flame.

Hydrogen atoms have also been detected in a premixed hydrogen/air flame, on the same burner. The line width showed the same dependence on the fuel/air ratio as that for the diffusion flame, and increased abruptly from 0·15 to 2·5 oersted as the fuel/air ratio was decreased through the stoichiometric value. Hydrogen atoms were also observed in methane/air and propane/air diffusion flames, but in both flames the line width was larger (0·8 oersted) for all values of the fuel/air ratio and showed no change across the stoichiometric point. The larger line width may be caused by interaction with elementary carbon species, such as C_2 and C_3, or even with soot particles.

The potential applications of this technique to kinetic and mechanistic studies in premixed and diffusion flames are currently being investigated.

J. E. BENNETT
B. MILE
R. SUMMERS

Shell Research Limited,
Thornton Research Centre,
Chester.

Received November 21, 1969.

[1] Bulewicz, E. M., James, C. G., and Sugden, T. M., *Proc. Roy. Soc.*, **A, 235,** 89 (1956).
[2] Panfilov, V. N., Tsvetkov, Yu. D., and Voevodskii, V. V., *Kinetika i Kataliz*, **1**, 333 (1960).
[3] Westenberg, A. A., and Fristrom, R. M., *Tenth Internat. Symp. on Combustion*, 473 (The Combustion Institute, Pittsburgh, 1965).
[4] Brown, J. M., and Thrush, B. A., *Trans. Faraday Soc.*, **63**, 630 (1967).
[5] Westenberg, A. A., and de Haas, N., *J. Chem. Phys.*, **43**, 1550 (1965).
[6] Bennett, J. E., and Blackmore, D. R., *Chem. Commun.*, 1521 (1968).

Catalytic Inhibition by Adsorbed Oxygen

THE effects of adsorbed oxygen on the properties of an initially clean tungsten surface have been investigated in detail by a wide range of experimental techniques. The changes in both the field emission microscope and the field ion microscope images of tungsten tips have been

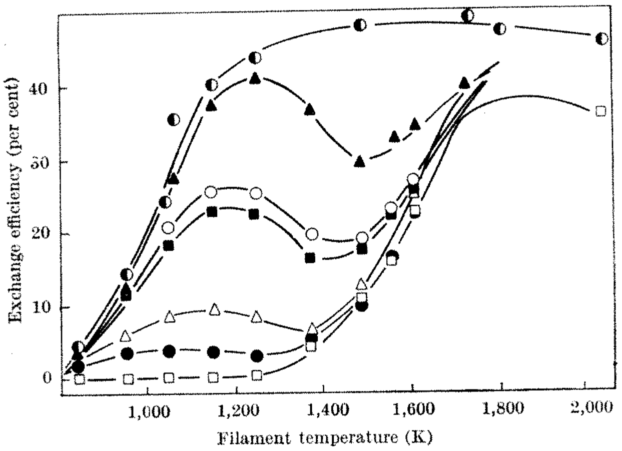


Fig. 1. The effect of adsorbed oxygen on the catalytic activity of tungsten for carbon monoxide isotope equilibration. Oxygen uptakes in units of 10^{14} atom/cm^2: ◐, 0; ▲, 0·51; ○, 0·97; ■, 1·7; △, 5·1; ●, 5·9; □, 8·3.

reported[1,2], as have the changes in the low energy electron diffraction patterns of some oriented tungsten ribbons[3,4]. The adsorption and desorption phenomena which occur when tungsten at high temperatures reacts with oxygen have also been studied extensively[5-7]. It is generally agreed that oxygen adsorbed on tungsten can be removed by heating *in vacuo* but that the desorbing species is never molecular oxygen. Measurements of the adsorption kinetics using the "flash filament" technique reached the expected conclusion that this is a highly reactive gas/metal surface system[8].

An alternative technique for studying the effect of adsorption on the properties of a surface is to monitor the change in chemical reactivity when adsorption takes place. A suitable measure of the reactivity of a tungsten surface is its efficiency as a catalyst for the equilibration of mixtures of isotopes of simple gases. By incorporating a mass-spectrometer as part of the reaction chamber, reliable and accurate measurements of the changes in the reactivity of the surface can be made. This technique has been used to study the effect of increasing oxygen adsorption on the catalytic efficiency of tungsten for the reaction $^{28}N_2 + {}^{30}N_2 = 2\ {}^{29}N_2$ (ref. 9). The complete correlation between the reduction in catalytic activity and the diminution in chemisorptive uptake of nitrogen as the oxygen coverage was increased suggested that the catalytic activity of this well annealed tungsten filament was distributed over much of the surface and was not concentrated at special sites of high activity.

Interesting new possibilities may arise when the reaction chosen to monitor the effect of the adsorbed oxygen itself involves the exchange of an oxygen atom. This situation occurs with the reaction $^{12}C^{18}O + {}^{13}C^{16}O = {}^{12}C^{16}O + {}^{13}C^{18}O$, which is here reported. The interaction of a single carbon monoxide isotope with an oxygenated tungsten surface has been investigated previously by flash filament[10] and oxygen isotope exchange experiments[11]. It is also known qualitatively that a hot tungsten surface is a very efficient catalyst for the carbon monoxide isotope equilibration reaction[12].

The ultra-high-vacuum apparatus of volume 2 l., and the tungsten wire of length 20·7 cm and geometric area 0·87 cm^2, have been used for a series of experiments in which the interaction of tungsten with a variety of gaseous environments has been investigated[9,11,13]. Before the first experiments were started the filament was freed from carbonaceous impurities by prolonged heating in oxygen, followed by removal of the resulting oxygen layer by heating to about 2,070 K *in vacuo*[13]. After this treatment the reactivity of the filament towards nitrogen, both in chemisorption[13] and in catalysis[9], was typical of a polycrystalline tungsten wire. For this filament, therefore, as for some tungsten ribbons recently studied[4], heating to 2,070 K *in vacuo* for a few minutes is sufficient to remove a layer of adsorbed oxygen. It is desirable to have a consistently maintained heating cycle for a tungsten wire, because its reactivity may depend on the temperature to which it is heated before an adsorption is started[8], and this filament was cleaned by heating to 2,070 K before each experiment was started. One feature of this series of experiments[9,11,13] has been the frequent oxygenation of the surface by reaction with nitrous oxide. This reaction has provided a convenient method of maintaining the surface in a carbon-free condition after the initial cleaning process, a particularly important consideration in carbon monoxide isotope exchange experiments.

Two bakable metal valves joining the UHV chamber to two gas storage volumes served as variable leaks for the admission of gases. A similar valve connecting the chamber to the pump allowed a suitable rate of flow of gas over the filament to be chosen. The isotopic composition of the gas was determined with an omegatron radiofrequency mass spectrometer which formed an integral part of the UHV system. Oxygenation of the filament to any desired coverage was carried out by "titrating" in the appropriate amount of nitrous oxide from the storage volume and removing the adsorbed nitrogen by heating the filament briefly to 1,300 K. The results of earlier experiments have shown that this sequence can provide a reproducible, known uptake of oxygen[9,13]. The highest coverages were obtained by allowing a further period of adsorption of nitrous oxide after the removal of the surface nitrogen. In this stage only the oxygen atom of the nitrous oxide was adsorbed and the nitrogen appeared in the gas phase. The $^{13}C^{16}O$ was prepared by the reduction of $^{13}C^{16}O_2$ liberated from Ba $^{13}CO_3$ (ref. 14) and the $^{12}C^{18}O$ by the oxidation of "analar" sugar charcoal with $^{18}O_2$. A mixture of approximately equal parts of $^{13}C^{16}O$ and $^{12}C^{18}O$ was made up in an ancillary gas preparation apparatus and kept in one of the gas-storage volumes. No appreciable equilibration of the mixture occurred during the period of the experiments.

The catalysis of the equilibration of the mixture of isotopes by the clean tungsten wire was measured by streaming the mixture through the apparatus and recording the mass spectrometer peak heights of $^{12}C^{16}O$, $^{13}C^{16}O$, $^{12}C^{18}O$ and $^{13}C^{18}O$. The total pressure of carbon monoxide was about 6×10^{-8} torr. The filament temperature was increased in steps and the isotopic composition was recorded at each temperature. The onset of catalysis was marked by an increase in $^{12}C^{16}O$ and $^{13}C^{18}O$ at the expense of $^{12}C^{18}O$ and $^{13}C^{16}O$. Catalysis by the oxygenated filament was studied similarly. The filament was cleaned after each set of experiments at a particular oxygen coverage. The oxygenation process covers the surface with ^{16}O which

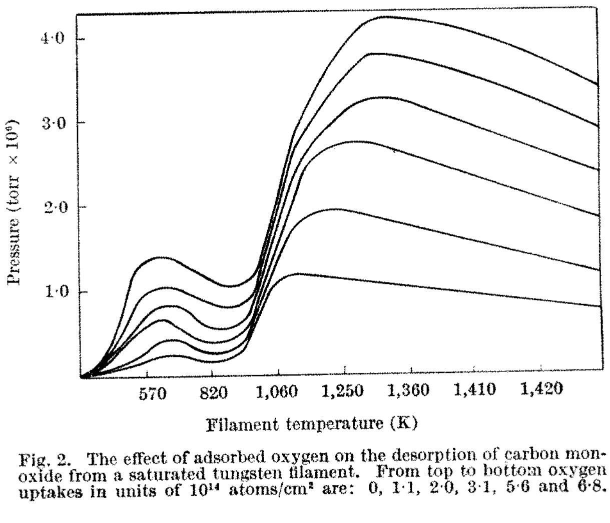


Fig. 2. The effect of adsorbed oxygen on the desorption of carbon monoxide from a saturated tungsten filament. From top to bottom oxygen uptakes in units of 10^{14} atoms/cm^2 are: 0, 1·1, 2·0, 3·1, 5·6 and 6·8.

can itself react with $C^{18}O$ (ref. 11). The effect of this reaction on the composition of the mixture of isotopes was to give an enhanced partial pressure of $^{12}C^{16}O$ when the filament was first heated above about 840 K. The surface, however, eventually achieved the same $^{16}O/^{18}O$ ratio as the gas phase and when this had happened there was detailed atomic balance between the adsorption and the desorption of the oxygen isotopes. It was then possible to measure the effect of the adsorbed oxygen on the catalytic efficiency of the tungsten.

The calculation of the catalytic efficiency of the tungsten requires a knowledge of the pumping constant of the aperture of the exit valve. This was therefore measured, but with nitrous oxide, which gives results concordant with nitrogen[13] rather than with carbon monoxide, which forms a "surface phase"[15]. A suitable pressure of nitrous oxide was established in the UHV chamber and the mass spectrometer was tuned to the peak at mass 44. The first order decay of this peak when the gas admittance valve was shut rapidly was recorded and the pumping constant was calculated. A small correction for the pumping effect of the mass spectrometer filament was made.

In a separate series of experiments the effect of pre-adsorbed oxygen on the chemisorptive uptake of carbon monoxide was measured. The filament, with a chosen coverage of oxygen, was allowed to saturate with carbon monoxide at an equilibrium pressure of 3×10^{-8} torr. The pressure burst when the filament was "flashed" to 2,000 K was recorded with an ionization gauge and fast writing recorder. The manufacturer's value for the sensitivity of the ionization gauge was used to calculate the uptakes. There may be considerable error in this number which would introduce a systematic error into the calculated uptakes. The catalytic efficiency, E, of the tungsten filament for the isotope equilibration reaction can be calculated from the mass spectrometer peak heights, using the previously derived formula[17]:

$$E = kcpV({}^{31}X - {}^{31}X_0)/AZ({}^{29}X\,{}^{30}X - {}^{28}X\,{}^{31}X)$$

In this equation k is the pumping constant of the aperture of the exit valve, c is a constant which relates the number density of molecules to the pressure, p is the pressure, V is the volume of the apparatus, A is the area of the filament and Z is the rate of collision of molecules at a pressure of 1 torr with one square cm of surface. ${}^{i}X$ is the mole fraction of any isotope when equilibration is occurring, ${}^{31}X_0$ is the mole fraction of $^{13}C^{18}O$ when the filament is cold and inactive. The dependence of E on filament temperature and on oxygen coverage is shown in Fig. 1. A consequence of the high maximum catalytic efficiency of the filament, about 50 per cent, is that the gas approached to within about 20 per cent of its equilibrium composition when the clean filament was above 1,400 K. The form of the equation is such that in these circumstances any uncertainty in the peak heights results in a much greater uncertainty in the calculated efficiency. There was therefore a random error in E which increased to about ±20 per cent in the high temperature, plateau region.

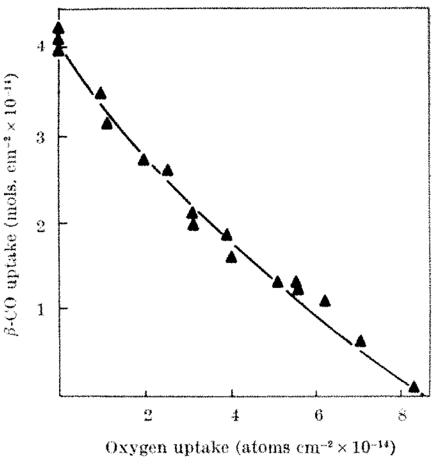


Fig. 3. The effect of adsorbed oxygen on the uptake of β-CO by tungsten.

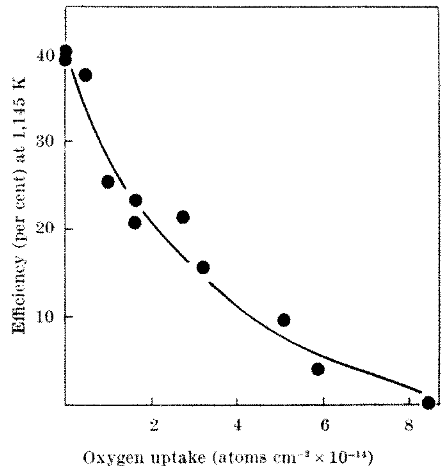


Fig. 4. The effect of adsorbed oxygen on the catalytic efficiency at 1,145 K.

The pressure record when the filament saturated with carbon monoxide was flashed to 2,000 K is shown in Fig. 2. It has the normal, well separated desorption regions which have been called α and β[18]. The unresolved fine structure in the higher temperature, β-peak, can be shown to consist of three sub-peaks when a heating programme designed for optimum resolution is used[19]. The decreased pressure bursts when some oxygen was adsorbed before saturation with carbon monoxide are also shown in Fig. 2.

It can be seen from Fig. 1 that the equilibration reaction did not start until the filament reached a temperature well above that at which the lower temperature, α-state had desorbed. The effect of adsorbed oxygen on the uptake of β-CO alone, therefore, is as shown in Fig. 3. In Fig. 4 the effect of the oxygen on the catalytic efficiency at 1,145 K is shown.

The temperature dependence of the efficiency of the clean tungsten filament for carbon monoxide isotope equilibration was very similar indeed to the temperature dependence of a clean molybdenum filament[17]. The similarity extends to the values of the efficiencies in the high-temperature, plateau regions which were the same for both metals. The absence of a contribution to equilibration from the lower temperature α-state and the onset of equilibration above a filament temperature of about 800 K are in complete agreement with an earlier, kinetic study of this reaction[12]. If it is correct to assume that every collision in which gas is absorbed also leads to full isotope equilibration on the surface, the measured exchange efficiency can be equated with the sticking probability. The equilibrium surface coverage in the high temperature plateau region was low, so that the calculated sticking probability, 0·5, was that of an essentially clean surface. This value agrees well with the initial sticking probability of 0·4 at 1,200 K measured by the flash filament technique[20]. The constancy of sticking probability over a 600 K temperature range above 1,400 K is typical of many systems at high temperatures[17].

The correlation, which was observed here, between the filament temperature at which isotope equilibration first became measurable and the temperature at which chemisorbed gas was desorbed has been taken, in other similar situations, as evidence that the equilibration reaction took place by adsorption into and desorption from this chemisorbed state[17]. Carbon monoxide is generally believed to be adsorbed without dissociation[18] and it has therefore been suggested that the chemisorbed molecules form a bimolecular, head-to-tail transition state complex[12]. The failure of the α-state to participate in equilibration may then be due to the relatively small perturbation of the C—O bond associated with the formation of a weak carbon to surface bond[17].

The most important effects of the pre-adsorbed oxygen were the smooth reduction in both the catalytic efficiency and in the uptake of β-CO as the oxygen coverage increased. As a comparison of Figs. 3 and 4 shows, however, the equilibration curve is noticeably more concave towards the abscissa than the uptake curve. This observation can be interpreted in the light of a theoretical treatment of the effect of a catalytic poison, which occupies single sites, on the reactivity of a uniform surface[21]. It was shown that, for a first order reaction, when the surface is almost bare, the shape of the poisoning curve depends on the number of surface sites required by the reactant. As the site requirement increases, so the curve becomes increasingly concave. The mechanism suggested for the carbon monoxide isotope equilibrium reaction involves the formation of a four-centre transition state complex on the surface[12]. The site requirement for such a mechanism would be expected to lead to a pronounced concavity of the poisoning curve, in agreement with the experimental results. It again seems probable therefore that the catalytic activity was distributed over much of the surface and that sites of especially high reactivity did not make a measurable contribution to the reaction. This regular decline in reactivity also suggests that once the surface oxygen had reached the same isotopic composition as the carbon monoxide it played no overall part in the equilibration process and that it did not affect the mechanism of the surface reaction. The role of the adsorbed oxygen as a catalytic poison appears therefore to be limited to reducing the area of the filament which is available for catalysis and chemisorption. These results and conclusions are, however, at variance with earlier work[22] in which an initial rapid decline in carbon monoxide uptake was observed. The origin of the discrepancy is not clear, but the considerable change in the topography of a tungsten surface which occurs in the presence of oxygen[9] may depend sensitively on the detailed history of the sample and this change may profoundly affect the chemical reactivity.

We thank the Science Research Council for the grant with which the omegatron was purchased and for a research grant to P. R. V.

R. P. H. GASSER
P. R. VAIGHT

Physical Chemistry Laboratory,
South Parks Road,
Oxford OX1 3QZ.

Received November 14, 1969; revised January 5, 1970.

[1] Bassett, D. W., *Trans. Faraday Soc.*, **64**, 489 (1968).
[2] Bell, A. E., Swanson, L. W., and Crouser, L. C., *Surface Sci.*, **10**, 254 (1968).
[3] Chang, C. C., and Germer, L. H., *Surface Sci.*, **8**, 115 (1967).
[4] Tracy, J. C., and Blakely, J. M., *Surface Sci.*, **13**, 313 (1968).
[5] McCarroll, B., *J. Chem. Phys.*, **46**, 86 (1967).
[6] Ptushinskii, Y. G., and Chuikov, B. A., *Surface Sci.*, **7**, 90 (1967).
[7] Lee, A. G., and Pethica, B. A., *Proc. Roy. Soc.*, A, **309**, 141 (1969).
[8] Singleton, J. H., *J. Chem. Phys.*, **47**, 73 (1967).
[9] Gasser, R. P. H., and Vaight, P. R., *Nature*, **221**, 166 (1969).
[10] Ageikin, V. S., and Ptushinskii, Y. G., *Sov. Phys. Solid State*, **10**, 1698 (1969).
[11] Gasser, R. P. H., and Vaight, P. R. (in the press).
[12] Madey, T. E., Yates, jun., J. T., and Stern, R. C., *J. Chem. Phys.*, **42**, 1372 (1965).
[13] Gasser, R. P. H., and Lawrence, C. P., *Surface Sci.*, **10**, 91 (1968).
[14] Gasser, R. P. H., Gowan, P. M., and Newman, D. G., *Trans. Faraday Soc.*, **64**, 2196 (1968).
[15] Hobson, J. P., and Earnshaw, J. N., *J. Vacuum Sci. and Tech.*, **4**, 257 (1967).
[16] Gasser, R. P. H., and Thwaites, R., *Vacuum*, **17**, 265 (1967).
[17] Gasser, R. P. H., and Marsay, C. J., *Trans. Faraday Soc.*, **64**, 516 (1968).
[18] Ehrlich, G., *Ann. Rev. Phys. Chem.*, **17**, 295 (1966).
[19] Redhead, P. A., *Trans. Faraday Soc.*, **57**, 641 (1961).
[20] Hill, M. P., and Pethica, B. A., *Trans. Faraday Soc.*, **65**, 876 (1969).
[21] Herington, E. F. G., and Rideal, E. K., *Trans. Faraday Soc.*, **40**, 505 (1940).
[22] Ageikin, V. S., and Ptushinskii, Y. G., *Ukr. Fiz. Zh.*, **12**, 1115 (1967).

Effect of Drag Reducing Additives on Pressure Loss across Transitions

THIS communication describes in brief some of the interesting points which came to notice from an experimental investigation into the behaviour of dilute aqueous solutions of commercial quality Guar Gum.

A good deal of information is available[1-3] on the drag reducing effects of these solutions when they are made to flow through straight pipes, but very few data on pressure gradients exist, when such fluids cross the transitions usually incorporated in a pipe system.

Data are presented here on one such transition, namely a sudden contraction. We have considered two different geometries, a reduction in pipe diameter from (*a*) 1·25 inch to 1 inch and from (*b*) 1·25 inch to 0·5 inch. Guar Gum was used as a solute in concentrations of 100, 200, 300, 400 and 500 parts per million by weight and ordinary tap water was used as a solvent.

The required solution was circulated through a close-loop type rig, chiefly consisting of a supply tank, a centrifugal pump, flow meters and a sudden contraction.

A piece of copper pipe six feet long and of 1·25 inch diameter preceded the contraction, which was followed by an eight feet length of the required smaller diameter pipe.

All the tests were conducted at 19° C. This temperature was maintained reasonably constant by means of either a cooling coil or a heater fitted inside the supply tank.

During any test the flow rate, temperature, viscosity and pressure drop across a transition and across the five feet length of straight pipe were noted. As soon as an abrupt increase in pressure loss across the straight length of the pipe was noticed, the solution was discarded as being degraded.

The viscosity of the solution was determined by means of a "U" tube viscometer at temperatures between 16° C and 25° C.

A plot, Fig. 1, of these observations shows that the ratio of the kinematic viscosity of the solution to the kinematic viscosity of water is independent of temperature and is dependent only on concentration. Similar results were obtained by Elata and Tirosh[1].

Figs. 2 and 3 show the variation in pressure loss coefficient, k_{cg} (which is the number of velocity heads lost), with Reynolds number based on the viscosity of the solution and its average velocity in the down stream pipe. They indicate that the pressure loss coefficient decreases

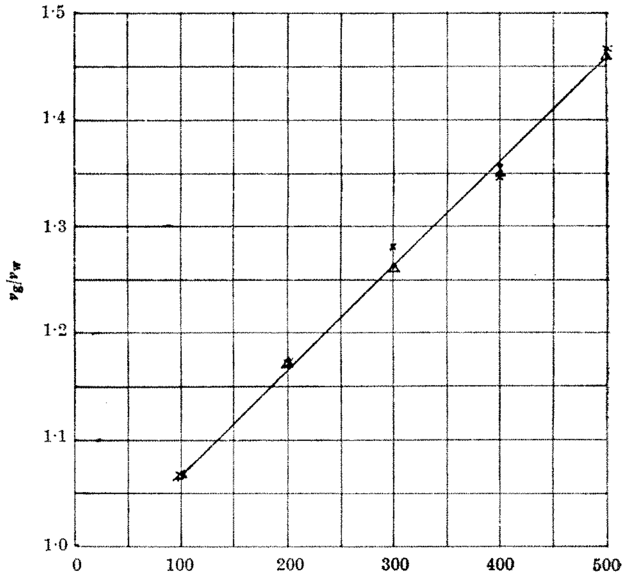


Fig. 1. Relative kinematic viscosity of Guar Gum. ●, 25° C; ×, 20° C; △, 16° C.

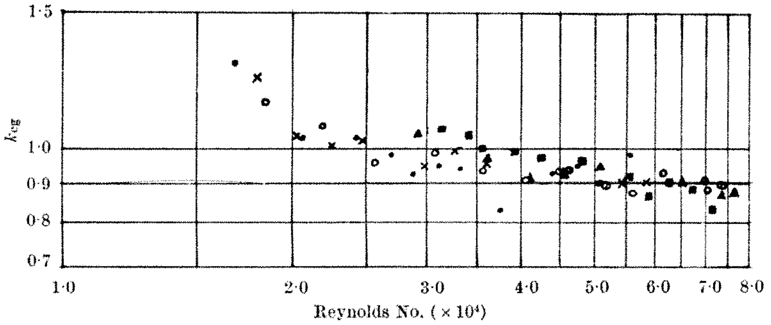


Fig. 2. Effect of Guar Gum concentration: sudden contraction 1·25 inch to 1 inch. ▲, 100; ■, 200; ○, 300; ×, 400; ●, 500 parts per million by weight.

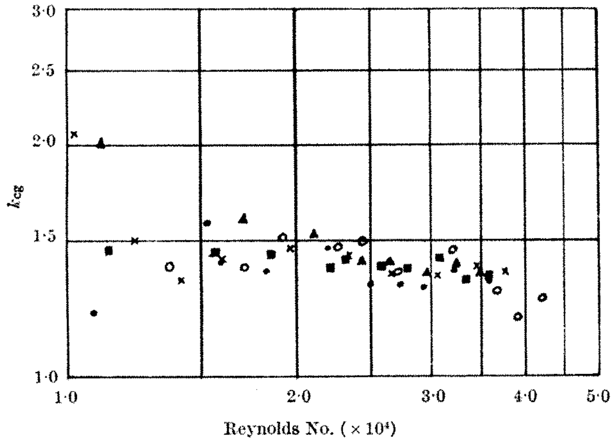


Fig. 3. Effect of Guar Gum concentration: sudden contraction 1·25 inch to 0·5 inch. ▲, 100; ■, 200; ○, 300; ×, 400; ●, 500 parts per million by weight.

with Reynolds number and is substantially independent of the concentration. The erratic nature of points for Reynolds number below 20,000 implies that the flow in this region is of a fluctuating type.

From these results, it is clear that the mechanism responsible for drag reduction in straight pipes breaks down completely when there is a sudden change in the flow pattern. The most important implication of this is that a polymeric additive that reduces drag in straight pipes will not cause reduction in pressure loss across fittings like valves, sudden contractions and so on, normally incorporated in a pipe system.

I thank the authorities of Hendon College of Technology for making available the facilities for this work.

V. G. Pisolkar

Hendon College of Technology,
London NW4.

Received November 5, 1969; revised January 9, 1970.

---

[1] Elata, C., and Tirosh, J., *Israel J. Tech.*, **3**, No. 1, p. 1 (1965).

[2] Elata, C., Lehrer, J., and Kahanowitz, A., *Israel J. Tech.*, **4**, No. 1, p. 87 (1966).

[3] White, A., *J. Mech. Engr. Sci.*, **8**, No. 4, p. 452 (1966).

Radiocarbon Dating of Mortars

Conflicting reports have been published in recent years on the reliability of radiocarbon dating of mortars. Delibrias and Labeyrie[1] found accurate dating to be possible, but Stuiver and Smith[2] found incorrect age assessments. The observed discrepancies were attributed to the dilution of ^{14}C activity by ancient carbon which may have been a residue of the limestone from which the "CaO" was initially prepared. In addition a minor dilution effect might result if calcareous sands were used in the manufacture of the original aggregate. We have investigated further the validity of the dating technique, using a number of British mortars of accurately known age and origin provided by the UK Ministry of Public Building and Works, Ancient Monuments Branch. Radiocarbon analyses of these samples were performed by methane gas counting following acid hydrolysis of the samples and conversion of the product carbon dioxide to methane by catalytic hydrogenation[3].

Isotope ratios of carbon-12 to carbon-13 were measured at the National Physical Laboratory with an *MS*3 mass spectrometer. Radiocarbon ages of samples (with 1σ standard deviations) were calculated using the Lamont VIII procedure[4] based on the 5,568 year half-life for ^{14}C. The results obtained are presented in Table 1 where δ^{13}C defined by

$$\delta^{13}\text{C} = \frac{{}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}} - {}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}}{{}^{13}\text{C}/{}^{12}\text{C}_{\text{standard}}} \times 1{,}000 \text{ per mil}$$

represents the per mil deviation of the sample ^{13}C/^{12}C ratio from that of the P.D.B. belemnite standard.

Table 1. RESULTS OF MORTAR ANALYSES

Sample	δ^{13}C (‰)	^{14}C age (years) ± 1σ	True age (years)
London Tower	−17·1	+5·39 (Δ) ± 0·61%	Recent
Hampton Court	−21·4	738 ± 52	425
Orford Castle	−10·9	7,370 ± 87	785
Conway Town Wall	−9·4	2,012 ± 53	665
Hampton Court	−13·6	370 ± 31	420
Carlisle Castle (total)	−34·8	2,002 ± 58	570
Carlisle Castle (first fraction)	−14·6	1,158 ± 57	570
Carlisle Castle (second fraction)	−37·0	2,936 ± 72	570

The London Tower sample, a repair mortar of 1953, was assigned a Δ value of +5·39, which represents a ^{11}C concentration 5·39 per cent above the natural atmospheric level. A positive Δ of this magnitude must have resulted from the incorporation of artificial ^{14}C from nuclear weapons tests. The ^{14}C content of this sample is in fact identical to 1956 atmospheric activities and so must have been relatively uninfluenced by the doubling of atmospheric ^{14}C concentrations which occurred between 1954 and 1963. Although the hardening of mortar can, on occasions, require several hundred years for completion it seems that in this case the setting had reached an advanced stage within the first few years.

Of the seven samples dated, six produced ^{14}C ages significantly older than the true ages, indicating contamination by old carbon. δ^{13}C data do not seem to reveal any correlation with the extent of contamination although in theory the percentage content of limestone ($\delta^{13}\text{C} = 0$) and shell carbonate ($\delta^{13}\text{C} = -2{\cdot}5$ ‰) might be estimable by observation of the sample δ^{13}C deviation from that of carbonated lime ($\delta^{13}\text{C} = -10$ ‰). Some fractionation effects may occur, however, during carbon dioxide fixation.

An indication of the extent of contamination by old carbon may be obtained through analyses of consecutive carbon dioxide fractions released during acid hydrolysis. The amorphous carbonate from atmospheric carbon dioxide fixation appears to be hydrolysed more rapidly than the crystalline carbonate of limestone and calcareous sands. Thus the first hydrolysis fraction of Carlisle Castle mortar contained considerably less old carbon than either the second or total fractions.

Accurate ^{14}C dating of mortars therefore depends on the extent of contamination by ancient carbon. The use of calcareous sands (as against silicaceous sands) and/or incompletely kilned limestone would seem to depend on geographical location and for the British mortars studied the technique seems unreliable.

We thank the National Physical Laboratory and the Ministry of Public Building and Works for their cooperation.

M. S. Baxter
A. Walton

Department of Chemistry,
University of Glasgow,
Glasgow W2.

Received November 27, 1969.

---

[1] Delibrias, G., and Labeyrie, J., *Proc. Sixth Intern. Conf. Radiocarbon and Tritium Dating, Pullman, Washington, 1965*, Conf. 650652, 344 (US Department of Commerce).

[2] Stuiver, M., and Smith, C. S., *Proc. Sixth Intern. Conf. Radiocarbon and Tritium Dating, Pullman, Washington, 1965*, Conf. 650652, 338 (US Department of Commerce).

[3] Baxter, M. S., Ergin, M., and Walton, A., *Radiocarbon*, **11**, 1, 43 (1969).

[4] Broecker, W. S., and Olson, E. A., *Radiocarbon*, **3**, 176 (1961).

BIOLOGICAL SCIENCES

Amino-acid Sequence of the Carboxyl Terminal Octapeptide of Human Haptoglobin β Chain

Haptoglobin (Hp) is an α_2-glycoprotein which exhibits the unique property of binding free haemoglobin stoichiometrically. The haemoglobin binding sites were found to be located mainly on the larger of the dissimilar polypeptide chains, the β chain[1-3]. The amino-acid sequence of the α chains has been reported[4]. We have determined the sequence of the carboxyl terminal octapeptide of the haptoglobin β chain.

Haptoglobin was purified by the method of Gordon and Bearn[2] and the β chain was isolated, after reduction and alkylation of Hp, by column chromatography using 'Sephadex *G*-200' equilibrated with 5 M guanidine HCl (ref. 5). The purified Hp β chains were subjected to enzymatic hydrolysis with trypsin or chymotrypsin, and the resulting peptides were purified by column chromatography on 'Aminex A-5' and 'AG-50W-X2' resins by the method of Jones[6].

Table 1. EDMAN DEGRADATION OF THE C-TERMINAL PEPTIDE OF Hp β CHAIN

Sequence: H_2N-Val-Glx-Lys-Thr-Ile-Ala-Glu-Asn-COOH

←——C5B——→

←——T2A——→

Edman step	0	1	2	3	4	5	6	7
Amino-acid								
Asn*	1·03	0·87	0·96	1·01	1·07	1·04	1·05	1·00
Thr	0·78	0·67	0·78	0·72	**0**	0	0	0
Glu	1·97	1·97	**0·96**	1·03	1·09	1·08	1·00	**0**
Ala	0·93	0·80	0·97	0·75	1·00	1·08	**0**	0
Val	1·13	**0·11**	0·07	0	0	0	0	0
Ile	1·00	1·00	1·00	1·00	1·01	**0·26**	0	0
Lys	0·92	0·35†	0·22	**0**	0	0	0	0
Dansyl-N-terminal:	Val	Glx	Lys	Thr	Ile	Ala	Glu	Asn

Values represent residues remaining after each Edman step. Bold type indicates residues missing after each step.

* When hydrolysed with leucine aminopeptidase this residue was eluted at the position characteristic for serine, glutamine and asparagine. After acid hydrolysis the elution position shifted to that characteristic for aspartic acid. No such shift was noted for the penultimate glutamic acid.

† Lysine is often destroyed during acid hydrolysis of phenylisothiocyanate treated peptides.

One tryptic peptide, designated T2A, contained neither lysine nor arginine. Furthermore, one chymotryptic peptide of similar amino-acid composition, designated C5B, did not contain tryptophan, tyrosine, phenylalanine or leucine. Sequential analysis of these two peptides was performed by Dansyl–Edman degradation[7] and subtractive Edman degradation (Table 1). The sequences of the two peptides were identical in the terminal five positions. The chymotryptic peptide C5B contained three additional residues, lysine, glutamic acid or glutamine and valine. The Dansyl (1-dimethylaminonaphthalene-5-sulphonyl chloride) derivative of the N-terminal residue present before each degradation step was identified by polyamide plate thin-layer chromatography[8].

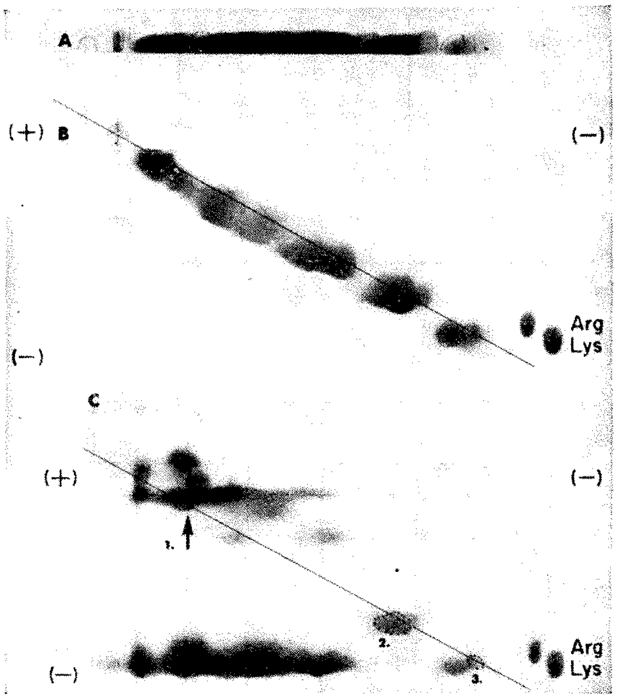


Fig. 1. Diagonal electrophoresis, *p*H 3·4 of the Hp β chain tryptic peptides. *A*, First dimension separation; *B*, second dimension separation; *C*, second dimension separation after digestion *in situ* with carboxypeptidase B.

Further evidence that this peptide is the carboxyl terminal sequence was obtained by carboxypeptidase B digestion in combination with two-dimensional high voltage electrophoresis[9]. Fig. 1 is a photograph of the diagonal electrophoresis at *p*H 3·4. After the first dimensional separation three strips were cut from the paper. One strip was stained with ninhydrin as a guide strip and appears in Fig. 1*A*. The second strip was sewn onto another paper and subjected to high voltage electrophoresis at a 90° angle and was used as a control to establish the diagonal migration pattern seen in Fig. 1*B*. The peptides contained in the third strip were digested *in situ* by spraying the strip with a solution of carboxypeptidase B (0·01 mg/ml. in 0·07 M ammonium carbonate). After 18 h in a moist chamber this strip was dried, sewn onto another paper, and subjected to second dimensional separation. The resulting pattern is shown in Fig. 1*C*.

Any peptide containing C-terminal lysine or arginine migrated off the diagonal because of the cleavage of these residues by carboxypeptidase B. The resulting free lysine or arginine also migrated off the diagonal. Three peptides unaltered by carboxypeptidase activity remained on the diagonal. These peptides were eluted from the remaining portion of the paper and further purified. The material eluted from area three was free lysine. Peptide 2 was shown to be H_2N-Thr-Pro-Lys-COOH, which proved resistant to carboxypeptidase B digestion, and had remained unaltered although it contained a C-terminal lysine. The slow migrating peptide indicated by the arrow was identical to peptide T2A.

Hydrazinolysis of three separate Hp β chain preparations failed to reveal any free amino-acids after extraction of the hydrazides. This would be expected in a protein having asparagine as the C-terminal residue as a result of the formation of the asparagine mono-hydrazide derivative.

When the C-terminal sequence of the human haptoglobin β chain is compared with the C-termini of other proteins, it is interesting that it is slightly similar to the Fc fragment (heavy chain) of IgG (ref. 10) and very similar to the C-termini of chymotrypsinogen[11] and trypsinogen[12] (Table 2). Sequential homology between the haptoglobin α chain and the κ and λ chains of Bence Jones proteins has been observed previously[4]. The striking similarity of the C-termini of the haptoglobin β chain, chymotrypsinogen and trypsinogen is surprising, and may possibly indicate a relationship of haptoglobin with the serine protease family.

Table 2. CARBOXYL TERMINAL SEQUENCE COMPARISON OF HUMAN Hp β, CHYMOTRYPSINOGEN A, TRYPSINOGEN AND HUMAN IgG H CHAIN

Hp β chain	-Val- Glx- Lys- Thr- Ile- Ala- Glu- Asn- COOH
Chymotrypsinogen A*	-*Val*- *Gln*- Gln- *Thr*- Leu- *Ala*- Ala- *Asn*- COOH
Trypsinogen*	-Ile- Lys- Glu- *Thr*- *Ile*- *Ala*- Ser- *Asn*- COOH
Human IgG H chain†	-Thr- *Gln*- *Lys*- Ser- Leu- Ser- Leu- Ser- Pro- Gly- COOH‡

* Bovine.

† Human myeloma γG1 and γG2.

‡ Two terminal gaps are introduced in the first three protein sequences to maximize homologies with IgG H chain.

We thank the US Public Health Service for research grants and Dr R. T. Jones for his instruction.

DON R. BARNETT
TONG-HO LEE
BARBARA H. BOWMAN

Department of Human Genetics,
University of Texas Medical Branch,
Galveston, Texas 77550.

Received November 7; revised December 23, 1969.

[1] Shim, B. S., Lee, T. H., and Kang, Y. S., *Nature*, **207**, 1264 (1965).

[2] Gordon, S., and Bearn, A. G., *Proc. Soc. Exp. Biol. and Med.*, **121**, 846 (1966).

[3] Shim, B. S., Jin, K. S., and Cleve, H., *Proc. Exp. Biol. and Med.*, **126**, 221 (1967).

[4] Black, J., and Dixon, G. H., *Nature*, **218**, 736 (1968).

[5] Gordon, S., Cleve, H., and Bearn, A. G., *Proc. Soc. Exp. Biol. and Med.*, **127**, 52 (1968).

[6] Jones, R. T., in *Technicon Symposium, Automation in Analytical Chemistry*, 414 (Mediad Inc., New York, 1967).

[7] Gray, W. R., in *Methods in Enzymology* (edit. by Hirs, C. H. W.), **11**, 469 (Academic Press, New York, 1967).

[8] Woods, K. R., and Wagn, K. T., *Biochim. Biophys. Acta*, **133**, 369 (1967).

[9] Naughton, M. A., and Hagopian, H., *Anal. Biochem.*, **3**, 276 (1962).

[10] Porter, R. R., *Biochem. J.*, **105**, 418 (1967).

[11] Hartley, B. S., Brown, J. R., Kauffman, D. L., and Smillie, L. B., *Nature*, **207**, 1157 (1965).

[12] Walsh, K. A., and Neurath, H., *Proc. US Nat. Acad. Sci.*, **52**, 884 (1964).

Mild "Unstable Haemoglobin Haemolytic Anaemia" caused by Haemoglobin Shepherds Bush (B74 (E18) Gly→Asp)

A 30 YEAR old South African woman of British extraction was investigated because of a persistent reticulocytosis. As a child she had been chronically anaemic, and at the age of six her spleen had been removed. After that time she had been well, but on one occasion she became "ill" after a course of sulphonamide. She was found to have a well compensated haemolytic anaemia; the haemoglobin level was 13 g/100 ml. of blood, and 5–8 per cent of her red cells were reticulocytes. Though of normal size the red cells showed target cell formation, basophilic stippling, and 75–80 per cent contained Heinz bodies. The test for abnormally heat labile haemoglobin[1] was positive. The patient's mother was found to carry the same haemoglobin abnormality but was not thought to suffer from severe haemolysis. She did, however, experience an episode of severe haemoglobinuria following sulphonamides.

The haemolysate contained 2·1 per cent Hb F as determined by alkali denaturation[2]. Paper[3] and starch gel[4] electrophoreses were used to demonstrate a higher than normal Hb A_2 fraction (3·5 per cent), a small amount (0·8 per cent) of a haemoglobin with the mobility of free α chains and a haemoglobin fraction moving more anodically than Hb A. Its separation from Hb A was incomplete by these methods, but chromatography on a 'DEAE–Sephadex' column using a *p*H gradient[5] yielded a well separated abnormal fraction amounting to 24 per cent which was subsequently used for the determination of the amino-acid substitution. On electrophoresis of the total globin in starch gel with 6·5 M urea at *p*H 8·6 (ref. 6), bands were found in the position of normal α and β chains, and in addition one band, amounting to about a quarter of the total globin, was seen which moved anodically to the β chain, indicating that the abnormal haemoglobin had an additional negative charge in the β chain. A quantitative separation of the globin chains obtained from the whole haemoglobin was made on a carboxymethyl–cellulose column in the presence of 8 M urea[7] and showed a new β chain peak, amounting to 24 per cent of the non α chains.

An unstable haemoglobin found previously at Hammersmith Hospital had already been named Hb Hammersmith[8], and the new variant has therefore been named Hb Shepherds Bush.

The purified abnormal haemoglobin fraction was converted into globin and digested with trypsin for 2 h at *p*H 8·6. The fingerprint[9,10] of the abnormal haemoglobin (Fig. 1) differed from that of Hb A by the absence of the spot representing β^ATpIX (residues 67–82 of the 146 of the β chain) and the presence of a new spot which stained for histidine[11]. It was displaced towards the anode relative to β^ATpIX and had a lower chromatographic mobility. All the other spots normally found on the fingerprint of Hb A were present in their usual positions and gave the expected specific staining reactions[11]. The insoluble "core" which remained after tryptic digestion was examined by peptic digestion and fingerprinting, but no evidence for any further abnormalities was obtained.

Table 1. AMINO-ACID COMPOSITION OF βTpIX (β67–82) OF Hb SHEPHERDS BUSH

Amino-acid	Hb SB			Hb A	Expected for β^ATpIX
Asp	3·4	3·6	3·7	3·0	3
Ser	0·9	1·5	1·25	1·0	1
Gly	1·0	1·3	1·3	2·0	2
Ala	1·9	2·2	2·1	2·1	2
Val	0·8	1·0	1·0	0·9	1
Leu	3·8	3·6	3·7	4·0	4
Phe	0·7	0·9	0·9	1·0	1
Lys	1·0	1*	1*	1*	1
His	1·2	1*	1*	1*	1
Yield (μmoles)	0·024	0·021	0·021	0·017	

* A lysyl is presumed to be present because the C terminal residues of tryptic peptides would either be lysyl or arginyl and the presence of arginine could be excluded by a negative staining reaction. A positive test for histidine was taken to indicate the presence of histidyl.

The peptide was isolated by preparative fingerprinting; the electrophoretic separation (1·5 h) was more prolonged than usual to ensure adequate separation from another nearby peptide (βXIIIa 121–130). Table 1 shows that the new peptide resembled β^ATpIX in composition except that it contained only one residue of glycine instead of two and approximately four residues of aspartic acid instead of three; this suggested a Gly→Asp substitution. Although asparagine also produces aspartic acid on hydrolysis, a Gly→Asn mutation would not alter the charge of βTpIX, and furthermore this substitution is not compatible with a single base change of the codons for glycine[13].

There are two glycine residues in β^ATpIX at positions 69 and 74; in Hb J Cambridge, β69 Gly–Asp[9], which is not unstable, the first of these is substituted. It therefore seemed likely that the new haemoglobin was β74 Gly→Asp, and in order to confirm this the mutant peptide was further degraded with thermolysin into smaller peptides. Thermolysin is a bacterial protease which hydrolyses peptide bonds on the amino side of amino-acids with bulky hydrophobic side chains[14–16]—for example, phenylalanine, leucine, valine and so on—but is inhibited by the

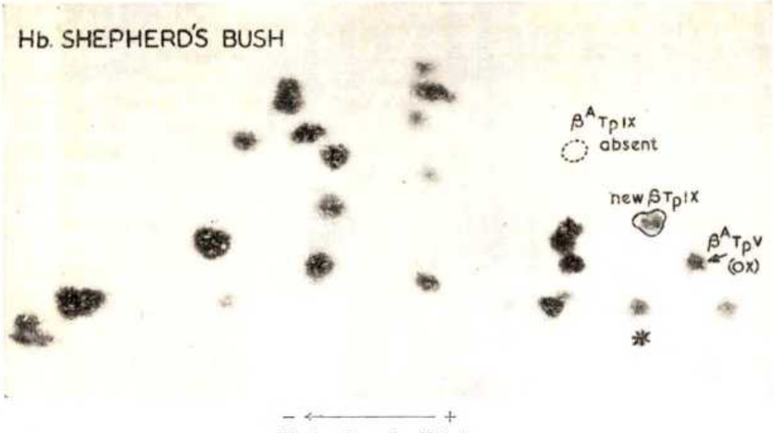
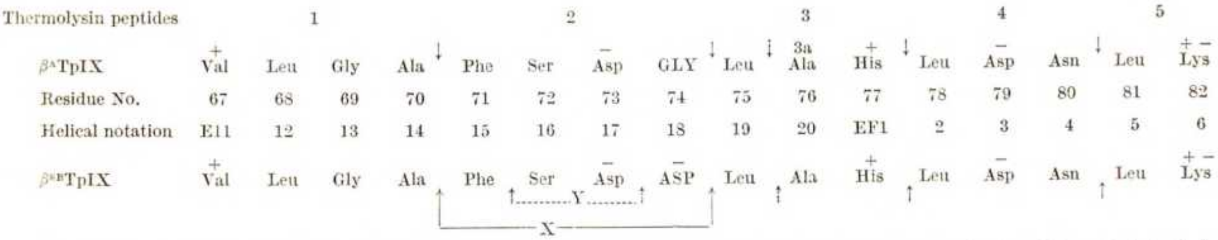


Fig. 1. Fingerprint of Hb Shepherds Bush. For details see text. An asterisk indicates the point of application.

immediate proximity of a charged amino group. Fig. 2 shows the amino-acid sequence of β^ATpIX and the expected points of thermolysin hydrolysis. Glycine 74 is in an acidic peptide which would become more acidic on substitution of glycine by aspartic acid.



Thermolysin peptides	1				2					3			4		5	
β^ATpIX	+ Val	Leu	Gly	Ala ↓	Phe	Ser	− Asp	GLY ↓	Leu ↕	3a Ala	+ His ↓	Leu	− Asp	Asn ↓	Leu	+ − Lys
Residue No.	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82
Helical notation	E11	12	13	14	15	16	17	18	19	20	EF1	2	3	4	5	6
β^SBTpIX	+ Val	Leu	Gly	Ala ↑	Phe ↑	Ser	− Asp ↑	− ASP ↑	Leu ↕	Ala	+ His ↑	Leu	− Asp	Asn ↑	Leu	+ − Lys

Fig. 2. Sequences of normal and abnormal βTpIX and expected thermolysin peptides 1–5. Full arrows represent main sites of thermolysin hydrolysis; dotted arrows, other sites of hydrolysis. Positive and negative signs denote electric charges at pH 6·4.

The abnormal βTpIX and normal β^ATpIX were eluted from preparative fingerprints and digested with thermolysin for 3–5 h at 37° C in 0·2 M ammonium acetate buffer (pH 7·4) containing 5 mM barium acetate. The barium was removed by precipitation as the sulphate and centrifugation, and the digests were evaporated to dryness *in vacuo*. Diagnostic and preparative fingerprints of the thermolysin digests were made in the same way as for tryptic digests, and the peptides were eluted, hydrolysed and analysed.

Fig. 3 is a drawing of a fingerprint of a thermolysin digest of β^ATpIX. In the corresponding fingerprint of the abnormal βTpIX, spot 2 was absent and two new spots X and Y were present. Amino-acid analysis of X (Table 2) showed that it corresponded to the expected product of a β74 Gly→Asp substitution. Peptide Y contained chiefly serine and aspartic acid and could be derived from X by further hydrolysis. The new unstable Hb Shepherds Bush can therefore be described as $\alpha_2\beta_2$ 74 (E18) Gly→Asp.

The glycine at position β74 is the eighteenth residue of the E helix[17,18] and is at an internal site which forms part of the hydrophobic lining of the haem pocket, but is not in direct contact with the haem group[19]. Position E18 is occupied by glycine in all the mammalian β chains so far tested[20] and in the δ chain of human Hb A_2, and by alanine in the γ chain of human Hb F. The equivalent position in the α chain (α69) is occupied by alanine in human and animal α chains. Position E18 corresponds to position 75 in myoglobin and this is occupied by isoleucine in human, whale, dolphin and seal myoglobins[21,22]. In all cases the amino-acid has a nonpolar side chain. Aspartic acid is an "antihelical" residue[23]; β73 (E17) is aspartic acid in normal haemoglobin and the presence of an additional β74 (E18) aspartic acid side chain can be expected to weaken the E helix further by interfering with the carbonyl part of the preceding β70 (E14) alanine residue.

Hb Shepherds Bush represents another instance where a charged amino-acid is introduced into the haem pocket and one has to explain why this does not cause complete disruption of the structure. Examination of a scale model shows that the β carboxyl group of the aspartic acid might be drawn out of the haem pocket towards the surface of the molecule possibly to form an ionic link with histidine β77 (EF1). Similar explanations have been suggested in the cases of Hb Zürich β63 (E7) His→Arg[24] and Hb Borås β88 (F4) Leu→Arg[25]. Using his model of horse haemoglobin[26] Dr M. F. Perutz showed us why a β74 (E18) Gly→Asp mutation would be expected to make the molecule unstable: the part of the haemoglobin where E18 is situated is tightly packed and the E and F

Table 2. AMINO-ACID COMPOSITION OF THERMOLYSIN PEPTIDES: NO. 2 FROM A, AND X AND Y FROM Hb SHEPHERDS BUSH IN FIG. 3

Amino-acids	Hb A Peptide 2 β71–74	Hb Shepherds Bush Peptide X β71–74	Hb Shepherds Bush Peptide Y β72–73
Asp	1·0 (1)	2·0 (2)	1·0 (1)
Ser	0·8 (1)	1·2 (1)	0·8 (1)
Glu		*0·3*	*0·4*
Gly	1·0 (1)	*0·3* (0)	*0·4*
Ala			*0·2*
Phe	0·75* (1)	0·6* (1)	
Yield (μmoles)	0·079	0·017	0·017

* Phenylalanine is the N-terminal residue and is partly destroyed by ninhydrin staining.

Figures in brackets are the expected numbers of residues; those in italics are considered to represent contamination.

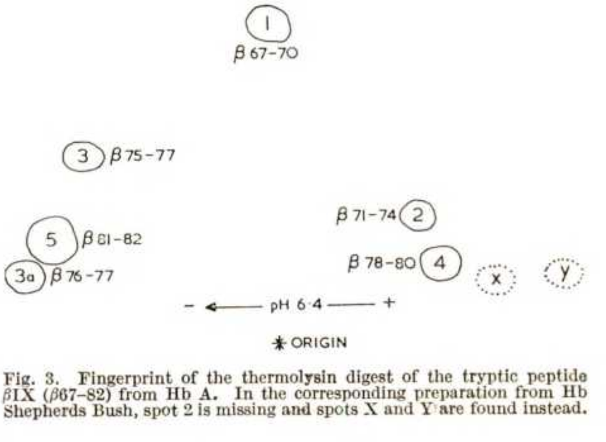


Fig. 3. Fingerprint of the thermolysin digest of the tryptic peptide βIX (β67–82) from Hb A. In the corresponding preparation from Hb Shepherds Bush, spot 2 is missing and spots X and Y are found instead.

helices are lying closely opposite each other. Although it can just accommodate an alanine instead of a glycine as in the α and γ chains, the insertion of an aspartic acid side chain at E18 in the β chain would interfere with the residues of threonine β84 (EF8) and phenylalanine β85 (F1) lying opposite and move the E and F helices apart, thereby weakening the hydrophobic forces which hold the haem pocket together.

J. M. WHITE
M. C. BRAIN

MRC Research Group on Haemolytic Mechanisms,
Royal Postgraduate Medical School, London.

P. A. LORKIN
H. LEHMANN

MRC Abnormal Haemoglobin Unit,
Department of Biochemistry,
University of Cambridge.

M. SMITH

Galton Laboratory,
University College,
London.

Received November 3; revised November 25, 1969.

[1] Dacie, J. V., Grimes, A. J., Meisler, A., Steingold, L., Hemsted, E. H., Beaven, G. H., and White, J. C., *Brit. J. Haematol.*, **10**, 388 (1964).
[2] Betke, K., Marti, H. R., and Schlicht, I., *Nature*, **184**, 1877 (1959).
[3] Cradock-Watson, J. E., Fenton, J. C. B., and Lehmann, H., *J. Clin. Pathol.*, **12**, 372 (1959).
[4] Poulik, M. D., *Nature*, **180**, 1477 (1957).
[5] Huisman, T. H. J., and Dozy, A. M., *J. Chromatog.*, **19**, 160 (1965).
[6] Chernoff, A. I., and Pettit, N. M., *Blood*, **24**, 750 (1965).
[7] Clegg, J. B., Naughton, M. A., and Weatherall, D. J., *J. Mol. Biol.*, **19**, 91 (1966).
[8] Dacie, J. V., Shinton, N. K., Gaffney, P. J., Carrell, R. W., and Lehmann, H., *Nature*, **216**, 663 (1967).
[9] Sick, K., Beale, D., Irvine, D., Lehmann, H., Goodall, P. T., and MacDougall, S., *Biochim. Biophys. Acta*, **140**, 231 (1967).
[10] Lehmann, H., and Huntsman, R. G., *Man's Haemoglobins*, 299 (North-Holland, Amsterdam, 1966).
[11] Smith, I., *Chromatographic and Electrophoretic Techniques*, **1** (Heinemann, London, 1969).
[12] Beale, D., *Biochem. J.*, **103**, 129 (1967).
[13] Nirenberg, M., Leder, P., Bernfield, M., Brimacombe, R., Trupin, J., Rotman, F., and O'Neal, C., *Proc. US Nat. Acad. Sci.*, **53**, 1161 (1965).
[14] Ambler, R. P., and Meadway, R. J., *Biochem. J.*, **108**, 893 (1968).
[15] Matsubara, H., *Biochem. Biophys. Res. Commun.*, **24**, 427 (1969).
[16] Matsubara, H., Singer, A., and Saski, R. M., *Biochem. Biophys. Res. Commun.*, **34**, 719 (1969).
[17] Perutz, M. F., *J. Mol. Biol.*, **13**, 646 (1965).
[18] Perutz, M. F., Kendrew, J. C., and Watson, H. C., *J. Mol. Biol.*, **13**, 669 (1965).
[19] Perutz, M. F., Muirhead, M., Cox, J. M., and Goodman, L. C. G., *Nature*, **219**, 131 (1968).
[20] Dayhoff, M. O., and Eck, R. V., *Atlas of Protein Sequence and Structure* (Biochemical Research Foundation, Silverspring, Maryland, 1968).
[21] Hill, R. L., Harris, C. M., Naylor, J. F., and Sams, W. M., *J. Biol. Chem.*, **244**, 2182 (1969).
[22] Bradshaw, R. A., and Gurd, F. N., *J. Biol. Chem.*, **244**, 2167 (1969).
[23] Guzzo, A. V., *Biophys. J.*, **5**, 809 (1965).
[24] Perutz, M. F., and Lehmann, H., *Nature*, **219**, 902 (1968).
[25] Hollender, A., Lorkin, P. A., Lehmann, H., and Svensson, N., *Nature*, **222**, 935 (1969).
[26] Perutz, M. F., *Proc. Roy. Soc.*, B, **173**, 113 (1969).

Allotype Polymorphism of Low Density β-Lipoproteins in Pig Serum (LDLpp I, LDLpp 2)

A GREAT variety of serum antigens have been found in a number of mammalian species[1,2].

A young pig 'Chester 16-4' was injected five times at 14 day intervals with 5 ml. pooled sera of five 'Hampshire' pigs to which 1·5 ml. Freund's complete adjuvant had been added. The Ouchterlony double-diffusion technique as modified by Rapacz *et al.*[3] was used to demonstrate isoprecipitins against serum antigens. The immune serum, C16-4, gave positive reactions with three of the donor sera. The precipitation lines in the agar were clearly visible, crescent shaped and curved towards the peripheral well as shown by the band between the centre well (*A*) and peripheral well 1 (Fig. 1). This indicates that the antigen is of relatively high molecular weight. Normal sera from other pigs were tested against the antiserum, and a second band was observed in several cases, for example, the band between the centre well (*A*) and well 2 (Fig. 1). None of the three donor sera mentioned here showed this specificity. Pigs of three breeds, 397 of the Hampshire, 431 of Chester and 305 of Poland China breeds (from the University of Wisconsin Experimental swine herd), were tested. Only in the Hampshire breed were antigenic specificities found, with the frequency of 37 per cent.

The following techniques were used in an effort to identify the antigens: (1) gel chromatography separation, (2) gel immunoelectrophoresis, (3) staining with protein and lipid dyes, (4) paper electrophoresis, and (5) ultracentrifugation. Normal sera of phenotypes Lpp 1, Lpp 2 and Lpp 1,2 (Fig. 1) were fractionated on a 'Sephadex *G*-200' column. Antigen activities were restricted to the first peak supporting previous observation that the antigens are of high molecular weight. When the sera were tested by immunoelectrophoresis, the antigens were found to migrate to the α-globulin position. The stronger precipitin line stained weakly with protein reagent 'Amido Black 10*B*' and strongly with lipid stain 'Oil Red O'. The second precipitate (the one closer to the central well) stained like the first, but much less intensely. The antigens were found in the β-globulin and the β-lipoprotein position when serum samples were run on paper electrophoresis.

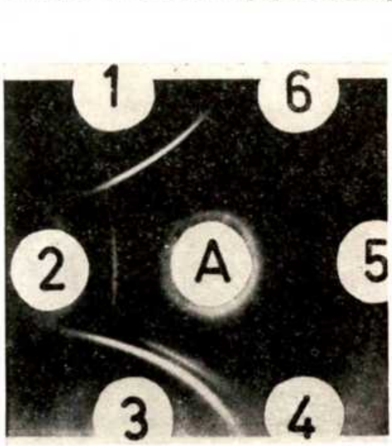


Fig. 1. Double diffusion precipitation test in agar gel. Precipitation reactions observed in this study between the immune serum placed in the centre well *A* and the normal sera placed in the peripheral wells 1–6 represent four phenotypes: Lpp 1 (reaction between *A* and 1); Lpp 2 (reaction between *A* and 2); Lpp 1,2 (reaction between *A* and 3); and Lpp 0 (lack of precipitate between *A* and 4, 5, 6).

Further identification of the lipoprotein allotypes was attempted according to the Cohen technique, using the preparative ultracentrifuge, by flotation based on selective solvent density. The antiserum reacted only with the 1·019–1·063 density material which indicates that the antigens are low density β-lipoproteins. Allotypic polymorphism of low density β-lipoproteins has been found in humans[4-8]. Absorptions were carried out to determine the relation between the antigens and to explain the unexpected presence of the second specificity in some sera. Lpp 1, Lpp 2, Lpp 1,2 and Lpp 0 are the symbols proposed for the four phenotypes observed in Fig. 1.

Absorption with all sera of Lpp 2 type removed only precipitins directed against the weaker antigen, leaving the antibodies for the stronger specificity. The Lpp 1,2 type serum absorbed all precipitating antibodies as did each Lpp 1 type serum used.

The ability of Lpp 1 serum to remove the antibodies for both types is consistent with its antigenic ability to stimulate production of both antibodies. Absorption results are evidence in support of the contention that: (1) the second low density β-lipoprotein antigen is present on the independent molecules, (2) the phenotype Lpp 1 represents the lipoprotein molecules which carry both antigenic specificities, and (3) immune serum C16-4 contains two types of isoprecipitins.

The genetic relationship between Lpp 1 and Lpp 2 became evident as a result of 179 matings, representing seven combinations, which produced 1,038 offspring. The most unequivocable result was obtained from the Lpp 1,2 × Lpp 0 mating: all offspring fell either into the Lpp 1 or the Lpp 2 allotype classes, neither parent belonging to either class. The data from all matings are consistent with the hypothesis that: (1) the allotypes Lpp 1 and Lpp 2 are controlled by codominant allelic genes which we have chosen to symbolize by *Lpp 1* and *Lpp 2*, leaving *Lpp 0* to designate the negative allele; (2) these genes seem to function independently of each other, that is, to be expressed as separate molecules; and (3) gene *Lpp 1* synthesizes two specificities, one unique to one allotype and the other common to both.

J. RAPACZ
R. H. GRUMMER
JUDITH HASLER
R. M. SHACKELFORD

Department of Meat and Animal Science,
University of Wisconsin,
Madison, Wisconsin 53706.

Received August 25; revised December 1, 1969.

[1] Stone, W. H., *Adv. in Immunogen.* (Lippincott, Phil., 1967).
[2] Lush, I. E., *Frontiers in Biology*, **3** (North-Holland, Amsterdam, 1966).
[3] Rapacz, J., Korda, N., and Stone, W. H., *Genetics*, **59**, 387 (1967).
[4] Allison, A. C., and Blumberg, B. S., *Prog. in Medical Genetics*, 176 (1966).
[5] Blumberg, B. S., Dray, S., and Robinson, J. C., *Nature*, **194**, 656 (1962).
[6] Allison, A. C., and Blumberg, B. S., *Lancet*, 634 (1961).
[7] Berg, K., *Acta Path. Microbiol. Scand.*, **59**, 369 (1963).
[8] Bütler, R., and Brunner, E., *Vox Sang.*, **14**, 230 (1968).

Biological Properties of Model Molecules for Vitamin B$_{12}$

CERTAIN tetradentate organo-cobalt complexes have physical and chemical properties which resemble those of vitamin B$_{12}$ (ref. 1). We have investigated whether the water-soluble complexes based on *bis*-(diacetylmonoxime-imino)-1,3-propane—abbreviated (DOH)$_2$propane (Fig. 1)—will also mimic some of the biological properties of vitamin B$_{12}$, particularly the promotion of growth in bacteria and the catalytic function of the vitamin as a coenzyme. A vitamin B$_{12}$-dependent strain of *Lactobacillus leichmanii* was used to assay the growth stimulating properties of the (DOH)$_2$propane compounds, with or without added vitamin B$_{12}$. The growth of *L. leichmanii* was only irregularly stimulated when the synthetic compounds were added to the medium alone, but in the presence of catalytic amounts of vitamin B$_{12}$ a constant stimulation was found (Fig. 2). Each compound is active in a narrow range of concentrations, with a characteristic optimum. In the series of cobalt–aquo complexes, for example, the values are 0·07 mM for the benzyl derivative, 0·4 mM for *n*-propyl and phenyl derivatives, 0·7 mM for methyl and ethyl derivatives (Fig. 2*a*).

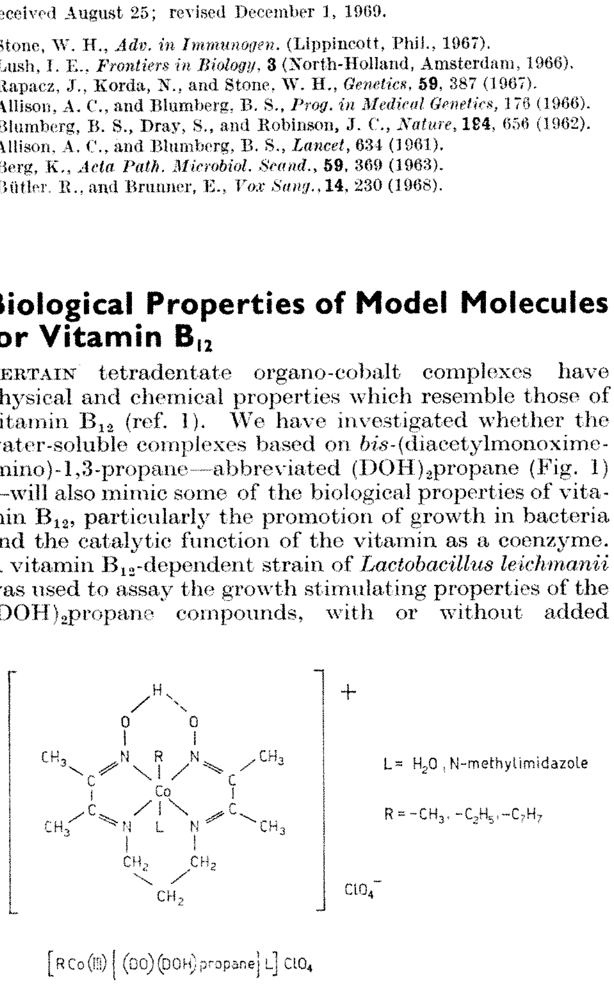

+
L= H$_2$O , N-methylimidazole
R = -CH$_3$, -C$_2$H$_5$, -C$_7$H$_7$
ClO$_4^-$
[RCo(III){(DO)(DOH)propane}L] ClO$_4$

Fig. 1. Structure of cobalt chelates of *bis*-(diacetylmonoximeimino)-1,3-propane.

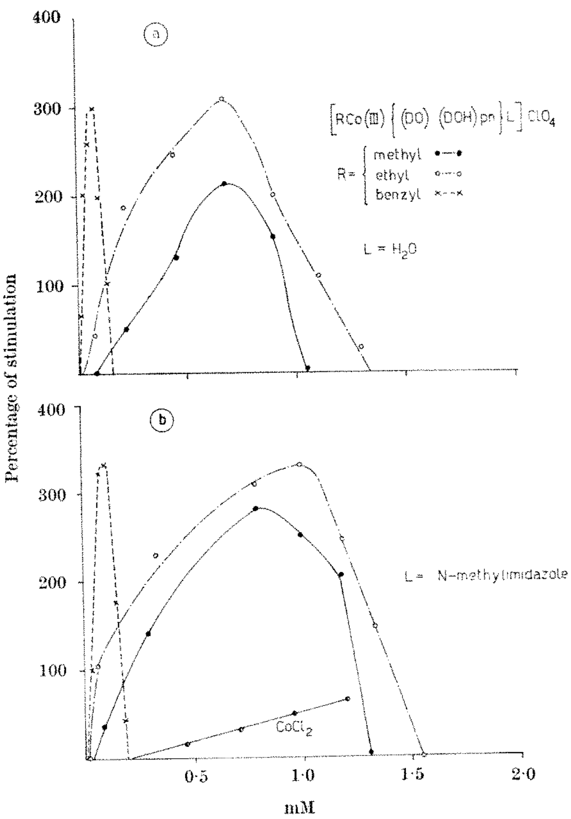


Fig. 2. Growth stimulation of a vitamin B$_{12}$-dependent strain of *L. leichmanii* by (DOH)$_2$propane compounds. Cyanocobalamin (3·6 × 10^{-12} M) was added to the assay medium. *L. leichmanii* (ATCC 7830) was grown in the LBS medium (Baltimore Biochemical Laboratories). The culture medium for the inoculum was the LBS broth. The basal medium stock solution, for the assays, was the Difco 'Bacto' B$_{12}$ assay medium. The activity of vitamin B$_{12}$ or model molecules was assayed according to the method reported in the *US Pharmacopeia*[2].

Complexes based on N-methylimidazole had virtually the same effect (Fig. 2*b*), the compound with a benzyl group being again active in the lowest concentration, at least ten times less than that for methyl or ethyl derivatives. Methyl and phenyl derivatives of the aquo-compounds, even at the optimum concentrations, are less active than the other compounds. By replacing the synthetic compounds with CoCl$_2$, all other experimental conditions being unchanged, only a negligible growth stimulation effect was observed (Fig. 2*b*).

The biochemical properties of these synthetic organo-cobalt derivatives were studied as coenzymes for bacterial propanediol dehydratase (EC 4.2.1.28) (ref. 3). We investigated whether the (DOH)$_2$propane complexes could replace the native cobamide coenzyme and also whether they otherwise influenced the enzymatic reaction. The enzyme was depleted of its native coenzyme by charcoal adsorption of a cell-free extract from *Aerobacter aerogenes* (ATCC 8724), kindly supplied by Dr Abeles, Waltham, Massachusetts. This was grown in a medium formed by: 23 mM K$_2$HPO$_4$; 7 mM KH$_2$PO$_4$; 10 mM (NH$_4$)$_2$SO$_4$; 0·8 mM MgSO$_4$·7H$_2$O; 200 mM glycerol; 54 mM propane-1,2-diol and 0·2 per cent Difco yeast extract, in deionized water. The inoculum was prepared in the same medium, but propane-1,2-diol was omitted. The bacteria were grown overnight at 37° C; harvesting and washing of cells, lyophilization, sonication and charcoal adsorption of the coenzyme were carried out as described by Lee and Abeles[4]. Sonication was performed for 1 min on aliquots of 3·5 ml. of cell suspension at 3A, 20 kHz, 0° C, using the Branson sonifier. The propanediol dehydratase activity was followed by determining propionaldehyde, the product of the reaction, as 2,4-dinitrophenylhydrazone, according to Böhme and Winkler[5]. The apoenzyme can be reassociated with the native coenzyme: a K_m value of $5{\cdot}76 \times 10^{-7}$ M has been found, using dimethylbenzimidazolylcobamide (Glaxo, Verona), in good agreement with data obtained in other laboratories[6]. One may wonder whether the term K_m is appropriate in this case because the combination of the apoenzyme with the coenzyme is practically irreversible.

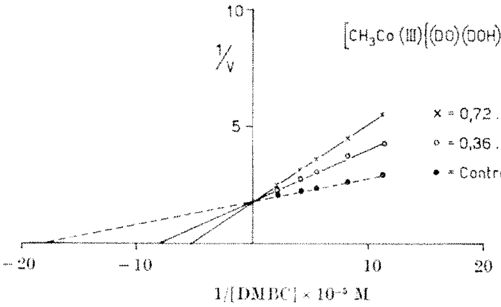


Fig. 3. Inhibition of the propanediol dehydratase activity by two different concentrations of $[CH_3Co(III)\{(DO)(DOH)propane\}H_2O]ClO_4$.

When the cobamide coenzyme was replaced by the $(DOH)_2$propane compounds the reaction was not catalysed, indicating that all compounds are inactive. All the compounds inhibited the B_{12}-coenzyme dependent propanediol dehydratase reaction and Lineweaver–Burk[7] plots indicate inhibition of the competitive type (Fig. 3). The K_i values for the different complexes are similar (Table 1). Our results open the possibility that these synthetic complexes may be promising model compounds for further studies on the mechanism of action of vitamin B_{12}.

Table 1. K_i VALUES ($\times 10^{-4}$ M) FOR SYNTHETIC COMPOUNDS IN THE PROPANEDIOL DEHYDRATASE REACTION

	$[RCo(III)\{(DO)(DOH)propane\}L]ClO_4$	
	L = aquo	L = N-methylimidazole
$R = -CH_3$	2·47–3·20	2·35–2·93
$R = -C_2H_5$	7·24–7·54	2·88–4·72
$R = -n\text{-}C_3H_7$	5·11–5·30	—
$R = -C_6H_5$	4·46–4·74	—
$R = -C_7H_7$	2·92–3·31	1·61–1·88

G. C. was the recipient of a grant from the Italian National Research Council.

N. STAGNI
B. DE BERNARD

Institute of Biochemistry,
University of Trieste.

G. COSTA
G. MESTRONI

Institute of Chemistry,
University of Trieste.

Received July 16, 1969; revised January 15, 1970.

[1] Costa, G., Mestroni, G., and de Savorgnani, E., *Inorg. Chim. Acta*, **3**, 323 (1969).

[2] *US Pharmacopeia*, **17**, 864 (1965).

[3] Abeles, R. H., and Lee, jun., H. A., *J. Biol. Chem.*, **236**, PC 1 and 2347 (1961).

[4] Lee, jun., H. A., and Abeles, R. H., *J. Biol. Chem.*, **238**, 2367 (1963).

[5] Böhme, H., and Winkler, O., *Z. Anal. Chem.*, **142**, 1 (1954).

[6] Friedrich, W., Heinrich, H. C., Königk, E., and Schulze, P., *Ann. NY Acad. Sci.*, **112**, 601 (1964).

[7] Lineweaver, M., and Burk, D., *J. Amer. Chem. Soc.*, **56**, 658 (1934).

Antitumour Polysaccharide derived Chemically from Natural Glucan (Pachyman)

WE have reported[1] that the polysaccharide lentinan obtained from *Lentinus edodes* (Berk.) Sing. (a popular edible mushroom in Japan) had a strong antitumour activity against sarcoma 180 implanted subcutaneously in mice, and that almost complete regression of the tumours had been observed after ten doses of 1 mg/kg. Lentinan is a β-(1→3)-linked linear glucan having a molecular weight of 950,000–1,050,000 according to light scattering methods.

On the other hand, pachyman[2], obtained from *Poria cocos* Wolf (Bukuryo) and hitherto considered to be a β-(1→3) linear glucan, is completely devoid of antitumour activity. Re-examination of the structure of pachyman has demonstrated, however, that the β-(1→3) chain has some β-(1→6)-linked branches. The gas chromatogram (0·75 per cent 'SE-30' on 'Gas-Chrom *Q*', 6 feet × 0·25 inch, 230° C) of the disaccharide portion, the product of partial acetolysis of pachyman, exhibited the peak for gentiobiose octaacetate, thus indicating the presence of β-(1→6)-glycoside linkages, besides that of laminaribiose octaacetate indicative of β-(1→3) glycoside linkages. The results of periodate oxidation and Smith degradation[3,4] also supported the presence of β-(1→6) linkages.

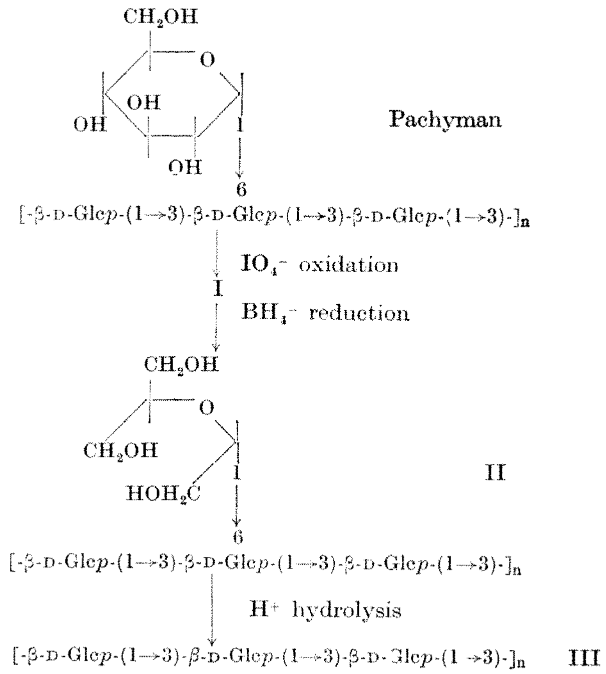

CH₂OH
O
OH
OH
OH
1
6
Pachyman
[-β-D-Glcp-(1→3)-β-D-Glcp-(1→3)-β-D-Glcp-(1→3)-]n
IO₄⁻ oxidation
I
BH₄⁻ reduction
CH₂OH
O
CH₂OH
HOH₂C
1
6
II
[-β-D-Glcp-(1→3)-β-D-Glcp-(1→3)-β-D-Glcp-(1→3)-]n
H⁺ hydrolysis
[-β-D-Glcp-(1→3)-β-D-Glcp-(1→3)-β-D-Glcp-(1→3)-]n
III

Fig. 1. Chemical conversion of pachyman to its oxidized/reduced derivative (II) and the final product of mild hydrolysis (III).

Experiments were carried out to sever the β-(1→6) linkages in pachyman by the method shown in Fig. 1 to convert it to a lentinan-like β-(1→3) linear glucan. By this conversion, a new polysaccharide with a strong antitumour activity was formed. A suspension of 3·7 g of pachyman in 450 ml. water was mixed with 150 ml. aqueous solution of 0·1 M sodium metaperiodate and stirred for 40 h in darkness at room temperature. The mixture was adjusted to *p*H 4·5 with acetic acid and centrifuged to yield a colourless paste (I). This substance was suspended in 200 ml. of water, and a solution of 800 mg of sodium borohydride in 100 ml. water was added with stirring; the mixture was stirred for 20 h to effect reduction. A further 300 mg of sodium borohydride was added and the stirring continued for another 20 h. The reaction mixture was adjusted to pH 6·0 with acetic acid and centrifuged, and the precipitate was collected, washed with ethanol and ether, and dried to 3 g of white powder (II).

This product was suspended in 200 ml. of 0·05 M sulphuric acid and stirred vigorously at room temperature for 24 h to effect mild hydrolysis. The mixture was centrifuged and the precipitate was washed consecutively with water, ethanol and ether, and dried to give 2·87 g of antitumour polysaccharide (III) having an optical rotation $[\alpha]_D^{25} + 23{\cdot}3°$ ($c = 1$, 1 M NaOH). This substance was insoluble in water but readily dissolved in alkalis, and has marked swelling properties. Its infrared spectrum lacked any absorptions corresponding to aldehyde or carboxyl groups.

Table 1. ANTITUMOUR EFFECT OF PACHYMAN, II AND III

Sample	Dose	Body weight change	Average weight of tumour (g)	Inhibition ratio (per cent)	Complete regression
Pachyman	5 mg/kg × 10	+4·4 g	6·6	0	0/8
	1 mg/kg × 10	+5·9 g	6·8	−3	0/9
	Control	+5·6 g	6·6		0/8
II	5 mg/kg × 10	+2·3 g	0·3	96	4/9
	Control	+0·9 g	6·7		0/8
III	5 mg/kg × 10	+3·3 g	0·8	88	2/6
	Control	+0·9 g	6·7		0/8

Antitumour activity was tested by the method described previously[1]. A 7 day old ascites sarcoma 180 was transplanted subcutaneously into the right groin of a Swiss albino mouse. The test sample was injected intraperitoneally once a day for 10 days starting 24 h after transplantation. The mice were killed after 5 weeks, the tumours were extirpated and weighed, and inhibition ratios were calculated and compared with the findings in control mice. Complete regression of the tumours was recorded (Table 1). Although pachyman has no antitumour activity, derivatives II and III exhibited pronounced activity, similar to that manifested by lentinan (Table 1). *Poria cocos* Wolf is one of the most important crude drugs in Japan and in other Asian countries. Pachyman is extracted from this material in a yield of about 75 per cent. The yield of II and III from pachyman is about 80 per cent. The methods seem to be the simplest and most convenient for obtaining a polysaccharide with a marked antitumour activity against transplanted tumour. Neither II nor III has any toxicity. We propose calling III "pachymaran".

The fact that such a marked difference in antitumour activity could result from a small change in fine structure, involving a small amount of β-(1→6)-linked branching or some related structural difference arising from it, is of interest with respect to the biological activities of polysaccharides in general. It is also of particular relevance to the question, raised in connexion with previous studies on antitumour polysaccharides, whether a minute amount of impurity might be the active principle. We are now studying the fine structure of pachyman, II and III, with a view to correlating structure with antitumour action in greater detail.

We thank Dr Waro Nakahara for his encouragement, and Miss Reiko Tokuzen for her cooperation.

GORO CHIHARA
JUNJI HAMURO
YUKIKO MAEDA
YOSHIKO ARAI
FUMIKO FUKUOKA

National Cancer Centre,
Research Institute,
Tokyo.

Received October 28, 1969.

[1] Chihara, G., Maeda, Y., Hamuro, J., Sasaki, T., and Fukuoka, F., *Nature*, **222**, 687 (1969).
[2] Warsi, S. A., and Whelan, W. J., *Chem. Ind.*, 1573 (1957).
[3] Goldstein, I. J., Hay, G. W., Lewis, B. A., and Smith, F., *Methods in Carbohydrate Chemistry* (edit. by Whistler, R. L.), **5**, 361 (Academic Press, New York, 1965).
[4] Saito, H., Misaki, A., and Harada, T., *Agric. Biol. Chem.* (Tokyo), **32**, 1261 (1968).

Self-annealing of Subgroup 2 Myxovirus RNAs

THE myxoviruses of subgroup 2, Newcastle disease virus (NDV)[1] and Sendai virus[2], have been shown to contain a homogeneous component of RNA which has a sedimentation coefficient ($S_{20,w}$) of 57S, and the properties of single stranded RNA. Cells infected with these viruses synthesize viral specific RNA, 70 to 90 per cent of which anneals exclusively with virion RNA to form highly ordered complexes suggesting that most of the intracellular RNA is complementary in base sequence to virion RNA[2-4]. Several distinct components of viral specific RNA are synthesized in infected cells and one of these (57S) is indistinguishable from virion RNA in sedimentation rate[2]. The 57S RNA in infected cells has been shown to be almost completely in the form of viral nucleocapsid (C. D. Blair and W. S. R., manuscript in preparation) and annealing studies have shown that this RNA component anneals extensively when incubated with virion RNA (unpublished results of W. S. R.). Because of this observation, annealing experiments were done with 57S RNA isolated from purified Sendai virus and Newcastle disease virus, and the results I report here indicate that a large fraction of the single stranded 57S RNA from these virusesself anneals.

The L-Kansas strain of NDV and the Harris strain of Sendai virus were grown in embryonated eggs[2]. Sendai virus was labelled with $^{32}PO_4$ in embryonated eggs and NDV and Sendai viruses were labelled with ^{3}H-uridine in tissue culture[2]. The viruses were purified as previously described[3] except that the concentrated virus was incubated with pancreatic ribonuclease (10 µg/ml.) and T_1 ribonuclease (1 µg/ml.) for 30 min at room temperature to digest all RNA not protected by the virus coat, before the final sucrose gradient sedimentation. Virion RNA was extracted as previously described[3] and the 57S viral RNA was further purified by sucrose density gradient sedimentation[1]. RNA fractions from the peak of the 57S band were used for annealing experiments.

Table 1. SELF-ANNEALING OF SENDAI AND NDV RNAS

Conditions	Radioactive RNA					
	57S Sendai				57S NDV	
	^{3}H		^{32}P		^{3}H	
	C.p.m.	Per cent	C.p.m.	Per cent	C.p.m.	Per cent
Unlabelled 57S Sendai RNA added to annealing mixture						
2·0 µg	956	43	—	—	69	4·9
1·0 µg	883	40	544	22		
0·2 µg	430	19				
0	98	4·5	130	5·4		
Unlabelled 57 S NDV RNA added to annealing mixture						
0·5 µg	96	4·4	95	3·9	305	22
0·25 µg					152	11
0					77	5·5
Unlabelled 18S ribosomal RNA from uninfected cells 10 µg	102	4·5	110	4·4	71	4·9
No annealing	64	2·9	36	1·4	39	2·8
RNA heat denatured before ribonuclease	36	1·6	25	1·0	35	2·5
Total radioactivity (no ribonuclease digestion)	2,216	100	2,431	100	1,396	100

For annealing, the radioactive and unlabelled RNAs were placed in 0·3 ml. buffer containing 0·1 M Tris · HCl (pH 7·4) and 0·1 M NaCl in sealed tubes in a water bath at 95° C and the bath was slowly cooled to 37° C over 12 h. One ml. of buffer containing 0·1 M Tris · HCl (pH 7·4), 0·1 M NaCl, 0·001 M $MgCl_2$, 13 µg pancreatic ribonuclease and 1 µg T_1 ribonuclease was added to each tube for incubation at 37° C for 1 h and then the trichloroacetic acid precipitable radioactivity was determined as previously described[3].

Table 1 shows the results of annealing radioactive NDV and Sendai virus RNAs with increasing amounts of the unlabelled viral RNA. The specific activities of the ^{3}H-labelled RNAs could not be precisely determined because of the small quantities available, but they were in excess of 50,000 c.p.m. per µg so that less than 0·04 µg of ^{3}H-labelled RNA was added to any annealing reaction. Increasing amounts of ^{3}H-labelled Sendai virus RNA became resistant to ribonuclease digestion when incubated in annealing conditions with increasing amounts of unlabelled Sendai virus RNA, and the plateau or maximum amount of labelled RNA that annealed was 43 per cent of the input radioactivity. When no unlabelled RNA was added to the reaction mixture, only a small amount of self annealing occurred (4·4 per cent of the ^{3}H-RNA became resistant to ribonuclease digestion). The same level of annealing occurred in the presence of heterologous NDV or of ribosomal RNAs, which indicates that the ^{3}H-labelled Sendai RNA does not anneal to NDV or ribosomal RNA. The viral RNA which had not been annealed was almost completely digested by ribonuclease in these experimental conditions suggesting that it has a single stranded structure[5-7].

Similarly, the ^{32}P-labelled Sendai virus RNA prepared in embryonated eggs and the ^{3}H-RNA from NDV grown in tissue culture annealed specifically in the presence of unlabelled RNA from the same virus but not with RNA from the heterologous virus. In several experiments with RNA from different preparations of Sendai virus, the plateau with excess unlabelled RNA was at the level of 20 to 60 per cent annealing of the radioactive RNA and the significance of this variability is not clear.

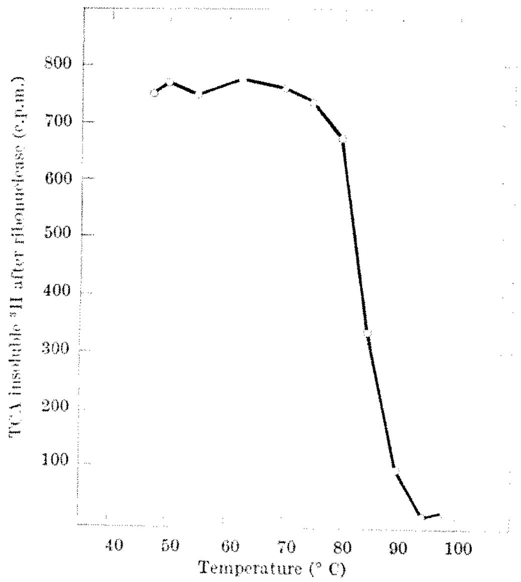


Fig. 1. Thermal transition of ribonuclease resistant product formed by self annealing viral RNA. Sendai virus RNA labelled with ^{3}H (21,000 c.p.m.) was annealed in the presence of 10 μg unlabelled Sendai virus RNA as described. The annealing mixture was then diluted 1 : 20 with water to a final salt concentration of 0·01 M. The entire solution was placed in a water bath at room temperature and the temperature increased in stages. At each of the points designated in the figure the temperature was held constant for 5 min and an aliquot of solution was removed and rapidly cooled in an ice bath. The water bath temperature was then increased. The radioactivity precipitable by trichloroacetic acid (TCA) in each aliquot after digestion with ribonuclease was determined as described in Table 1.

Fig. 1 shows the thermal transition of the ribonuclease resistant ^{3}H-RNA product of an annealing reaction with Sendai virus RNA to a ribonuclease susceptible form. The relatively sharp transition at approximately 85° C in 0·01 M salt indicates that the annealed RNA is in a highly ordered form consistent with base pairing of RNA strands with complementary base sequences[5-7].

The observation that the extent of annealing is greatly dependent on the concentration of viral RNA (Table 1) strongly suggests that intermolecular annealing rather than intramolecular annealing is occurring. This suggests that these viral RNAs consist of mixtures of two different RNA molecules which are partially or completely complementary in base sequence. The base compositions of the viral RNAs[1,2] indicate, however, that complementary sequences are not present in equal amounts.

The finding of the self annealing of these viral RNAs alters the interpretation of previous experiments showing that 70 to 90 per cent of the viral specific RNA synthesized in infected cells anneals to viral RNA[3,4]. Because both strands seem to be present in RNA recovered from virus it is not possible to determine the strandedness of the RNA synthesized in infected cells by annealing to viral RNA.

This work was supported by a US Public Health Service research grant.

WILLIAM S. ROBINSON

Department of Medicine,
Stanford University,
Stanford, California 94305.

Received August 25; revised November 14, 1969.

[1] Duesberg, P. H., and Robinson, W. S., *Proc. US Nat. Acad. Sci.*, **54**, 794 (1965).

[2] Blair, C. D., and Robinson, W. S., *Virology*, **35**, 537 (1968).

[3] Bratt, M. A., and Robinson, W. S., *J. Mol. Biol.*, **23**, 1 (1967).

[4] Kingsbury, D. W., *J. Mol. Biol.*, **18**, 204 (1966).

[5] Geiduschek, E. P., Moohr, J. W., and Weiss, S. B., *Proc. US Nat. Acad. Sci.*, **48** 1078 (1962).

[6] Robinson, W. S., Hsu, W. T., Fox, C. F., and Weiss, S. B., *J. Biol. Chem.*, **239**, 2944 (1964).

[7] Weissman, C., and Ochoa, S., in *Progress in Nucleic Acid Research* (edit. by Davidson, J. N., and Cohn, W. E.), **6**, 353 (Academic Press, New York, 1967).

Three Types of Virus Particle in *Penicillium stoloniferum*

IT has been demonstrated recently that virus particles and double stranded viral RNA isolated from *Penicillium stoloniferum* (ATCC 14586) are potent inducers of interferon in animals[1-3]. We now report that this strain of *P. stoloniferum* contains three polyhedral viruses: two serologically distinct particles of similar size (25–30 nm diameter) and small numbers of a third larger particle (40–45 nm diameter).

Virus preparations were obtained essentially as described previously[2], either from 2 day old mycelium or 5 day old culture filtrates, and were purified by rate zonal centrifugation on 10–45 per cent sucrose density gradients at 69,000*g* for 1·2 h. Only one virus specific band was obtained. When such preparations were examined on Ouchterlony gel diffusion plates against antiserum prepared previously[2] against a partially purified virus preparation two virus specific precipitin lines were formed. Electrophoresis of such preparations either on 0·5 per cent agarose gels or 4 per cent polyacrylamide gels (containing 0·04 per cent methylene *bis*-acrylamide) using TAE buffer (0·04 M Tris, 0·05 M acetate, 0·0016 M EDTA, *p*H 8·0) resulted in the formation of two well separated virus bands (slow and fast), which could be located by staining either for protein with amido black[4] or for RNA with acridine orange[5]. The slow band was more diffuse than the fast band and showed a tendency to separate into two components (Fig. 1). That the slow band was not an aggregate of the fast band was indicated by examination of unseparated preparations in the Beckman Model 'E' analytical ultracentrifuge using the ultraviolet scanner. Two incompletely separated boundaries were observed, both having sedimentation coefficients in the range 90–110S. Greater differences in *s* values would be anticipated between monomeric and aggregated forms.

The slow and fast viruses were separated by preparative electrophoresis on columns of 0·5 per cent agarose at

pH 8·0 (0·05 M Tris-HCl buffer) using 16 V/cm for 1 h. The two virus bands, located by staining a portion of the gel as described here, were cut out and the virus particles were eluted by vigorous stirring with 0·03 M sodium phosphate buffer, pH 7·6. Viruses from each band were examined with the electron microscope using negative staining with phosphotungstic acid, and found to consist of polyhedral particles, both of similar diameter (25–30 nm). In addition, the slow virus preparation contained small numbers of larger polyhedral particles (40–45 nm diameter).

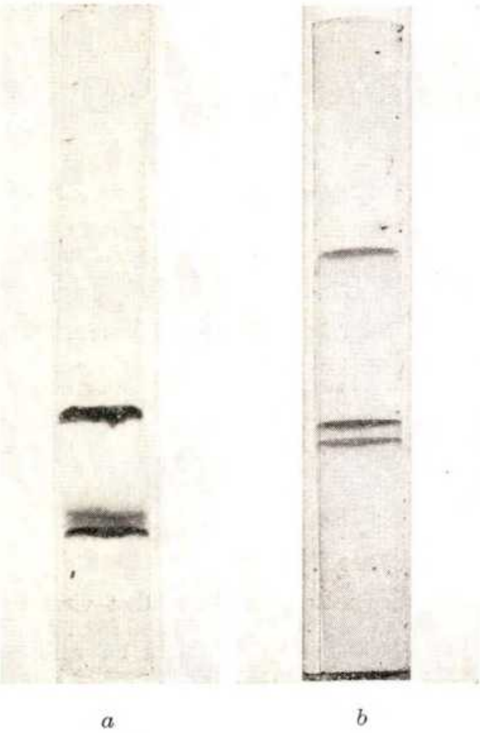


Fig. 1. Polyacrylamide gel electrophoresis in TAE buffer. Anode at top of gel. 6 mA/tube (internal diameter 5 mm). *a*, Unseparated *P. stoloniferum* virus (2·5 h) stained with amido black; *b*, RNA prepared from *a* (1·6 h) stained with acridine orange.

The two smaller viruses were serologically distinct. In gel diffusion tests, using the antiserum described here, two precipitin lines were obtained, both with the unfractionated virus preparations and with mixtures of the separated viruses. Separately the slow and fast viruses each yielded a single precipitin line. The two lines crossed over each other indicating non-identity of the two immunogens (Fig. 2). Examination of each precipitin line electron microscopically revealed the presence of only the smaller virus particles (25–30 nm diameter). The larger particles (40–45 nm diameter) did not give rise to a precipitin line with the antiserum used, and further investigation is required to determine if they are serologically related to either of the two smaller viruses.

Previous work has established that the viral RNA from *P. stoloniferum* is double stranded[2,3] with a sedimentation coefficient of 10–12S. Because this RNA was prepared without separation of the individual viruses it was suspected that it may have been a mixture. RNA prepared from the natural mixture of the three viruses by the phenol method[6] has now been examined by polyacrylamide gel electrophoresis (4 per cent acrylamide, 0·04 per cent methylene *bis*-acrylamide, TAE buffer) and found to give three principal bands (Fig. 2). Further characterization of the RNAs from the individual viruses is in progress.

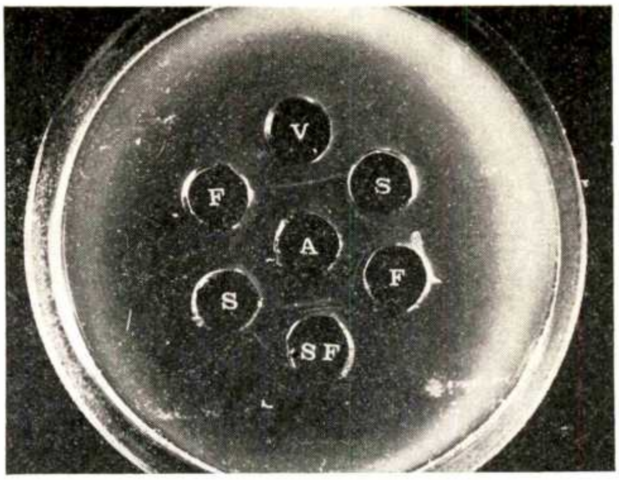


Fig. 2. Serological gel-diffusion plate. A, Antiserum; V, unseparated virus; F, fast virus; S, slow virus.

We thank Professor Sir Ernst Chain for his interest and advice, Mr G. T. Banks for pilot plant fermentations and Mrs Joan E. Darbyshire for electron microscopy.

K. W. BUCK
GILLIAN F. KEMPSON-JONES

Department of Biochemistry,
Imperial College of
Science and Technology,
London SW7.

Received January 2, 1970.

[1] Ellis, L. F., and Kleinschmidt, W. J., *Nature*, **215**, 649 (1967).

[2] Banks, G. T., Buck, K. W., Chain, E. B., Himmelweit, F., Marks, J. E., Tyler, J. M., Hollings, M., Last, F. T., and Stone, O. M., *Nature*, **218**, 542 (1968).

[3] Kleinschmidt, W. J., Ellis, L. F., Van Frank, R. M., and Murphy, E. B., *Nature*, **220**, 167 (1968).

[4] Smith, I., *Chromatographic and Electrophoretic Techniques*, **2**, 382 (Heinemann, London, 1968).

[5] Richards, E. G., Coll, J. A., and Gratzer, W. B., *Anal. Biochem.*, **12**, 452 (1965).

[6] Franklin, R. M., *Proc. US Nat. Acad. Sci.*, **55**, 1504 (1966).

Autoradiographic Detection of Molecular Hybrids between rRNA and DNA in Tissue Sections

WE describe here a method for DNA–RNA hybridization which permits one to localize, at the microscopical level, molecular hybrids formed between a purified ^{3}H-labelled RNA species and DNA in the cell structures of tissue sections.

Chinese hamster cells of the CHEF 125 strain were grown in monolayer cultures in Eagle's minimal essential medium supplemented with 10 per cent calf serum. In order to label the rRNA to a high specific activity, ^{3}H-5-uridine (Radiochemical Centre, Amersham, 19 Ci/mmole) was added to the cultures at 30 µCi/ml. for three generations; a half-generation chase with non-radioactive uridine followed. For the same purpose the *Escherichia coli* 500 *Hfr U*$^-$ uracil requiring strain was grown for several generations in synthetic medium[1] in the presence of ^{3}H-5-uridine (10 Ci/mmole; 20 µCi/ml.); a hundred-fold excess of non-radioactive uridine was then added for about half a generation. Hamster rRNA was extracted from total cells and purified by sucrose gradient centrifugation[2]. *E.*

coli rRNA was extracted from cells, by grinding with alumina, following the same procedure.

The hamster ^{3}H-RNA (equimolar mixture of 28S and 18S components) studied here has been tested in conventional hybridization experiments with purified DNA extracted from hamster cells. The temperature and time of incubation (65° C for 14 h) and ribonuclease treatment of the hybrids (10 μg/ml. at room temperature for 1 h) were the same as those for the hybridization experiments with tissue sections. In these conditions, saturation was reached at an RNA concentration of about 2 μg/ml.; about 0·02 per cent of the DNA was hybridized at saturation. ^{3}H-rRNA from *E. coli* hybridized negligibly with hamster DNA, but the expected level of hybridization (0·2 per cent at saturation) was obtained with DNA from *E. coli*.

To minimize the effects of the fixative on the DNA structure, golden hamster brain and liver were usually fixed for 3 h in a mixture of absolute alcohol and chloroform (2 : 1); in some cases 10 per cent acetic acid was added. The tissues were then embedded in paraffin and sectioned at 5 μm. Following removal of the paraffin and hydration, the sections were mounted on slides with glycerin–albumin or collected on membrane filters (Millipore, *GS* type, pore size 0·22 μm).

Denaturation of DNA was carried out by heating the tissue sections, on filters or slides, in SSC/10 at 100° C for 10 min (SSC = 0·15 M NaCl–0·015 M sodium citrate). Annealing was then performed at 65° C for 14 h in 2 × SSC containing 3 μg/ml. of purified ^{3}H-rRNA from Chinese hamster (1,250,000 d.p.m./μg). From then on the sections on filters were treated as described by Gillespie and Spiegelman[3] for conventional DNA–RNA hybridization. The specimens were thoroughly washed by filtration with 2 × SSC and then treated with ribonuclease (10 μg/ml.) for 1 h at room temperature in 2 × SSC. After another washing, by filtration with 6 × SSC, the specimens were air-dried and prepared for autoradiography. For sections on slides the same procedure was followed except that the washing was performed by several changes of SSC solutions. After the hybridization procedure, the sections were air-dried and the filters glued on slides. All slides were dipped in undiluted Kodak *NTB* 2 emulsion, air-dried and exposed in a dry atmosphere at 0°–4° C for periods from 20 to 60 days. Processing was carried out with Kodak *D*-19 developer. The slides were usually stained with haematoxylin. The sections, attached directly to slides, were mounted with a coverslip. The membrane filters were air-dried and clarified with immersion oil.

Microscopic examination of the autoradiographs obtained in this way revealed that most nuclei were labelled. In brain sections this labelling was chiefly restricted to the nucleolar regions (Fig. 1*C*). Neural cells are particularly suitable for this kind of observation because of the considerable size of their nuclei and nucleoli (Fig. 1*A*). Similarly, liver cells showed a grain concentration in the nucleoli (Fig. 1*D*) but silver grains sometimes appeared also in other nuclear regions. This may result from the presence of several smaller nucleoli (Fig. 1*B*) in these nuclei which are known frequently to be polyploid. In all cases the cytoplasmic labelling was very weak or absent, especially in the case of sections on filters. This is probably because the washing by filtration is more efficient. On the other hand, the nuclear labelling was greater in sections mounted on filters, probably because of the binding of the denatured DNA to the filters which might result in a decrease in the loss of DNA from sections and in less renaturation of DNA. For these reasons and for its simplicity the filter method was preferred in our experiments.

To verify that the binding of ^{3}H-RNA to the nuclear structures was the consequence of specific DNA–RNA hybridization, a heterologous radioactive rRNA (*E. coli* ^{3}H-rRNA; 1,200,000 d.p.m./μg) was used in hybridization experiments on tissue sections following the method described. Fig. 1*E* and 1*F* demonstrate that there is no labelling in the cells. Further evidence for the specificity of the hybrids obtained in tissue sections comes from competition experiments. The annealing reaction was carried out as described but in the presence of non-radioactive RNA in a ten-fold excess with respect to hamster ^{3}H-rRNA. When the non-radioactive RNA was homologous (hamster rRNA) to the radioactive RNA, the labelling of the nuclei and nucleoli was drastically diminished, while the addition of non-radioactive heterologous RNA (*E. coli* rRNA) lowered the nuclear and nucleolar labelling only slightly.

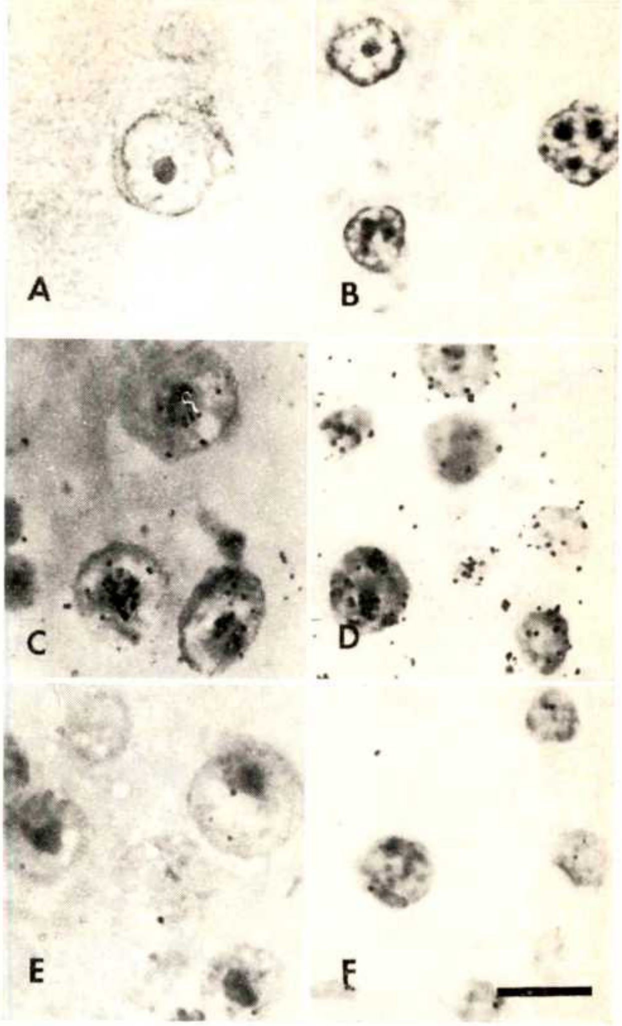


Fig. 1. Sections from hamster brain (*A*) and liver (*B*) fixed in alcohol–chloroform and stained with haematoxylin. Autoradiographs (exposure time 30 days) of brain (*C* and *E*) and liver (*D* and *F*) sections after hybridization with hamster ^{3}H-rRNA (*C* and *D*) and *E. coli* ^{3}H-rRNA (*E* and *F*). Fixation and staining as for (*A*) and (*B*). The bar represents 10 μm.

In all the experiments we have described the ribonuclease treatment following the hybridization was performed at a concentration of 10 μg/ml. at room temperature for 1 h. The suppression of this step resulted in a net increase of the grain number in the nuclei and nucleoli; a ribonuclease concentration of 100 μg/ml. (1 h at room temperature) reduced partially (30–50 per cent) the labelling as compared with the 10 μg/ml. ribonuclease treatment. In experiments with *E. coli* ^{3}H-rRNA, even in the absence of ribonuclease treatment, the grain count was at background level.

If the DNA thermal denaturation step (100° C in SSC/10 for 10 min) is omitted some hybridization still occurs on alcohol–chloroform fixed tissues. It is possible that the prolonged heating at 65° C during the annealing incubation is sufficient to loosen somewhat the DNA structure and to allow a certain degree of hybridization; a more likely explanation is that, during the histological procedure for paraffin embedding, conditions are such as to cause DNA denaturation. When there was acetic acid in the fixative solution, the hybridization seemed to be about the same with or without the thermic denaturation step, probably because of the denaturing action of acetic acid.

The number of silver grains found in hybridization experiments with hamster ^{3}H-rRNA averaged fifty per nucleus in 30 days' exposure time, when the 10 μg/ml. ribonuclease treatment was adopted. There is, however, considerable variability—from ten to ninety grains per nucleus—because only part of the nucleus is present in each section. These values are of the order of magnitude expected on the basis of the number of rRNA genes per cell, the specific activity of the ^{3}H-rRNA used and the efficiency of the autoradiographic method (about 100 grains per nucleus in 30 days' exposure time).

While this manuscript was in preparation two articles by Gall and Pardue[4] and by John, Birnstiel and Jones[5] appeared which describe similar *in situ* hybridization techniques.

We thank Professor A. Lima-de-Faria for assistance in the preparation of this manuscript.

M. Buongiorno-Nardelli

Istituto di Istologia ed Embriologia,
Facoltà di Scienze,
Università di Roma,
Italy.

F. Amaldi

Centro degli Acidi Nucleici del C.N.R.,
Istituto di Fisiologia Generale,
Università di Roma,
Italy.

Received August 8; revised October 14, 1969.

[1] Cohen, S. S., *J. Biol. Chem.*, **177**, 607 (1949).

[2] Attardi, G., Parnas, H., Hwang, M.-I. H., and Attardi, B., *J. Mol. Biol.*, **20**, 145 (1966).

[3] Gillespie, D., and Spiegelman, S., *J. Mol. Biol.*, **12**, 829 (1965).

[4] Gall, J. G., and Pardue, M. L., *Proc. US Nat. Acad. Sci.*, **63**, 378 (1969).

[5] John, H. A., Birnstiel, M. L., and Jones, K. W., *Nature*, **223**, 582 (1969).

Rare Bases in Animal DNA

The rare bases which occur in animal DNA deserve special attention because their presence is directly relevant to questions concerning the cellular, subcellular and species specificity of DNA methylation. In animal DNA, the base 5-methylcytosine (5-MeCyt) is usually detected[1,2]. Numerous attempts to find N^6-methyladenine (6-MeAde)—which is characteristic of the DNA of various microorganisms[3-5]—in animal DNA proved unsuccessful[3,6-9]. This may be accounted for by the fact that animal cells do not contain the enzymes required for methylating DNA at adenine residues[7-9]. Nevertheless, 6-MeAde and certain N^2-methylated derivatives of guanine have been recently discovered in appreciable amounts in bovine and human sperm DNA[10]. Our present knowledge on the specificity of DNA methylation in animal cells is not only scanty but also contradictory.

We report here a comparative study of the content of 5-MeCyt in the DNA of invertebrate and vertebrate animals belonging to different taxonomic groups. Further, we have studied the degree of methylation of the DNA in the sperm and different somatic tissues of the same animal. Special attention has been given to the detection of 6-MeAde in animal DNA.

DNA preparations from different cells and tissues[11,12] were hydrolysed (72 per cent $HClO_4$, 1 h, 100°) and bases were separated by paper chromatography as described earlier[4].

5-MeCyt was found in the DNA of all animals studied regardless of their taxonomic position (Table 1). The DNA of *Helix pomatia* is somewhat different from that of other molluscs[2] with respect to the content of 5-MeCyt. DNA preparations from the sperm cells of various species of sea urchin differ: the quantity of 5-MeCyt in the DNA of *Strongylocentrotus intermedius* is three-fold less than that in the DNA of *Echinus esculentus*[1]. The content of 5-MeCyt in the DNA of various species of mammals (somatic cells of rabbit and bull) also seems to be different but not so markedly. It is of interest that the DNAs of the rodents studied (rat[1], rabbit, mouse) are similar and have lower 5-MeCyt contents as compared with other mammals. A large proportion of 5-MeCyt is specific for fish DNA (about 1·5–2 moles per 100 moles).

Table 1. CONTENT OF 5-METHYLCYTOSINE IN DIFFERENT ANIMAL DNA

Source of DNA	Moles per 100 moles			
	5-MeCyt			G + C +
	N	(σ)	n	5-MeCyt
Invertebrates				
Porifera				
Suberites domuncula: whole animal	1·3		3	38·5
Coelenterata				
Metridium senile: whole animal	0·9		4	39·0
Mollusca				
Helix pomatia: liver	0·6		5	41·0
Echinodermata				
Strongylocentrotus intermedius: sperm	0·6		4	41·4
Vertebrates				
Osteichthyes				
Cottus sp: sperm	1·7		4	41·0
Trichiurus japonicus: sperm	1·5		3	41·3
Spheroides sp: sperm	1·5		3	41·0
Cyprinus carpio (carp): liver	1·41	0·02	7	38·6
sperm	1·17	0·02	7	38·6
Oncorhynchus gorbuscha (salmon): muscles	1·80	0·03	9	44·0
liver	2·13	0·06	9	44·1
kidney	1·98	0·03	9	44·0
spleen	2·05	0·06	9	44·0
sperm	1·64	0·04	6	44·0
Amphibia				
Rana temporaria: liver	1·6		3	41·0
red cells	1·5		3	
Reptilia				
Testudo horsfieldi: liver	1·5		4	44·8
Mammalia				
Mouse: spleen	1·0		4	42·2
liver	1·1		4	42·2
Rabbit: liver	0·91		4	42·6
kidney	0·93		4	
spleen	0·88		4	
Pig: kidney	1·20	0·07	7	44·0
spleen	1·10	0·11	8	44·1
sperm	0·77	0·03	8	44·1
Sheep: liver	1·13	0·09	6	42·6
kidney	1·15	0·05	6	42·8
spleen	1·07	0·06	8	42·4
sperm	0·76	0·02	6	42·0
Bull: liver (bull I)	1·40	0·10	6	44·0
kidney (bull I)	1·30	0·07	6	44·0
lung (bull I)	1·38	0·02	6	44·0
spleen (bull I)	1·66	0·02	6	44·0
spleen (bull II)	1·39	0·07	6	44·1
spleen (bull III)	1·07	0·02	5	44·0
kidney (bull II)	1·34	0·02	6	44·0
sperm	0·75	0·06	11	44·2

N = Mean or $(\bar{x})$; σ = standard deviation or $\sigma\{x\}$; n = number of determinations.

We observe that the content of 5-MeCyt of DNA varies from 0·5 to 2 moles per 100 moles depending on the species. Taking into consideration that some insects and Crustacea have about 0·1 mole per 100 moles of 5-MeCyt in their DNA (refs. 1, 2), it may be stated that in general the degree of methylation of animal DNA differs more than twenty-fold. This favours a marked species specificity in the methylation of animal DNA. As a rule, the more distant the species are taxonomically, the greater is the difference of the proportion of 5-MeCyt in their DNA. Animal DNA is less methylated than that of higher plants[13], but in animal DNA the variability of 5-MeCyt content is much greater within a particular species. In animal DNA, 5-MeCyt occurs predominantly in Pu-m^{5}C-Pu or Pu-m^{5}C-G sequences[9,14] and is evidently concentrated chiefly in GC-rich DNA. The possibility that the observed differences in 5-MeCyt content may be ascribed not only to the specificity of DNA methylases but also to the peculiarities of the genome structure in different animals cannot therefore be entirely excluded.

Of special interest for the study of methylation specificity is the search for other rare bases in animal DNA, particularly 6-MeAde. We failed to detect 6-MeAde in hydrolysates of DNA from different invertebrate and vertebrate animals by either paper chromatography or ultraviolet spectrophotometry. We have found it only in the DNA of total sponge mass, without actually isolating individual cells from this animal: it is therefore possible that the 6-MeAde arose from the DNA of symbiotic organisms—bacteria or especially algae, which have a substantial amount of this base in their DNA (ref. 5). We did not find it in the sperm DNA of mammals (bull, boar, sheep) or fish (salmon, carp), analysing quantities of these DNA which after hydrolysis and base separation yielded up to 100 μmoles of adenine. This means that even if 6-MeAde does exist in the DNA of animals, its quantity—by contrast with the data of Unger and Venner[10]—in the sperm DNA of the bull or other mammals and fish does not exceed 0·01–0·03 moles per 100 moles of adenine.

We also tried to detect 6-MeAde in animal DNA by means of radioactivity assay. After introducing 8-^{14}C-adenine (specific activity 5·5 Ci/mole) into different animals (mouse, frog, chicken embryo) labelled adenine is incorporated into the various DNAs (up to 2×10^5 c.p.m./μmole adenine of DNA) but no appreciable radioactivity in the paper chromatographic areas corresponding to 6-MeAde and N^6-dimethyladenine was detected. Adenine is not methylated to any appreciable degree, therefore, and 5-MeCyt seems to be the only rare base found consistently in all animal DNA.

DNA preparations from different somatic cells of the same animal are similar in total base composition and degree of methylation. Nevertheless, in bovine spleen DNA the content of 5-MeCyt can vary markedly in different bulls (for example, bull I and bull III in Table 1). In bovine DNA from other organs (for example, kidney) no fluctuation in the 5-MeCyt content was observed. No individual variation in the degree of DNA methylation in spleen and other organs of the rabbit was noted. As is evident from Table 1, spleen DNA can be partially undermethylated (bull III). This might account for the fact that rat liver DNA is incapable of being methylated *in vitro* by homologous enzyme[7] whereas spleen DNA can accept methyl groups in the presence of spleen DNA methylase[8]. Generally, however, the spleen DNAs of all the animals studied so far are methylated more or less normally as compared with DNA from other tissues, in spite of the fact that spleen DNA methylase may have a very low activity[7].

Changes of degree of methylation with age, in different animal tissues, are also of interest. We find a decrease in 5-MeCyt content in the DNA of all salmon somatic tissues in the course of spawning migration[15]. No noticeable accompanying changes (including degradational ones) are evident in the primary and secondary structure of salmon DNA (ref. 16). We suggest that this decrease in 5-MeCyt during spawning migration may result from the diminution of DNA methylase activity or from the enzymatic demethylation of DNA, or both.

We have found that sperm DNA contains less 5-MeCyt as compared with that of somatic cells. This is observed regularly both in fish and mammals. The 5-MeCyt content of the sperm DNA of different mammals is similar; the 5-MeCyt/cytosine ratio in sperm cells is usually 0·037 (mean value) and in somatic cells, 0·06–0·07. A lower degree of methylation of sperm DNA as compared with that of the somatic cells of vertebrates may be explained by at least two factors: (1) sperm DNA contains fewer sequences capable of accepting methyl groups, or (2) sperm DNA is partially undermethylated. The first alternative cannot be ruled out but it is, in our opinion, less probable, because the DNA of sperm and somatic cells have practically identical total base compositions (Table 1), frequencies of different pyrimidine clusters[17] and nearest neighbour base sequences[18]. These DNA therefore have similar nucleotide sequences and closely related molecular populations. In this connexion we believe the sperm DNA of vertebrates to be partially undermethylated and that the deficiency of methyl groups is made up on or after oocyte fertilization.

B. F. VANYUSHIN
S. G. TKACHEVA
A. N. BELOZERSKY

Laboratory of Bioorganic Chemistry,
Moscow State University,
Moscow, USSR.

Received April 25; revised September 23, 1969.

[1] Wyatt, G. R., *Biochem. J.*, **48**, 584 (1951).

[2] Antonov, A. S., Favorova, O. O., and Belozersky, A. N., *Dokl. Akad. Nauk SSSR*, **147**, 1480 (1962).

[3] Dunn, D. B., and Smith, J. D., *Biochem. J.*, **68**, 627 (1958).

[4] Vanyushin, B. F., Belozersky, A. N., Kokurina, N. A., and Kadirova, D. X., *Nature*, **218**, 1066 (1968).

[5] Pakhomova, M. V., Zaitseva, G. N., and Belozersky, A. N., *Dokl. Akad. Nauk SSSR*, **182**, 712 (1968).

[6] Brown, G. M., and Attardi, G., *Biochem. Biophys. Res. Commun.*, **20**, 298 (1965).

[7] Sheid, B., Srinivasan, P. R., and Borek, E., *Biochemistry*, **7**, 280 (1968).

[8] Kalousek, F., and Morris, N. R., *J. Biol. Chem.*, **244**, 1157 (1969).

[9] Grippo, P., Iaccarino, M., Parisi, E., and Scarano, E., *J. Mol. Biol.*, **36**, 195 (1968).

[10] Unger, G., and Venner, H., *Z. Physiol. Chem.*, **344**, 280 (1966).

[11] Marmur, J., *J. Mol. Biol.*, **3**, 208 (1961).

[12] Kirby, K. S., *Biochim. Biophys. Acta*, **36**, 117 (1959).

[13] Vanyushin, B. F., and Belozersky, A. N., *Dokl. Akad. Nauk SSSR*, **129**, 944 (1959).

[14] Doscocil, J., and Sorm, F., *Biochim. Biophys. Acta*, **55**, 953 (1962).

[15] Berdishev, G. D., Korotaev, G. K., Boyarskikh, G. V., and Vanyushin, B. F., *Biokhimia*, **32**, 988 (1967).

[16] Vanyushin, B. F., Korotaev, G. K., Mazin, A. L., and Berdishev, G. D., *Biokhimia*, **34**, 201 (1969).

[17] Mazin, A. L., Vanyushin, B. F., and Korotaev, G. K., *Dokl. Akad. Nauk SSSR*, **186**, 1434 (1969).

[18] Schwartz, M. N., Trautner, T. A., and Kornberg, A., *J. Biol. Chem.*, **237**, 1961 (1962).

Association of Murine Leukaemia Virus from a Mouse Lymphoma (2731/L) with Reovirus Type 3 Infection

STANLEY *et al.* reported the production of a lymphoma (2731/L) in Prince Henry (PH) mice after inoculation with spleen cells from a PH mouse with chronic (272 days) reovirus type 3 infection[1]. They suggested a relationship between reoviruses and the tumour. From recent data on the prevalence of murine leukaemia virus in most mouse strains[2], we would conclude that lymphomas induced in mice can, with considerable certainty, be attributed to murine leukaemia (C-type RNA) viruses in the passage materials. We have therefore tested for the presence of a murine leukaemia virus in the 2731/L tumour as well as other mouse lymphomas developing after various procedures (Table 1) in Stanley's PH line.

Primary NIH Swiss mouse embryo cells were used for tissue culture isolation; they were not found to harbour any murine leukaemia virus antigen[2]. BALB/c embryo cultures and Fisher rat embryo cultures (supplied by Microbiological Associates, Bethesda) were also used to test virus host specificity. All cells were pretreated with DEAE cellulose to increase the sensitivity of the assay[3]. The methods of inoculation and maintenance of the cultures have been previously described[2,4]. Complement fixation testing for the presence of leukaemia virus antigen was conducted under the direction of Horace C. Turner[4]. Four out of five PH mouse tumours (including the 2731/L tumour), kindly provided as 10 per cent suspensions by Dr Stanley, contained a murine leukaemia virus. On one occasion the virus was detected only after a blind passage of the originally inoculated cells. No foci of alteration in the tissue culture suggesting a sarcoma virus were seen[5]. In one specimen (LA5/T) polyoma virus was also isolated and identified by specific haemagglutination inhibition testing using guinea-pig red blood cells. Neutralization experiments were done on three isolates and all were found to have Gross–AKR type specific coat (Table 1). This finding is consistent with those of Hartley *et al.* who found all naturally occurring leukaemia viruses to be members of this subgroup[2]. The host specificity of the virus is consistent with many of the Gross–AKR type isolates which grow more readily in NIH Swiss cells than in BALB/c or rat embryo cells (J. W. Hartley, W. P. Rowe, R. J. H., in preparation). One isolate, for example, had a titre more than two logs higher in NIH Swiss than in BALB/c embryo cultures. The failure of the viruses isolated from the 2731/L and 553/L specimens to grow in the BALB/c and rat embryo cells is probably the result of titres too low to become established in the more resistant cells. In none of the original specimens could reovirus type 3 be found either by haemagglutination tests, complement fixation tests, or the mouse antibody production and rat antibody production tests (performed by Drs Michael Collins and John Parker, Microbiological Associates) (J. Parker, in preparation).

Table 1. ISOLATION OF LEUKAEMIA VIRUSES FROM TUMOURS OF PH MICE

Tumour No.	Method of tumour induction	Embryo cell cultures: NIH Swiss mouse	BALB/c mouse	Fisher rat	Virus identity	Other viruses isolated
2731/L	Inoculation of spleen cells from reovirus runt	+	–	–	Gross-AKR	–
553/L	Inoculation of spleen cells from graft versus host animal	+*	–	–		–
4261/T	Reovirus type 3 infection	–	–	–		–
1175/T	Endotoxin inoculation	+	+*	+*	Gross-AKR	–
LA5/T	X-irradiation	+	+*	+*	Gross-AKR	Polyoma

* Positive only after blind passage of original tissue culture harvests.

Keast and Stanley note that 52 per cent of the PH line develop lymphomas after irradiation[6]. The isolation of a Gross–AKR type leukaemia virus from one of these irradiated tumours, as well as other tumours induced in this PH line (Table 1), strongly suggests that this mouse strain does harbour a leukaemia virus. One would expect that as animals grow older, similar to the findings in BALB/c mice, they would have a greater frequency of virus detectable in the spleen[2]. It is therefore our impression that the mouse with chronic reovirus infection had by 272 days murine leukaemia virus in its spleen cells and inoculation of these cells into infant mice led to the 2731/L tumour induced by this C-type RNA virus.

The runting syndrome which accompanied the reovirus infection may have helped in the emergence of the leukaemia virus because the normal immune mechanism is affected[7]. In fact the tumours occurring in the runting mice of Schwartz and Beldotti[7] were probably also induced by a murine leukaemia virus. It is possible that reovirus type 3 played some part in activating or potentiating the leukaemia virus genome, similar to the findings reported with guaroa virus[8]. Reovirus type 3, however, has not enhanced the activity of other viruses[9]. Preliminary attempts to increase leukaemia virus production or expression in tissue culture with reovirus type 3 have been negative. Reovirus antigen present in the 2731/L tumour as reported by Stanley *et al.*[10] may then represent a latent carrier state of reovirus type 3 (ref. 8).

JAY A. LEVY
ROBERT J. HUEBNER

Viral Carcinogenesis Branch,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014.

Received October 14, 1969.

[1] Stanley, N. F., Walters, M. N-I., Leak, P. J., and Keast, D., *Proc. Soc. Exp. Biol. and Med.*, **121**, 90 (1966).
[2] Hartley, J. W., Rowe, W. P., Capps, W. I., and Huebner, R. J., *J. Virol.*, **3**, 126 (1969).
[3] Duc-Nugent, H., *J. Virol.*, **2**, 643 (1968).
[4] Hartley, J. W., Rowe, W. P., Capps, W. I., and Huebner, R. J., *Proc. US Nat. Acad. Sci.*, **53**, 931 (1965).
[5] Hartley, J. W., and Rowe, W. P., *Proc. US Nat. Acad. Sci.*, **55**, 780 (1966).
[6] Keast, D., and Stanley, N. F., *Proc. Soc. Exp. Biol. and Med.*, **122**, 1091 (1966).
[7] Schwartz, R. S., and Beldotti, L., *Science*, **149**, 1511 (1965).
[8] Turner, W., Chirigos, M. A., and Scott, D., *Cancer Res.*, **28**, 1064 (1968).
[9] Levy, J. A., Henle, G., Henle, W., and Zajac, B. A., *Nature*, **220**, 607 (1968).
[10] Stanley, N. F., and Keast, D., *Austral. J. Exp. Biol. Med. Sci.*, **45**, 517 (1967).

Histocompatibility and Human Placentation

HISTOCOMPATIBILITY antigens were recently suggested by Jones[1] to affect human placentation. His observation was based on data of maternal ABO blood groups and placental weights. The immunological disparity of the mother and her foetus was found to be associated with a relatively smaller placenta and vice versa. The expected proportion of ABO incompatible pregnancies for each maternal blood group was calculated from the ABO gene frequencies in the population[2].

Further studies on the influence of ABO incompatibility on human placentation were carried out by determining the maternal and infant ABO blood groups from 3,045 mother–child pairs. Rh-immunized mothers were excluded. These pairs were classified as ABO compatible, pseudocompatible and incompatible in primiparous and multiparous groups (Table 1). Placental and infant's birth weights and the duration of gestation were recorded. A comparison similar to that of Jones was made between maternal blood groups and placental weight.

According to the results in this series placental weights were not affected by ABO blood group compatibility or incompatibility between the mother and her child in either primiparous or multiparous groups (Table 1). No effect of ABO incompatible pregnancy on the weight of the child (Table 2) or the duration of gestation (Table 3) could be observed. Contrary to the observation of Jones, the mean placental weight of blood group O mothers was not lower than that of combined groups A, B and AB mothers (Table 4).

In human trophoblastic tumours a shift of the host's blood group distribution away from group O towards

Table 1. ABO BLOOD GROUP COMPATIBILITY AND MEAN PLACENTAL WEIGHT IN 3,030 MOTHER–CHILD PAIRS

Family	Mean placental weight (g) and No. of pairs: Compatible (O–O, A–A, B–B, AB–AB)	Pseudocompatible (A–O, B–O, AB–A, AB–B)	Incompatible (O–A, O–B, A–B, B–A, A–AB, B–AB)
Primiparae	605·7 (775)	595·4 (265)	610·9 (335)
Multiparae	630·2 (933)	635·3 (311)	635·3 (411)
Total	619·1 (1,708)	617·0 (576)	624·3 (746)

Table 2. ABO BLOOD GROUP COMPATIBILITY AND BIRTH WEIGHT OF THE CHILD IN 3,080 MOTHER–CHILD PAIRS

Family	Mean birth weight of the child (g) and No. of pairs: Compatible (O–O, A–A, B–B, AB–AB)	Pseudocompatible (A–O, B–O, AB–A, AB–B)	Incompatible (O–A, O–B, A–B, B–A, A–AB, B–AB)
Primiparae	3406·7 (771)	3416·1 (265)	3396·3 (327)
Multiparae	3546·3 (939)	3547·9 (353)	3549·5 (425)
Total	3483·4 (1,710)	3491·0 (618)	3482·9 (752)

Table 3. ABO BLOOD GROUP COMPATIBILITY AND DURATION OF GESTATION (WEEKS) IN 3,045 MOTHER–CHILD PAIRS

Family	Compatible (O–O, A–A, B–B, AB–AB)	Pseudocompatible (A–O, B–O, AB–A, AB–B)	Incompatible (O–A, O–B, A–B, B–A, A–AB, B–AB)
Primiparae	39·9 (774)	40·3 (262)	40·0 (335)
Multiparae	40·0 (922)	40·1 (345)	40·1 (407)
Total	40·0 (1,696)	40·2 (607)	40·0 (742)

Table 4. MEAN PLACENTAL WEIGHT ACCORDING TO MATERNAL ABO BLOOD GROUP

Maternal blood group	No. of cases Primiparae	No. of cases Multiparae	Mean placental weight Primiparae	Mean placental weight Multiparae
O	404	517	612·5	637·3
A	650	778	595·6*	631·0*
B	231	255	595·4*	633·0*
AB	102	138	585·1*	649·1*

* Mean placental weight of combined groups A, B and AB is 594·4 in the primiparous group and 633·6 in the multiparous group. Both values are smaller than that of the respective group O mothers.

A, B and AB has been observed[3,4] indicating a favourable influence of histocompatibility between the host and the tumour in the aetiology of the disease. The same is reflected by similarity in HL-A antigens[5] and by the elevated incidence of choriocarcinoma in women with consanguinous reproductive partners[6,7]. But in the growth of normal human trophoblast an analogous effect has not been found. An electronegative sialomucin coat on human trophoblast may protect the trophoblast from the immunological attack of the host's negatively charged lymphocytes[8].

MARKKU SEPPÄLÄ

Department of Gynaecology and Obstetrics,
University Central Hospital,
Helsinki,
and
Department of Serology and Bacteriology,
University of Helsinki.

MATTI TOLONEN

Department of Gynaecology and Obstetrics,
University Central Hospital,
Helsinki.

Received October 13, 1969.

[1] Jones, W. R., *Nature*, **218**, 480 (1968).

[2] Dobson, A. M., and Ikin, E., *J. Path. Bact.*, **48**, 221 (1946).

[3] Llewellyn-Jones, D., *J. Obst. Gynaec. Brit. Comm.*, **72**, 242 (1965).

[4] Scott, J. S., *Amer. J. Obst. Gynec.*, **83**, 185 (1962).

[5] Mogensen, B., and Kissmeyer-Nielsen, F., *Scand. Symp. Immunol.*, 19 (Swedish Soc. Microbiol., Stockholm, 1969).

[6] Azar, H. A., *Cancer*, **15**, 66 (1962).

[7] Iliya, F. A., Williamson, S., and Azar, H. A., *Cancer*, **20**, 144 (1967).

[8] Currie, G. A., and Bagshawe, K. D., *Lancet*, i, 708 (1967).

Effect of Heparin on the Detection of Human Isoantigens by the Cytotoxicity Technique

IT has been suggested that the anticoagulant heparin might block antigenic sites or otherwise interfere with the expression of antigen–antibody interaction[1-6]. Of particular relevance is the effect of heparin on transplantation behaviour and cytotoxicity reactions in mice[7,8], and on human mixed lymphocyte cultures[9]. We have studied the possible influence of heparin on human isoantigen typing by the cytotoxicity technique.

Two human lymphoid tissue culture lines, IM-1 and RPMI 4265, were used as target cells in the cytotoxicity reactions[10]. IM-1 cells were washed twice in Eagle's medium with 10 per cent foetal bovine serum and incubated as in the procedure of Lippman[7] with 0–1,000 μg sodium heparinate (Hynson, Westcott and Dunning) per 10^6 cells in 0·3 ml. medium for 20 min at 37° C. The cells were washed twice in fresh medium and then mixed with serial two-fold dilutions of serum Kessinger, which recognizes HL-A 7 and other antigens (P. I. Terasaki and G. N. R., unpublished data). The cytotoxic activity of the serum was determined, using trypan blue dye exclusion as the criterion of cell lysis[11]. An endpoint titre of 15 per cent greater cytotoxicity than the complement control was taken as positive[12]. The titres obtained (Table 1) were all quite similar and bore no obvious relationship to heparin concentration.

The results were confirmed using RPMI 4265 target cells incubated with sodium heparinate in solution (Upjohn), serum Christiansen to recognize 4a and other antigens (P. I. Terasaki and G. N. R., unpublished data) and ^{51}Cr release[12] as the measure of cytotoxicity. Titres were again similar (Table 1), and were without relation to the amount of heparin used. The results failed to demonstrate blocking of the cytotoxicity reaction.

The effects of heparin were also tested in the conditions used for HL-A typing of human peripheral lymphocytes. Two normal individuals, whose lymphocytes had been previously HL-A typed using a microcytotoxicity technique[13], were selected and retyped twice, using 2,000 USP units of heparin per 10 ml. of blood (200 units are normally used) in the first instance and defibrination in the other (Table 2). In both conditions, the same specificities were recognized as in the original assay. No previously recognized specificities were blocked by ten times the usual heparin dose.

Table 1. EFFECT OF HEPARIN ON CYTOTOXIC ANTIBODY TITRES

Heparin incubated with 1×10^6 cells (μg)	Cytotoxic titre* Serum Kessinger versus IM-1 cells	Cytotoxic titre* Serum Christiansen versus RPMI 4265 cells
0	11	10
10	12	14
30	13	14
100	18	11
300	11	9
1,000	10	10

* Reciprocal of dilution giving 15 per cent or more greater cytotoxicity than a control containing complement but no test serum.

Table 2. COMPARISON OF HL-A SPECIFICITIES RECOGNIZED ON LYMPHOCYTES OBTAINED FROM PERIPHERAL BLOOD ANTICOAGULATED BY DEFIBRINATION OR BY HEPARIN

Subject	Antiserum	Specificity	Per cent stained cells Defibrination	Per cent stained cells Heparin (2,000 units/10 ml. blood)
A.M. (HL-A1, HL-A6 [4b], HL-A8 Lc 20)	Morrison	HL-A1*	100	100
	TO/10/I	HL-A6 (4b)*	40	45
	Oshita	HL-A6 (4b)*	100	100
	Daly	HL-A8*	100	100
	Anderson	Lc 20	100	100
	Normal human serum	—	5	5
N.R. (HL-A3, HL-A7 Lc 20)	Storm	HL-A3*	100	90
	Cutten	HL-A7*	100	100
	D66–15903	HL-A7*	30	35
	Anderson	Lc 20	25	35
	Normal human serum	—	5	5

* Several antisera in addition to those listed were used in determining these specificities, with similar results.

Lippman's work[7,8] in a variety of mouse systems suggested that heparin might block antigen–antibody interactions. Untreated mouse L cells were rejected by host animals in doses as high as 10^9 cells, but 10^5 heparin-treated cells grew into large, metastasizing transplantable tumours[8]. Moloney tumour cells in allogenic hosts behaved similarly[7]. Lippman also demonstrated that treatment of Moloney target cells with heparin inhibited cytotoxicity in both anti-Moloney and anti-H2 systems. Possible mechanisms suggested for the action of heparin included cell surface coating and resultant sequestration from host immune defence mechanisms, or perhaps some kind of permanent induced surface change. Blocking was not felt to arise from anticomplementary effects of heparin.

Taylor, Culling and McDonald[3] showed that heparin in high doses (700 units per 10^5 cells) protected L strain fibroblasts against the damaging effects of specific guinea-pig cytotoxic antisera when mixed with the antiserum. Pretreatment of cells with heparin followed by washing did not protect against later exposure to complement and serum. Fluorescent antibody studies demonstrated that heparin did not prevent the attachment of antibody to cells, and its effect was felt to be anti-complementary. On the basis of this and other work[4] Taylor and Culling postulated interference of aggregation of immune cells around their targets, perhaps secondary to an alteration of cell surface charge or to an anti-complementary effect.

Although previous reports[1-8] seem to indicate that heparin can block certain immunological reactions both *in vivo* and *in vitro*, we have been unable to demonstrate an effect on anti-HL-A cytotoxicity reactions or on HL-A typing of lymphocytes. Heparin, by coating the cell surface or some other mechanism, may have a significant influence on immunological reaction involving mouse cells, but does not seem to have significant effects on human lymphocytes.

Mark E. Oren
Ronald B. Herberman
G. Nicholas Rogentine, jun.

Immunology Branch,
National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014.

Received August 21; revised December 24, 1969.

[1] Svejcar, J., Pekárek, J., and Johanovský, J., *Z. Immunforsch.*, **132**, 352 (1967).

[2] Gasic, G., and Baydak, T., in *Biological Interactions in Normal and Neoplastic Growth* (edit. by Brennan, M. J., and Simpson, W. L.) (Little, Brown, Boston, 1961).

[3] Taylor, H. E., Culling, C. F. A., and McDonald, T. J., *Amer. J. Pathol.*, **48**, 921 (1966).

[4] Taylor, H. E., and Culling, C. F. A., *Lab. Invest.*, **15**, 1960 (1966).

[5] Schröpl, F., and Eckert, C., *Arch. Klin. Exp. Derm.*, **229**, 326 (1967).

[6] Druyan, B. D., and Shatz, V. Ya., *Pat. Fiziol. Eksp. Ter.*, **10**, 69 (1966).

[7] Lippman, M., *Nature*, **219**, 33 (1968).

[8] Lippman, M., in *Proc. Symp. Epithelial Mesenchymal Interactions* (edit. by Fleischmajer, R., and Billingham, R. E.), 208 (Williams and Wilkins, Baltimore, 1968).

[9] Currie, G. A., *Nature*, **215**, 164 (1967).

[10] Rogentine, jun., G. N., and Gerber, P., *Transplantation*, **18**, 28 (1969).

[11] Herberman, R. B., and Fahey, J. L., *Proc. Soc. Exp. Biol. and Med.*, **127**, 938 (1968).

[12] Rogentine, jun., G. N., and Plocinik, B. A., *Transplantation*, **5**, 1323 (1967).

[13] Mittal, K. K., Mickey, M. R., Singal, D. P., and Terasaki, P. I., *Transplantation*, **6**, 913 (1968).

Inhibition of Primary Antibody Response by *E. coli* Asparaginase

Astaldi *et al.*[1] reported that *Escherichia coli* L-asparaginase inhibited the blast transformation characteristic of cultures of lymphocytes stimulated by phytohaemagglutinin, a finding which was promptly confirmed by the investigation of McElwain and Hayward[2]. In view of this observation and the known inhibition of protein synthesis[3] by asparaginase, it seemed likely that this anti-tumour agent might also inhibit antibody formation during the primary immune response. To examine this possibility, mice receiving asparaginase were immunized with human erythrocytes (RBC), and their haemagglutinin titres were compared with those of untreated mice.

C3H mice, 8–16 weeks of age, were used in these experiments because older mice of this strain may exhibit rather high agglutinin titres with human RBC (for example, 1:512 with papain treated cells). Mice were bred in this laboratory by litter mate matings. The *E. coli* asparaginase (Merck, Sharpe and Dohme) had a specific activity of 300–400 international units/mg protein. Four groups of mice were used for each experiment: (1) untreated, (2) immunized with RBC, (3) treated with asparaginase only, and (4) treated with asparaginase and immunized with RBC. In a number of experiments asparaginase was administered 5–8 days before giving a single immunizing dose of 10^8 human RBC; asparaginase treatment was then continued for up to an additional 9 days. Serum from the three mice in each group was then pooled for evaluation by serologic titration in saline and with papain treated RBC. All incubations were at 37° C, and RBC were from the same donor that was used for immunization. Saline agglutination was performed by incubating 1 drop of 2 per cent RBC suspension with 1 drop of mouse serum for 30 min. The tubes were then centrifuged and read microscopically for agglutination. Before titering with papain treated cells, 1 mg enzyme (Nutritional Biochemicals, crystalline) was activated for 1 h in 5 ml. 0·9 per cent NaCl containing 12 mg cysteine and 34 mg disodium ethylenediaminetetraacetate. One volume packed cells was incubated with 3 volumes papain solution for 5 min; cells were removed by centrifugation and washed once with 0·9 per cent NaCl. A 2 per cent suspension of the treated RBC was then incubated for 5 min with mouse antiserum, centrifuged, and read microscopically for agglutination.

Table 1. EFFECT OF ASPARAGINASE ON DEVELOPMENT OF C3H MOUSE AGGLUTININS TO HUMAN ERYTHROCYTES

Experiment	Asparaginase* (units/mouse/day)	RBC injection on day†	Serum collected on day†	Agglutinin titre			
				RBC only ‡		RBC + asparaginase	
				Saline	Papain	Saline	Papain
1	5	5	11	32	512	16	64
2	5	8	14	256	2,000	4	32
			17	64	2,000	8	512
3	12·5	6	12	32	1,000	8	64
			15	16	1,000	8	64

* Asparaginase given daily until serum collected.
† Day 1 was the day of the first asparaginase injection.
‡ Control mice not receiving asparaginase.

The protein content of the sera for the experimental groups varied from 58–66 mg/ml., but there was no systematic decrease in asparaginase treated animals. Untreated mice and those receiving asparaginase alone exhibited only low haemagglutinin titres, 1:1–1:4, although occasionally a papain titre of 1:8 was observed. In order to determine that asparaginase was not directly influencing agglutination the enzyme was added to mouse serum in a concentration of 25 units/ml. It had no effect on either saline or papain tests. The RBC donor for experiments 1 and 2 (Table 1) was group B, Rh negative; for experiment 3, group O, Rh positive. The titres which resulted from the RBC immunization were markedly reduced by asparaginase treatment (Table 1). The saline titres were decreased to just above the background level seen in the groups of mice receiving no treatment or asparaginase alone. Papain titres were also significantly reduced. The dose level of asparaginase used in these experiments, namely 200–500 units/kg/day, is at the lower end of the range now being used clinically in the treatment of human acute leukaemia. Although a number of questions concerning immunosuppression by asparaginase remain unanswered, those now using the drug clinically should be aware of this capacity to inhibit antibody formation.

This investigation was supported by a grant from the US Public Health Service.

Morton D. Prager
Ina Derr

Departments of Surgery and Biochemistry,
University of Texas Southwestern Medical School,
Dallas, Texas.

Received October 1, 1969.

[1] Astaldi, G., Burgio, G. R., Krč, J., Genova, R., and Astaldi, A. A., *Lancet*, i, 423 (1969).

[2] McElwain, T. J., and Hayward, S. K., *Lancet*, i, 527 (1969).

[3] Sobin, L. H., and Kidd, J. G., *J. Exp. Med.*, **123**, 55 (1966).

Positive α_1-Foetoprotein Tests in Pyridoxine Deprived Baboons: Relevance to Liver Carcinoma in Africans

A previous report[1] has described significant histological changes occurring in the livers of baboons that had been deprived of pyridoxine for periods of five months to three years. It was suggested that these changes might be "pre-neoplastic" and that specific dietary deficiencies could be involved—as well as plant or chemical carcinogens or other unknown factors—in the reported high incidence of primary carcinoma of the liver in Africans.

We report here the results of the α_1-foetoprotein (AFP) test on baboons fed a completely balanced artificial diet deprived only of either riboflavin or pyridoxine. The artificial diet, methods of feeding and care of these animals have been fully described previously[2]. The AFP test is highly specific for human and sub-human hepatocellular carcinoma, although there are certain unexplained anomalies[3-6]. The reagents used in our test were supplied by Dr Abelev from the Gamaleya Institute, Moscow. The antiserum was prepared by immunizing rabbits with human α_1-foetoglobulin. Using this rabbit antiserum, we found a "reaction of identity" with the sera of 50 per cent of our pyridoxine deficient baboons, indicating that AFP was present in the serum of these animals. The AFP test was done using both the conventional and triangular technique. All tests were done "blind" and read separately by three different workers; duplicate or triplicate estimations were done on all animals. The test was performed on twenty-five baboons of which ten were pyridoxine deprived, five riboflavin deprived, two pair-fed and eight on their natural diet.

Of the ten pyridoxine deprived animals, five had positive AFP tests. One of these had been on a pyridoxine free diet for three years, although during this period it had several times been given pyridoxine to keep it alive, and when sufficiently recovered, again put back on pyridoxine deficiency. It finally died and was autopsied. The liver had a whitish-grey nodule at the base of the central lobe; the nodule was 3 cm in diameter with a crenated edge and penetrated about 1 cm into the substance of the liver. Similar but smaller nodules were found in other parts of the liver. The AFP test done on serum three weeks before death was positive. The other four baboons with positive AFP tests have been on pyridoxine deficiency for 118 to 440 days and are still alive. Of the five AFP negative pyridoxine deficient baboons, one died after 507 days deprivation and at post mortem was found to have a grossly fatty liver with histological changes similar to those described previously in other pyridoxine deprived animals[1]. The remaining four AFP negative baboons are still alive and have been on pyridoxine deficiency for 146 to 700 days. The testes in all the animals, although juvenile—as in most pyridoxine deprived animals—had no macroscopic or histological abnormalities.

The five riboflavin deprived animals, on which the AFP tests were performed concurrently with the pyridoxine deprived baboons, were all negative and had been on riboflavin deficiency for 152 to 516 days. Although these animals developed gross skin lesions and in some marrow erythroid aplasia, their livers exhibited none of the changes seen in the pyridoxine deprived animals.

The AFP test was also negative in the two pair-fed animals which had been on the synthetic diet for 566 days. The eight naturally fed baboons had been on their normal diet continuously. None of these animals showed any skin, marrow or liver changes: they gained weight normally and were healthy, and their AFP tests were all negative.

We believe this is the first time that a positive α_1-foetoprotein test has been found in sub-human primates made deficient of a single dietary substance—pyridoxine. Pyridoxine is thought to be marginal in the diet of Africans, and if this is so, then its relevance to the high incidence of primary carcinoma of the liver in Africans could be considerable. Our results indicate that pyridoxine, but not riboflavin deprivation, is associated with a positive AFP reaction. Whether the positive reaction precedes, accompanies or succeeds the histological liver damage in the pyridoxine deficient baboons is being investigated. It is not at present known whether treatment with pyridoxine reverses a positive AFP test. What effect upsets in tryptophan metabolism caused by pyridoxine deprivation[7] have on the AFP test is not known at present. Certain metabolites of tryptophan, such as anthranilic and kynurenic acid and 5-hydroxytryptamine, are considered to be associated with carcinogenesis.

There does not seem to be a very close relationship between the duration of the pyridoxine deficiency and a positive AFP test. In some cases, the test was positive after 118 days of deprivation; in others, it was negative after 700 days. The fall in the serum pyridoxine levels, however, was always closely correlated with the duration of the deprivation or the time that had elapsed from the last dose of pyridoxine. These findings may indicate that the relationship between pyridoxine deficiency and a positive AFP test is not a direct one.

There are substantial changes in the serum pyridoxine, vitamin B_{12} and folate levels in the pyridoxine and riboflavin deprived animals, compared with those that were pair fed and those on a natural diet. It is significant that the level of vitamin B_{12} in the serum of members of the pyridoxine deprived group rises eight-fold during the course of the deprivation. Whether these changes in folate and vitamin B_{12} levels have any significance in the development of hepatocellular carcinoma or the positivity of the AFP test is unlikely, but unkown.

Table 1. MEAN SERUM PYRIDOXINE, VITAMIN B_{12} AND FOLATE LEVELS IN GROUPS OF BABOONS

Group	Pyridoxine (ng/ml.)	B_{12} (pg/ml.)	Folate (ng/ml.)
Natural diet	119·0	70·0	7·6
Range	(50–220)	(31—145)	(1·4–22·7)
Pair fed	220·0	119·0	5·0
Range	(127–300)	(80–175	(2·8–8·0)
Riboflavin	52·0	134·0	5·0
Range	(25–107)	(64–146)	(3·3–7·1)
Pyridoxine	9·1	531·0	6·9
Range	(2·2–22)	(64–557)	(3·4–7·6)

Our results indicate that if the α_1-foetoprotein test is indeed specific for hepatocellular carcinoma, then our baboons developed this condition as a result of pyridoxine deprivation. On the other hand, pyridoxine deficiency may produce a positive AFP test *per se*, and whether or not this is associated with histologically acceptable hepatocellular carcinoma is now under investigation. A full report of this work will be published later with histological details of the liver changes.

HENRY FOY
ATHENA KONDI
Wellcome Trust Research Laboratories,
P.O. Box 30141, Nairobi, Kenya.

C. A. LINSELL
International Agency for Research on Cancer,
P.O. Box 6831, Nairobi, Kenya.

ALISON M. PARKER
Department of Medical Physiology,
University College, Nairobi,
P.O. Box 30197, Nairobi, Kenya.

P. SIZARET
International Agency for Research on Cancer,
16 Avenue Maréchal Foch, 69 Lyon, France.

Received October 24; revised December 15, 1969.

[1] Foy, H., Gillman, T., Kondi, A., and Preston, J. K., *Nature*, **212**, 150 (1966).
[2] Foy, H., Kondi, A., and Mbaya, V., *Brit. J. Nutrit.*, **18**, 307 (1964).
[3] Tatarinov, Yu. S., *Vop. Med. Khim.*, **11**, 20 (1965); translated in *Fed. Proc.*, **25**, T344 (1966).
[4] Abelev, G. I., *Cancer Res.*, **28**, 1344 (1968).
[5] Hull, E. B., Carbone, P. O., Gitlin, D., O'Gara, R. W., and Kelly, M. G., *J. Nat. Cancer Inst.*, **42**, 1035 (1969).
[6] Van Gool, J., and Ladiges, N. C. J. J., *J. Pathol.*, **97**, 115 (1969).
[7] Foy, H., and Kondi, A., *Vitamins and Hormones*, **26**, 653 (1968).

Failure to detect an Antigen associated with Hepatitis in a Community Epidemic

HEPATITIS associated antigen (HAA) (Australian antigen[1], SH antigen[2]) is often demonstrable in the serum of cases of serum hepatitis. HAA is also reported in infectious hepatitis, although with lesser frequency[3-5]. The latter

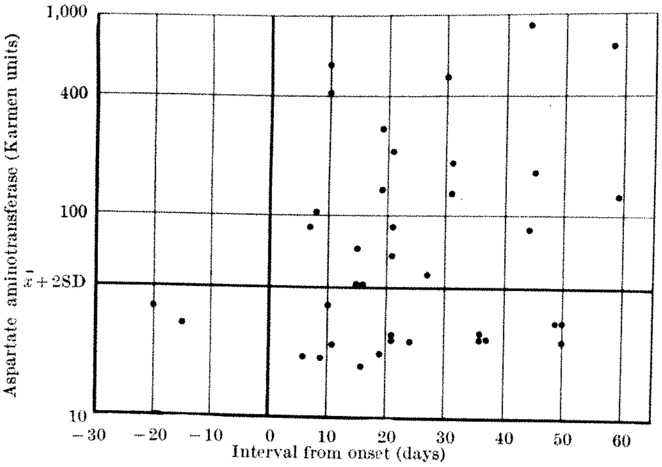


Fig. 1. Scattergraph showing interval in days from onset to collection as well as aminotransferase level of sera from Ely, Nevada. All were negative for hepatitis associated antigen.

observations, however, are in contrast with the finding by Giles and co-workers[6]. None of the children at Willowbrook State School developed HAA in their sera after inoculation with an agent (MS–1) having the characteristics of IH virus. The apparent discrepancy can be resolved only by additional observations using specimens from epidemiologically well characterized cases.

Availability of sera collected in 1958 during a community-wide epidemic of infectious hepatitis prompted the present study. During the 6 month period from October 1957 through March 1958 there were seventy-eight icteric cases of infectious hepatitis reported in White Pine County, Nevada. The epidemic involved the town of Ely (population 6,300), and the smaller communities of Ruth (660) and Spring Valley (330). Most of the cases (68 per cent) were in persons less than 20 years of age. The pattern of the disease in households and the community was consistent with person to person spread; thirty-four of seventy-eight cases had onset of symptoms 2 to 6 weeks after known contact. Immune serum globulin (ISG) was given to members of some households as soon as the index case was recognized, usually in a dose approximating 0·02 ml./kg. Only one of ninety-one persons receiving ISG had clinically recognized hepatitis; there were fourteen icteric cases among eighty contacts not given ISG. After it was recognized that an epidemic of infectious hepatitis was occurring, the National Communicable Disease Center (NCDC) began an intensive study. Serum specimens were collected every 2 weeks from recognized cases, from household contacts, and from families in which illness had not occurred. Assays for L-aspartate : 2-oxoglutarate aminotransferase activity were carried out to determine the extent to which inapparent infections and non-specific illnesses could be diagnosed, especially in household contacts who remained well after ISG was given. The close follow-up of contacts resulted in recognition of anicteric and inapparent infections[7].

The sera collected during the epidemic were retained for evaluation of subsequently developed procedures. The sets of specimens tested in the present study were selected: (1) if one or more sera were collected at a time when HAA was likely to be present, as judged from previous reports[4,9]; or, (2) if there was persistent elevation of aminotransferase. The selected sera were tested for the presence of HAA by the immunodiffusion procedure at both the National Institutes of Health (NIH) and NCDC, and by the complement-fixation test at NIH. The techniques are described elsewhere[5,8].

Fig. 1 records thirty-eight observations of twenty-one patients, thirteen with icteric and eight with anicteric hepatitis. These observations were made from 20 days before to 59 days after onset of symptoms. Eight of the specimens were within 12 days of onset of symptoms; eleven of those taken 21 days or more after onset showed elevated aspartate aminotransferase activities. All specimens were negative for HAA by the immunodiffusion and complement-fixation techniques. Table 1 records five observations of four household contacts with inapparent (asymptomatic) hepatitis. These specimens, all collected while aspartate aminotransferase levels were elevated, were likewise negative for HAA by both techniques.

Table 1. INAPPARENT (ASYMPTOMATIC) INFECTIOUS HEPATITIS, ELY, NEVADA, 1958

Serum No.	Household contact	Bilirubin in urine	Aspartate aminotransferase (Karmen units)	HAA
1	A	+	3,150	—
2	B	+	1,490	—
3	C	−	205	—
4	D	−	154	—
5*	B	−	67*	—

* 14 days after serum No. 2.

These HAA-negative cases meet all the usual requirements for a diagnosis of infectious hepatitis: (1) transmission was predominantly, if not entirely, contact-associated; (2) incubation periods were in the range usually assigned to infectious hepatitis; and (3) a high level of protection was attained with ISG in small doses. It is uncertain whether HAA-positive cases previously reported as infectious hepatitis would also meet these criteria. In sporadic cases, however, differentiation between infectious and serum hepatitis is particularly difficult.

Lack of a history of exposure to a parenteral source of infection is not an adequate basis for designating a case as infectious hepatitis. Obscure exposures to parenteral sources of infection (such as piercing of the ear lobes) may be more important than is usually appreciated. Track-finder's hepatitis[10] could not have been documented if the number of infections acquired by participants in that sport had not been so large. A diagnosis of infectious hepatitis is justified only when there is known contact or the case occurs in an epidemic setting. Patients with probable infectious hepatitis give a history of raw shellfish ingestion or foreign travel more frequently than controls[11], but both events are sufficiently frequent in the general population to limit their value as an indication of the source of infection in any individual.

Our failure to observe HAA positive specimens from patients with typical infectious hepatitis is similar to the experience at Willowbrook State School[6]. The persons studied in the 1958 epidemic in Ely share with those tested by Giles *et al.* the characteristic that the source of their infection could be defined with greater clarity than for patients in the usual hospital setting. There seems little doubt, therefore, that the complete absence of HAA in serum distinguishes at least some groups with infectious hepatitis from patients with serum hepatitis.

JAMES W. MOSLEY
Epidemiology and Research Analysis Branch,
Foreign Quarantine Program,
National Communicable Disease Center,
Atlanta, Georgia.

LEWELLYS F. BARKER
Laboratory of Viral Immunology,
Division of Biological Standards,
National Institutes of Health,
Bethesda, Maryland.

N. RAPHAEL SHULMAN
Clinical Haematology Branch,
National Institute of Arthritis and Metabolic Diseases,
National Institutes of Health,
Bethesda, Maryland.

MILFORD H. HATCH
Enteric Virology Unit,
Laboratory Division,
National Communicable Disease Center,
Atlanta, Georgia.

Received October 13, 1969.

[1] Blumberg, B. S., Gerstley, B. J., Hungerford, D. A., London, W. T., and Sutnick, A. I., *Ann. Intern. Med.*, **66**, 924 (1967).

[2] Prince, A. M., *Proc. US Nat. Acad. Sci.*, **60**, 815 (1968).

[3] Blumberg, B. S., Sutnick, A. I., and London, W. T., *Bull. NY Acad. Med.*, **44**, 1566 (1968).

[4] Gocke, D. J., and Kavey, N. B., *Lancet*, i, 1055 (1969).

[5] Hirschman, R. J., Shulman, N. R., Barker, L. F., and Smith, K. O., *J. Amer. Med. Assoc.*, **208**, 1667 (1969).

[6] Giles, J. P., McCollum, R. W., Berndtson, jun., L. W., and Krugman, S., *New Eng. J. Med.*, **281**, 115 (1969).

[7] Schneider, A. J., and Mosley, J. W., *Pediatrics*, **24**, 367 (1959).

[8] Shulman, N. R., and Barker, L. F., *Science*, **165**, 304 (1969).

[9] Wright, R., McCollum, R. W., and Klatskin, G., *Lancet*, ii, 117 (1969).

[10] Ringertz, O., and Zetterberg, B., *New Engl. J. Med.*, **276**, 540 (1967).

[11] Koff, R. S., Grady, G. F., Chalmers, T. C., Mosley, J. W., Swartz, B. L., and the Boston Inter-Hospital Liver Group, *New Engl. J. Med.*, **276**, 703 (1967).

Effect of Pregnancy Sera on Isolated Lysosomes

INFLAMMATORY diseases, notably rheumatoid arthritis[1], frequently subside during the last 3 months of pregnancy and recur several weeks after parturition. Evidence implicating lysosomes in the pathogenesis of inflammation[2] suggested that the subsidence of this phenomenon in pregnant human females could be mediated by an effect on these subcellular organelles. We present here evidence that serum from pregnant females stabilizes leucocyte lysosomes. In addition, we have data suggesting that hydrocortisone is not responsible for the stabilizing activity observed.

Table 1. LEUCOCYTE LYSOSOME STABILIZATION BY INDIVIDUAL PREGNANCY SERA*

Control	Per cent of control serum effect †
Matched sera six weeks after parturition	115, 85, 83, 83, 82, 73, 60, 47, 47
Age-matched non-pregnant female sera	79, 75, 67, 64, 60, 50, 47, 47, 45, 23
Matched umbilical cord sera	51, 45, 44, 42, 39, 30, 29

* Pregnancy sera were obtained from patients in their last three months of pregnancy. None was receiving medication. All sera were diluted to a final concentration of 1 : 10 and mixed at 20° C with a suspension of intact rabbit leucocyte granules. The change in absorbance at 520 nm was measured every minute for 3 min following the addition of 'Triton *X*-100'.

† The absorbance change of the lysosome suspension in pregnancy serum is expressed as a percentage of the change noted in the control serum 3 min after the addition of 'Triton'. Shown are the mean values of four–eight determinations for each serum.

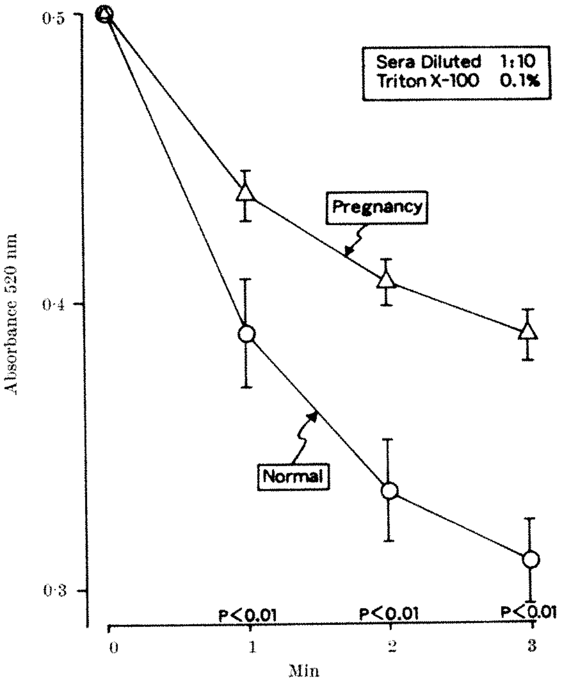


Fig. 1. Effect of pregnancy sera on isolated leucocyte lysosomes. A serum pool composed of sera from six pregnant females in their third trimester (△) is here compared with a serum pool obtained from seven age-matched normal females (○). Sera were mixed with an intact lysosome suspension in 0·3 M sucrose containing 30 units heparin/ml. and absorbance 520 nm change measured following the addition of 'Triton'. Shown are the mean values ± 1 *S.D.* of twelve determinations. Student's *t* test was used for statistical analysis of data.

Lysosomes isolated from glycogen-induced rabbit peritoneal polymorphonuclear leucocytes[3] were suspended in 0·3 M sucrose containing 30 units aqueous heparin/ml. at 20° C. The absorbance at 520 nm of the suspension of intact lysosomes is stable until the addition of 'Triton *X*-100'. This nonionic detergent disrupts organelle membranes releasing their soluble contents, resulting in a rapid decrease in absorbance of the lysosome suspension[4]. A serum pool obtained from six patients in their last 3 months of pregnancy was diluted 1 : 10 in the lysosome suspension before the addition of detergent. The effect of this serum pool on the absorbance of isolated detergent-treated lysosomes was compared with that of a serum pool obtained from seven normal, age-matched females (Fig. 1). It can be seen that the pregnancy serum pool retarded the absorbance change caused by detergent. The absorbance change of the lysosome suspension in pregnancy serum at 3 min is 57 per cent of the change noted in normal serum. This effect was significantly different from normal serum at each time measured. In addition to diminishing the effect of the detergent on absorbance of lysosomes, pregnancy sera also retarded the release of β-glucuronidase from the organelles by the detergent (K. H. H., L. A. F. and R. H. P., in preparation), thus confirming the presence of a lysosome stabilizer in pregnancy serum. The activity of individual sera can be seen in Table 1. The lysosome-stabilizing activities in twenty-six different pregnancy sera are compared individually with either a matched serum from women 6 weeks after parturition, a normal female adult control serum or a matched umbilical cord serum. The rate of absorbance change of all control sera was set as 100 per cent activity. The data indicate not only that varying amounts of lysosome stabilizer are present in most pregnancy sera, but also that this substance does not cross the placental barrier and that it is no longer present 6 weeks after the termination of pregnancy.

The elevated serum concentrations of adrenocorticosteroids present in the latter stages of pregnancy (relative to concentrations usually observed in either non-pregnant females or females 6 weeks after parturition or in umbilical cord samples) was originally thought to account for our observations. Hydrocortisone has been shown to stabilize lysosomal membranes subjected to a variety of stresses[5,6]. We determined the 11-hydroxycorticoid concentrations in the test sera using a fluorimetric method[7]. As expected, pregnancy sera contained high concentrations of cortisol (range = 17–92 μg/ml.), but the stabilizing activity in each serum was not related to its cortisol content. Furthermore, added hydrocortisone did not affect serum stabilizing activity, nor could we demonstrate an effect of this steroid on isolated leucocyte lysosomes stressed by detergent (K. H. H., L. A. F. and R. H. P., in preparation). These findings suggest that hydrocortisone is not responsible for the lysosomal stabilizing property of pregnancy serum. Studies in which corticosteroids have been shown to stabilize membranes have been confined to organelles derived from liver homogenates[5,6]. Evidence has not been presented indicating that cortisol interacts with membranes of the lysosomes from the cells most concerned with acute inflammation, the polymorphonuclear leucocytes.

We conclude that serum obtained from females in their last 3 months of pregnancy contains a factor other than hydrocortisone which stabilizes membranes of leucocyte lysosomes. How this substance regulates lysosomal membrane permeability is not yet known. Nor is the nature of the factor understood although studies in this direction are currently in progress in our laboratory. It appears possible that the development of this substance in the course of pregnancy, capable of interacting with leucocyte organelles to prevent the discharge of their lytic contents, could account for the subsidence of inflammatory disease.

This study was supported by grants from the San Antonio Area Foundation and from the Medical Research Foundation of Texas.

KARL H. HEMPEL
LINDA A. FERNANDEZ
ROBERT H. PERSELLIN

Department of Physiology and
Internal Medicine,
Section of Rheumatology,
University of Texas Medical School,
San Antonio, Texas.

Received September 16, 1969.

[1] Hench, P. D., Kendall, E. C., Slocumb, C. H., and Polley, H. F., *Ann. Rheum. Dis.*, **8**, 97 (1949).
[2] Persellin, R. H., *Med. Clin. N. Amer.*, **52**, 635 (1968).
[3] Cohn, Z. A., and Hirsch, J. G., *J. Exp. Med.*,**112**, 983 (1960).
[4] Keiser, H., Weissmann, G., and Bernheimer, A. W., *J. Cell Biol.*, **22**, 101 (1964).
[5] deDuve, C., Wattiaux, R., and Wibo, M., *Biochem. Pharmacol.*, **9**, 97 (1962).
[6] Weissmann, G., *Biochem. Pharmacol.*, **14**, 525 (1965).
[7] Mattingly, D., *J. Clin. Pathol.*,**15**, 374 (1962).

Features of the Synovial Fluid Film in Human Joint Lubrication

THE mechanism of lubrication in human joints depends on the bearing material (articular cartilage), the joint lubricant (synovial fluid) and the operating conditions (sliding or rolling speeds, load and time of load application). Cartilage is a composite of collagen fibre bundles set at various orientations within a ground substance of chondroitin sulphate, and containing a high proportion of watery fluid[1-4]. Its surface, which is very important to the lubrication behaviour, has been found to be rough by engineering standards. The centre line average value has been measured at 30 μinch for healthy cartilage, the roughnesses taking the form of undulations with small ones superimposed on others of larger wavelength[5,6].

Synovial fluid consists of a long-chain high molecular weight polymer, hyaluronic acid, bound to protein, within a dialysate of blood plasma, the resulting fluid being clear, yellow and tacky to the touch[7]. It is well established that the fluid is shear thinning, the sort of viscosities measured being 100 poise at 10^{-2} s shear rate, and 0·1 poise at 10^3 shear rate. A small degree of elasticity has been measured, which is probably very significant in terms of the concentration, molecular weight and "interlinking" of the polymer[8]. The loads and sliding speeds are variable depending on the activity, but typically, in the hip and the knee joints during walking, the loads fluctuate, peaks of three to four times body weight acting during "heel-strike" and "toe-off"[9,10].

Work on the ankle joints of dogs[11] has confirmed the low coefficient of friction, 0·002–0·01, and a mixture of lubrication mechanisms was postulated. We have suggested that a lubricant film between cartilage surfaces is established during joint movements under light load, and maintained under loaded conditions by a squeeze film action. The term "boosted lubrication" by fluid entrapment and enrichment was used to describe this phenomenon[12].

Studies with the scanning electron microscope have given some indication of the appearance of these protecting fluid films. Films were studied in two situations under pure squeezing loads, and under sliding and squeezing combined. In the first, specimens of cartilage covered with synovial fluid were loaded against a glass plate so that the contact loads and times under load represented a range of joint conditions. The situation was frozen by plunge immersion in liquid nitrogen, followed by drying under vacuum at −50° C. Contact loads used were from 3–15 kg/cm², and times from 2–90 s. In the second case, cartilage specimens sliding on glass were immersed within 1 s into liquid chlorodifluoromethane ('Arcton 22', ICI) close to its freezing temperature of 160°, to be followed again by vacuum drying.

The surfaces of the specimens were coated with about 500 Å of silver and examined in the stereoscan, scanning electron microscope.

With comparable conditions of load and time under load, the deposits observed on the cartilage surfaces pre-

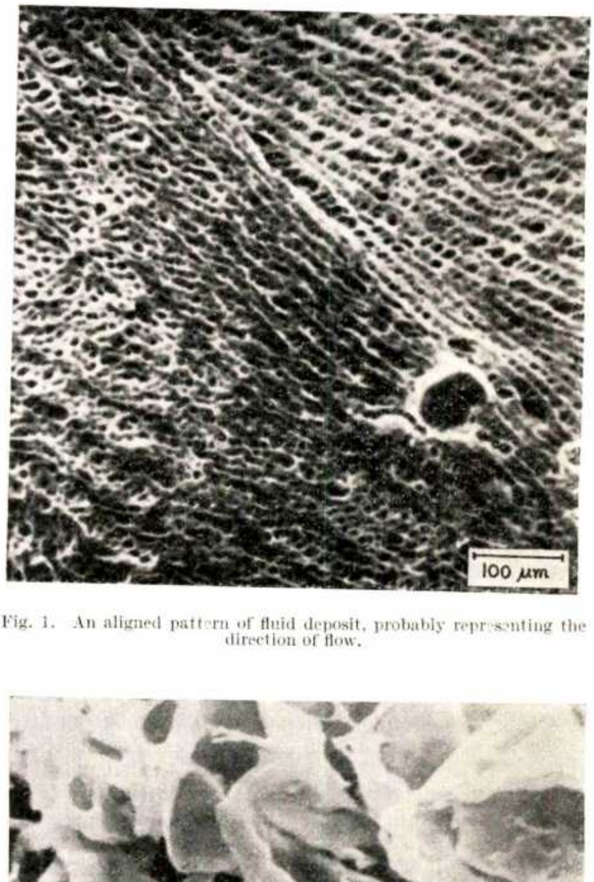


Fig. 1. An aligned pattern of fluid deposit, probably representing the direction of flow.

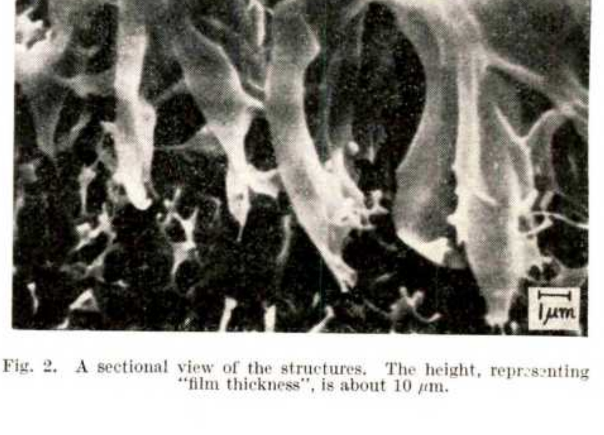


Fig. 2. A sectional view of the structures. The height, representing "film thickness", is about 10 μm.

pared by both methods displayed similar characteristics. The thickness of the deposit, which would represent roughly the film thickness betweeen the cartilage and glass, ranged from a maximum of about 10 μm to a minimum of about 0·25 μm, the former resulting, for instance, from a contact load of 3 kg/cm² acting for 2 s, and the latter for 15 kg/cm² acting for 5 s.

Fig. 1 shows that as the fluid was just being squeezed from between the surfaces, the flow was in preferred directions leaving a strongly orientated pattern of deposit. A sectional view at high magnification showed that there was an arcade-like structure formation (Fig. 2), the height of about 400 μinch representing film thickness. After longer times under load, the structures were collapsed (Fig. 3), until the limiting condition was reached of a complete skin over the surface (Fig. 4). A number of studies showed that the skins could be of double or treble thickness, particularly where there were trapped pools of fluid in the undulations on the cartilage. It seemed that the polymer had tended to be separated from the watery dialysate, the water having flowed out sideways and perhaps into the pores of the cartilage at low film thickness. This latter mechanism has been shown to be feasible by Maroudas[13].

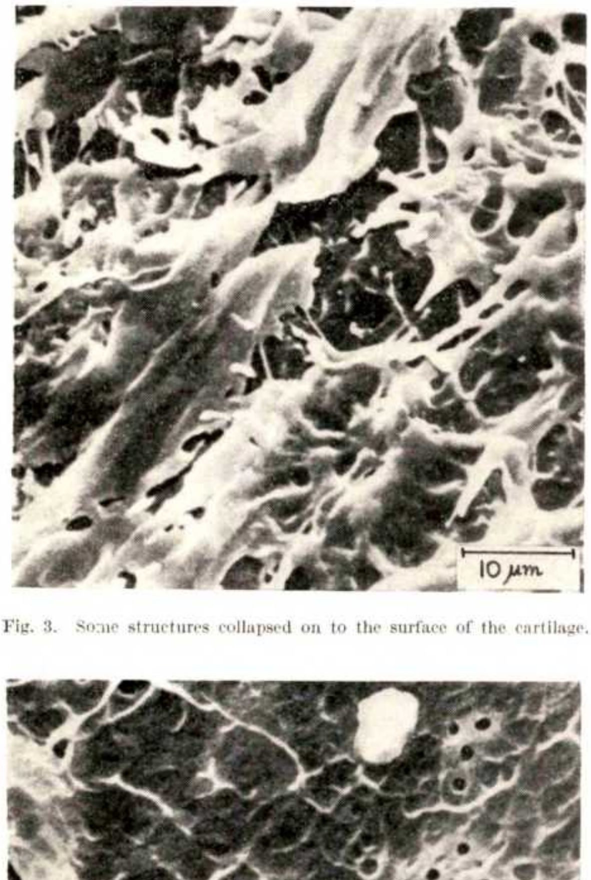


Fig. 3. Some structures collapsed on to the surface of the cartilage.

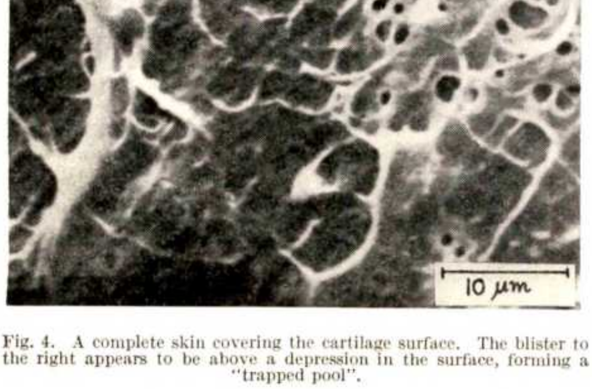


Fig. 4. A complete skin covering the cartilage surface. The blister to the right appears to be above a depression in the surface, forming a "trapped pool".

It is suggested that in activities such as walking the load-time combination is such that hip and knee joints will operate in a fairly thick film condition many times greater than the minimum of 0·25 μm, the film being recharged on swings of the joint under light load. Even when the thin film condition is reached, however, this will be an efficient protection mechanism for the cartilage surfaces. It will be very resistant to further thinning because of the very small flow rate of the thin layers of watery dialysate which would probably exist between the skins; but also, these thin watery layers would offer little resistance to sliding, accounting for the very low coefficient of friction.

Substitute polymers in solution which may possibly equal the remarkable lubricating properties of synovial fluid are currently being studied.

P. S. Walker

Bio-Engineering Group for the Study of Human Joints,
Rheumatism Research Unit,

J. Sikorski

Department of Textile Physics,

D. Dowson

Department of Engineering, Fluid
Mechanics and Tribology,
Institute of Tribology,

M. D. Longfield

Institute of Tribology,

V. Wright

Department of Medicine,
University of Leeds.

Received July 3, 1969; revised January 9, 1970.

[1] Weiss, C., Rosenburg, L., and Helfet, A. J., *J. Bone Joint Surg.*, **50**-A, 663 (1968).
[2] Mankin, H. J., *Bull. Rheum. Dis.*, **17**, 447 (1967).
[3] Bullough, P. G., and Goodfellow, J., *J. Bone Joint Surg.*, **50**-B, 852 (1968).
[4] McCall, J., *Lubrication and Wear in Joints* (edit. by Wright, V.), 39 (Sector, London, 1969).
[5] Walker, P. S., Sikorski, J., Dowson, D., Longfield, M. D., Wright, V., and Buckley, T., *Ann. Rheum. Dis.*, **28**, 1 (1969).
[6] Gardner, D. L., and Woodward, D., *Ann. Rheum. Dis.*, **28**, 379 (1969).
[7] Hamerman, D., Rojkind, M., and Sandson, J., *Fed. Proc.*, **25**, 1040 (1966).
[8] Davies, D. V., and Palfrey, A. J., *J. Biomech.*, **1**, 79 (1968).
[9] Paul, J. P., *Proc. Inst. Mech. Eng.*, **181** (3J), 8 (1967).
[10] Morrison, J. B., *Biomed. Eng.*, **3**, 164 (1968).
[11] Linn, F. C., *J. Biomech.*, **1**, 193 (1968).
[12] Walker, P. S., Dowson, D., Longfield, M. D., and Wright, V., *Ann. Rheum. Dis.*, **27**, 512 (1968).
[13] Maroudas, A., *Proc. Inst. Mech. Eng.*, **181** (3J), 122 (1968).

Scanning Electron Microscope Studies of Human Trabecular Bone

We wish to draw attention to the advantages of the scanning electron microscope in investigating the architecture of trabecular bone. This tissue, which contains about 25 per cent by weight of bone and 75 per cent of marrow[1,2], is important in the dosimetry of bone-seeking radionuclides. We have accordingly begun our work by using

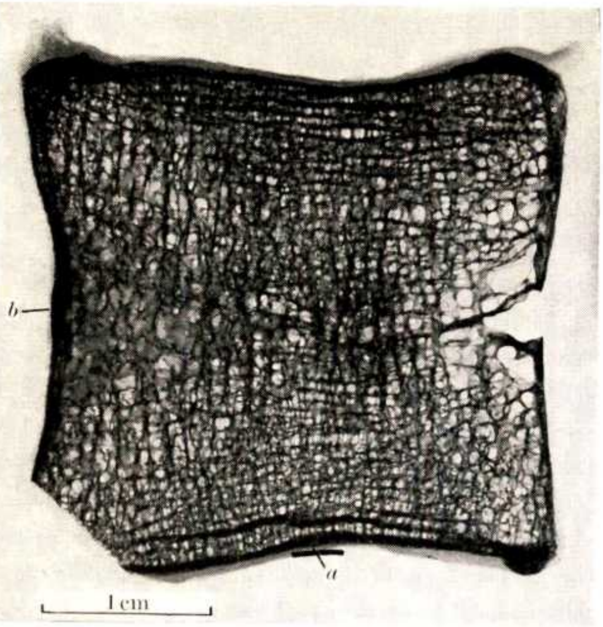


Fig. 1. Print of contact radiograph of sagittal section of vertebral body (one corner removed during preparation). The posterior vascular foramen is to the right.

the Stereoscan microscope at its lowest powers of magnification (×10 to ×100) to elucidate the general arrangement of the bony elements and to make estimates of their volume and surface area.

Two sections 3–5 mm thick were sawn, one a complete sagittal section and the other at right angles to it, passing through the foot of the neural arch. The surfaces were polished under liquid nitrogen so that the hard-frozen marrow protected the bone from damage or distortion. The marrow was washed out at room temperature with a water jet. The sections were then dried and treated with a thin coat of Duron[3] antistatic agent.

Fig. 1 is an enlarged print of a contact radiograph of the sagittal section. The structure inside the vertebral body varies considerably and, notably, there are dark lines indicating concentrations of bone at right angles to the section, especially near the intervertebral disks.

The Stereoscan photographs in Fig. 2*a* and *b* show the areas adjacent to the opaque markers in Fig. 1. The surface electron micrographs give almost no indication of underlying chemical composition; for example, uncalcified tissue can be identified only by its morphological appearance.

In Fig. 2*a* the cortical wall is seen to contain narrow channels, presumably vascular channels. A second region of thicker bone, with channels, can be correlated with the most conspicuous dark line in Fig. 1. There are many short sturdy rods, about 750–1,000 µm long and from about 100–200 µm thick at their centres. There are also a number of small laminae which appear to be about 75–100 µm thick. There is nothing which could be described as an enclosed marrow space, but rather a marrow continuum intersected by a coherent framework of bone.

Fig. 2*b* is a complete contrast. It is composed exclusively of thin curved laminae about 75–100 µm thick, enclosing large interconnecting marrow spaces. In many other regions of the vertebral body the rod-like and plate-like structures exist in close proximity.

Measurements of the area of the polished bone surface and of the boundary between bone and marrow were made in twelve small regions, six from each section, excluding cortical bone. The relative area, by Delesse's theorem[4,5], is numerically equal to the relative volume of bone. The relative weight can thus be calculated. Further, from the perimeter, with certain assumptions about the isotropy of the structure[5], the area of the surface separating bone and marrow can be deduced. Table 1 gives details.

Table 1. SUMMARY OF MEASUREMENTS

Relative area of polished bone surface	$0{\cdot}13_5$	cm^2/cm^2 surface area of section
(Near intervertebral disks, approx.	0·18	cm^2/cm^2 surface area of section)
Calculated fraction by weight of bone in trabecular bone tissue	0·23	
(Near intervertebral disks, approx.	0·30)	
Perimeter of polished bone surface	22	cm/cm^2 surface area of section
Calculated area on interface between bone and marrow	28	cm^2/cm^3 trabecular bone tissue

(Specific gravity of bone, 2 g cm^{-3}; of marrow, 1 g cm^{-3})

In spite of the very different appearance of the structure at different parts of the vertebral body, the measured areas and perimeters are fairly close to the average except very near the intervertebral disks. The exact definition of the polished surface means that systematic errors should not be large.

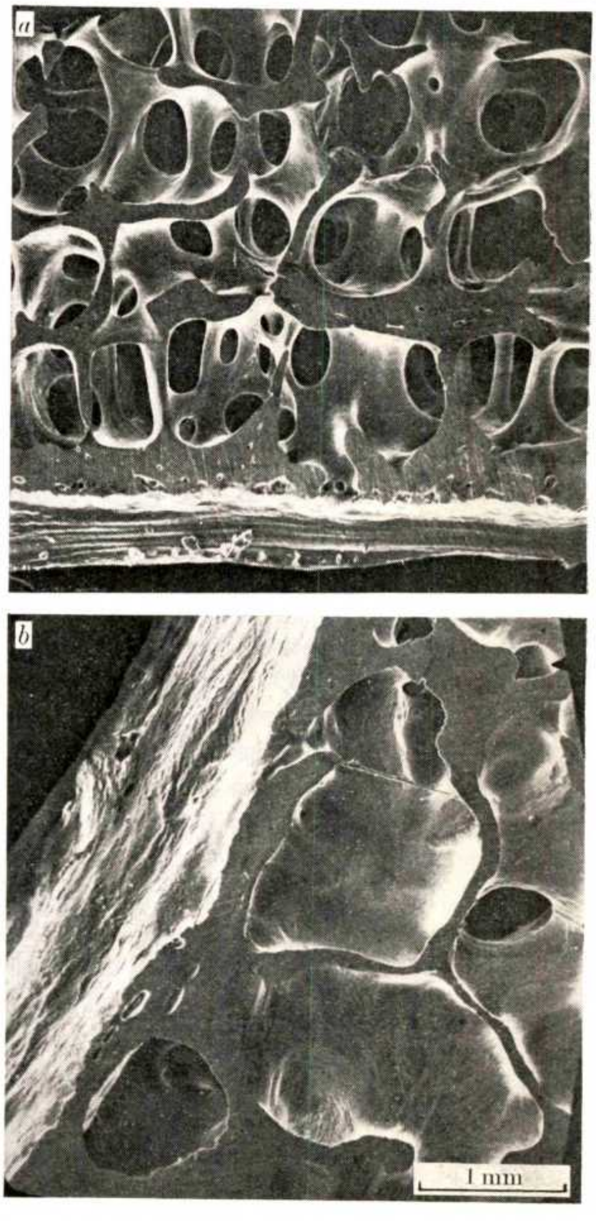


Fig. 2. Stereoscan pictures of regions *a* and *b*, opposite the positions of the markers in Fig. 1. The upper surface of the bone has been polished and the marrow washed out. There are remnants of soft tissue adhering to the outer surface of the bony cortex. Same scale for *a* and *b*.

Our relative area measurements agree quite well with those of Robertson and Godwin[6], who found 11·6 per cent of bone area. They also lead to a relative weight which agrees with our own chemical analyses. Dunnill *et al.*[7] found 25·3 ± 2·6 per cent for the percentage volume of bone, and 30·0 ± 6·1 cm^2 per ml. for the surface area in lumbar vertebral bodies from males in the 20–29 age group. They included cortical bone, which would have a much greater effect on the volume ratio than on the surface area.

Lloyd *et al.*[8] have published automatic measurements of bone perimeters in micrographs of bone sections 100 μm thick. For a lumbar vertebra they find 90 cm/cm^2 of bone surface. In our sections the corresponding figure (perimeter/relative area of bone) is 22/0·135 = 165 cm/cm^2 polished bone surface.

We hope to publish a more detailed account of this work elsewhere. We thank Dr G. M. Ardran and Mr H. E. Crooks for taking the X-radiograph and also Dr Ardran for obtaining the bone sample.

E. D. DYSON
Radiological Protection Division,
C. K. JACKSON
Solid State Division,
W. J. WHITEHOUSE
Health Physics and Medical Division,
AERE, Harwell.

Received October 20; revised December 12, 1969.

[1] Dyson, E. D., and Whitehouse, W. J., *Nature*, **217**, 576 (1968).
[2] Dyson, E. D., and Whitehouse, W. J., *Proc. Symp. Ossium Europ. Assoc. Radiology* (in the press).
[3] Sikorski, J., Moss, J. S., Newman, P. H., and Buckley, T., *J. Sci. Instrum.*, **1**, 29 (1968).
[4] Chayes, F., *Petrographic Modal Analysis* (Wiley, New York, 1956).
[5] Hennig, A., *Zeiss Werkz.*, **30**, 78 (1958).
[6] Robertson, J. S., and Godwin, J. T., *Brit. J. Radiol.*, **27**, 241 (1954).
[7] Dunnill, M. S., Anderson, J. A., and Whitehead, R., *J. Path. Bact.*, **94**, 276 (1967).
[8] Lloyd, E., Rowland, R. E., Hodges, D., and Marshall, J. H., *Nature*, **218**, 365 (1968).

Cyclical "On Demand" Oral Intake by Adults

RESEARCH into cyclical events of sleep[1] has dealt notably with the appearance every 90 min of rapid eye movement (REM, paradoxical or dreaming) sleep. REM sleep was first seen with an hourly periodicity in small infants. If the infant was feeding "on demand" the demands were related to the hourly cycle[2]. The oral activity (eating, drinking, smoking) of ten unsuspecting adults[3], mostly alone, during periods of 6 h or more, has also revealed a cyclical pattern with a periodicity of just over one and a half hours. Different weightings were given to different oral activities in accordance with psychoanalytic tenets: milk, for example, had extra weight because of its "basic" oral quality. Even without the weighting, however, a cyclical pattern was evident. Seven schizophrenic patients showed similar patterns during one 6 h session each[4].

In an attempt at confirmation J. M. and I. O. observed eight young adults for from one to ten sessions each; there were twenty-seven sessions in all. As a preliminary each subject had electroencephalographic electrodes and a small box fixed to the head and was shown biotelemetry equipment. The subjects drew their own conclusions: in fact none realized the purpose of the experiment. It was agreed in advance that each session should be of 6 h duration; they could occupy it as they chose and would not be interfered with.

The subjects were observed alone through a one-way screen for 6 h (in some cases boredom reduced the time to 5 h 45 min) in a comfortable room containing a coffee table with coffee powder, milk, apples, saccharin, still and carbonated fruit drinks, chocolate, water and utensils. To reduce possible digestive or sugar-metabolism factors, only low-calorie fruit drinks and Cadbury's diabetic chocolate were provided. No subject recognized the special nature of these foods. They occupied their time in reading, studying, napping and so on.

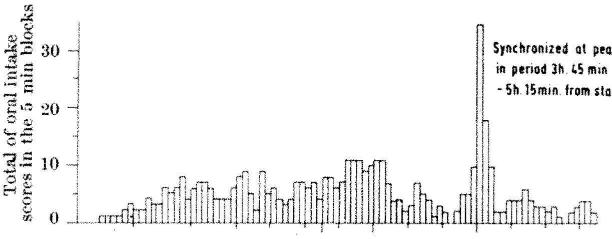


Fig. 1. Distribution of oral intake scores when peak intakes were synchronized for the period 3 h 45 min to 5 h 15 min from the start. The figure shows the two groups of eight 5 min blocks, around the 90 min and the 135 min marks, which were used in the analysis of results.

The 6 h period was divided into 5 min blocks or intervals on a scoring sheet and the observer scored each oral act, namely, each bite of chocolate, each sip of drink, each puff of smoke, and every instance where pencil, pen or fingers went on or into the mouth or nose.

The great amount of contact around the snout area was surprising and we can only suppose that it passes unremarked in normal life because of its ubiquity. Owing to its continual presence we have not used it in our analysis of data. Nor have we used the smoking scores, for it proved impossible to be sure whether a tobacco pipe was still alight and when a puff of smoke had actually been drawn. Our eventual scoring was confined to feeding and drinking.

In analysing the data each 5 min block was given a score based on the number of different forms of eating or drinking in the 5 min. In this first procedure the number of individual sips of drink or bites of apple was not taken into account, for there seemed no grounds for considering eight sips of coffee to be worth more than one bite of chocolate. Thus a 5 min block containing six bites of apple only scored 1, but a 5 min block with two bites of chocolate and three sips of lemon drink scored 2.

To synchronize the peaks of oral activity in the different sessions, we adopted a procedure resembling that of Friedman and Fisher[3]. When they entered the experimental room, the subjects tended in the first minutes to sample the food available. Our synchronization procedures, however, have in all cases avoided both the first and last 30 min of the sessions. In the first place the peak of oral activity in the 90 min around the 4·5 h mark in each session was determined, namely between 3 h 45 min and 5 h 15 min. As Friedman and Fisher found, there was little difficulty in selecting a peak. When there were two equal scores the number of sips of drink or bites of food served as a guide; when two adjacent 5 min blocks were identical the first was arbitrarily taken as the peak. The peak score was noted at a central zero mark on a graph and the scores in the 5 min blocks on either side throughout the whole session were marked at appropriate distances. This was done for all records and the scores accumulated (Fig. 1). In two cases there had been no oral intake in the key period so that these sessions had to be omitted. Consequently the accumulated scores are based on twenty-five records.

Similarly the peak 5 min block of oral activity between 30 min and 2 h from the start of every session was deter-

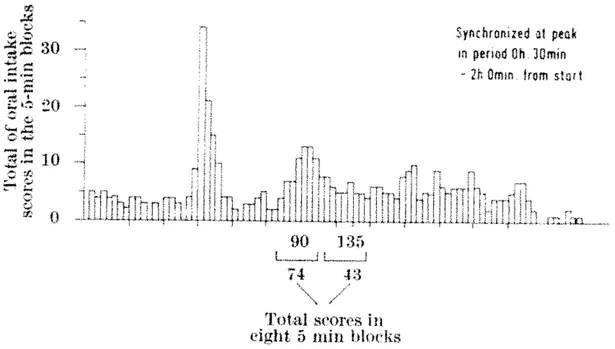


Fig. 2. Distribution of oral intake scores when peak intakes were synchronized for the period 0 h 30 min to 2 h from the start. The two groups of eight 5 min blocks used in the analysis are again shown.

mined. Again two records had to be omitted because they showed no oral intake in the key period. The other twenty-five were synchronized and the scores for the sessions again accumulated (Fig. 2).

In selecting peaks we were *ipso facto* selecting "lows" and, because the peaks were evenly spread over the 90 min periods from which they were selected, we should expect "lows" to extend for one and a half hours on either side of the synchronized peaks in Figs. 1 and 2, with a rise therefore expected at 90 min before and after the synchronization point. If oral activity were random the curve should then level off until diminishing data caused a fall. In particular, in Fig. 1 a "low" should be present between the synchronization peak and the 90 min mark before that peak, and there should be no "low" around the 135 min mark before the synchronization peak. If, on the other hand, a 90 min cycle of oral activity were present, then a "high" would be hypothesized around the 90 min mark and a "low" around the 135 min mark. In this connexion the four 5 min blocks on each side of that 90 min mark which lies before the synchronization peak in Fig. 1 were found to contain the eight scores shown in Table 1. The eight 5 min blocks are separated in Fig. 1 by a single 5 min block from the eight blocks grouped on either side of the 135 min mark. These last eight scores are also shown in Table 1.

Table 1. THE SCORES IN THE GROUPS OF EIGHT 5 MIN BLOCKS AROUND EACH HYPOTHESIZED "HIGH" AND "LOW"

	Hypothesized "highs"	Hypothesized "lows"
Fig. 1.	11, 11, 9, 10, 11, 11, 7, 4 (total 74)	7, 6, 7, 4, 8, 8, 6, 7 (total 53)
Fig. 2.	4, 7, 7, 11, 13, 13, 11, 8 (total 74)	6, 5, 5, 7, 5, 5, 4, 6 (total 43)

Similarly for Fig. 2, if oral activity were random there should be no "low" around the 135 min mark after the synchronization peak, but if a 90 min cycle were present one would hypothesize a "high" around the 90 min mark after the synchronization peak and a "low" around the 135 min mark. The scores for the eight 5 min blocks around each of these two marks are given in Table 1.

If we examine the possibility that the sixteen scores of "high" 5 min blocks in Table 1 could be drawn from the same distribution as the sixteen scores of "low" 5 min blocks, $t = 3.64$ ($P < 0.001$).

The phenomenon of adult, oral intake occurring cyclically has thus been observed in the conditions of our own study, as in the conditions of Friedman and Fisher's study. To determine variations in periodicity within and between individual subjects the scores were later examined by an independent mathematician (H. L.) and subjected to periodogram analysis[5].

An approximate period of n units (1 unit $= 5$ min) was adopted and the observations for each individual set up in arrays of n columns. The correlation ratio (the ratio of the *S.D.* of the weighted means of the vertical arrays to the *S.D.* of all the observations in the distribution) was calculated for period n in each trial for each individual. This procedure was carried out for various values of n and graphs of the correlation ratio against the period n were plotted. From these graphs, the value of n with the maximum correlation ratio was the required number of units in the period. For example, a maximum correlation ratio of 1·23 for $n = 19$ gives a period of 95 min. The periodicity of oral activity for each trial, to the nearest 5 min, is shown in Table 2 and it can be seen that values cluster around 90 min.

Table 2. PERIODICITY OF ORAL ACTIVITY

Subject	Activity in the 27 sessions (min)	Hour of commencement of session
S	105, 90, 95, 95, 95, 85, 85, 90, 85, 90	1415, 1320, 1355, 1125, 1330, 1205, 1520, 1415, 1245, 1025
M	100, 90	1515, 0930
R	95, 95	1340, 1210
C	80, 80, 90	1415, 1410, 1035
P	105	1415
N	90, 100	1355, 1355
L	80, 85, 90	1415, 1400, 1320
W	80, 90, 95, 80	1110, 1045, 1445, 1325

The rate of rise to the peak value of the correlation ratio was found from (1) the correlation ratios for the two periods immediately preceding the period containing the maximum correlation ratios and (2) the maximum correlation ratio. The best straight line through these points was found by least squares. The slope of this line gave an indication of the speed of attaining the maximum correlation ratio. These gradients are shown in Table 3.

Table 3. RATE OF RISE TO MAXIMUM CORRELATION RATIO FOR THE TWENTY-SEVEN SESSIONS

Subject	
S	0·10, 0·45, 0·00, 0·03, 0·15, 0·09, 0·10, 0·07, 0·05, 0·04
M	0·04, 0·06
R	0·09, 0·13
C	0·11, 0·06, 0·12
P	0·06
N	0·06, 0·02
L	0·28, 0·05, 0·14
W	0·01, 0·05, 0·05, 0·14

Our subjects began their sessions at times varying from 0930 to 1520 h (Table 2). We can see nothing in our data that would allow of an explanation of the findings in terms of any learned periodicity of intake. The conventional British sequence of breakfast, morning coffee, lunch, afternoon tea and evening meal does not conform to a 90 min pattern.

It seems to us probable that a one and a half hour cycle of oral events in waking life would be related in the adult, as in the infant, to the cycle of events that follow a similar periodicity within sleep. The periods of cyclically recurring REM sleep are accompanied by dreaming, penile erections, irregularity of the heart and breathing, and other changes characteristic of behaviour governed more by emotion than by reason. A cyclical pattern of oral intake, which we have demonstrated, and the continual touching of the nose and mouth, on which we have remarked, would serve to illustrate how covert urges may shape our waking lives.

IAN OSWALD
JUDITH MERRINGTON

University Department of Psychiatry,
Royal Edinburgh Hospital,
Morningside Park,
Edinburgh.

HEATHER LEWIS

Department of Mathematics,
Napier College of Science and Technology,
Colinton Road,
Edinburgh.

Received April 14; revised December 3, 1969.

[1] Oswald, I., *Sleep* (Penguin, Harmondsworth, 1966).
[2] Aserinsky, E., and Kleitman, N., *J. Appl. Physiol.*, **8**, 11 (1955).
[3] Friedman, S., and Fisher, C., *J. Amer. Psychoanal. Ass.*, **15**, 317 (1967).
[4] Friedman, S., *Amer. J. Psychiat.*, **125**, 743 (1968).
[5] Tippett, L. H. C., *Methods of Statistics*, 328 (Dover, New York, 1952).

Effects of 6-Hydroxydopamine on CNS Catecholamines, Spontaneous Motor Activity and Amphetamine Induced Hyperactivity in Rats

THE compound 6-hydroxydopamine (6-OHDA) produces a long-lasting depletion of brain noradrenaline (NA) and dopamine (DA) when injected into the lateral ventricle of the rat brain[1]. This treatment also results in a reduction in the activities of the catecholamine biosynthetic enzymes, tyrosine hydroxylase and DOPA decarboxylase, in the hypothalamus and corpus striatum. In addition there is a marked reduction in the rate of uptake of ^{3}H-NA into tissue slices from these regions[1]. These findings are consistent with the evidence from morphological studies[2,3] which suggest that the compound causes an acute degeneration of adrenergic nerve terminals in the sympathetic nervous system when administered systemically. The effects of 6-OHDA in the CNS are restricted to NA and DA containing neurones, and there is no recovery for periods of up to 142 days after treatment[1]. The doses of 6-OHDA injected into the brain are not sufficiently large to have any effects on the peripheral sympathetic nervous system[2].

In a preliminary study of the effects of 6-OHDA on animal behaviour, amphetamine-induced hyperactivity was investigated before and after drug treatment. Male albino Wistar rats (weighing initially 150 g) were acclimatized to photocell activity boxes on 4–6 successive days, until a stable baseline of spontaneous motor activity was attained. This was accompanied by a reduction in defaecation to a stable level while in the box. (+)-Amphetamine in saline was injected intraperitoneally (0·5 and 1·5 mg/kg; expressed as free base) and the rats were placed in a holding cage for 55 min. They were then transferred to the activity boxes and scored individually for a 10 min test period, previous experiments having established that the peak amphetamine effect occurred at this time. Individual cumulative records showed that the rate of activity during the sampling period was constant, after an initial burst in the first minute. Precautions were taken to exclude extraneous stimuli from the boxes, and the recordings were made between 1100 h and 1500 h to reduce variations arising from diurnal changes in motor activity. Control motor activity was measured 55 min after the injection of a saline placebo. The investigation of high doses of (+)-amphetamine (>3·0 mg/kg) was discontinued, because the stereotyped behaviour predominating at these doses interfered with the measurement of motor activity.

Rats were anaesthetized with ether, and 6-OHDA, dissolved in 20 µl. of cooled Merlis solution, was injected into the lateral ventricle of the brain[1]. The solution contained ascorbic acid (1·0 mg/ml.) to retard autooxidation of the drug. One group of sixteen animals received 100 µg of 6-OHDA (free base) per animal; a second group received two doses of 250 µg per animal (with an interval of two days between doses). During the second week after 6-OHDA treatment, testing was resumed, and the responses to the same doses of amphetamine and to a saline placebo were again measured.

Animals were then killed by stunning and decapitation, and the brains were rapidly removed and assayed for NA and DA. The results obtained from rats treated with 6-OHDA were expressed as percentages of control values for animals treated with the vehicle solution alone (Table 1). The results were in agreement with those previously described for rats treated with these doses of 6-OHDA (ref. 1).

Rats treated with 100 µg of 6-OHDA did not exhibit a significant change in control motor activity (before 6-OHDA = 156 ± 12; after 6-OHDA = 149 ± 17 counts per 10 min per rat). Burkard, Jalfre and Blum[4] also found no change in spontaneous motor activity after administration of 250 µg of 6-OHDA in rats. In animals treated with 2 × 250 µg, however, control motor activity was significantly reduced (before 6-OHDA = 181 ± 14; after 6-OHDA = 120 ± 12, $P<0{\cdot}01$) (Fig. 1).

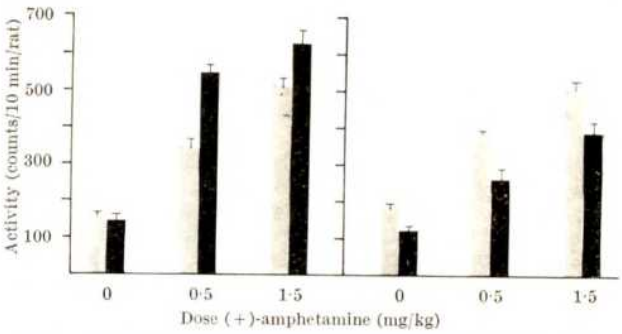


Fig. 1. The effects of (+)-amphetamine on motor activity in rats, before (stippled columns) and after treatment (black columns) with 6-OHDA. Two groups of sixteen animals received 100 µg (on the left) and 2 × 250 µg (on the right) of 6-OHDA respectively. Values are means ± *S.E.*

The effects of 6-OHDA treatment on (+)-amphetamine induced hyperactivity are summarized in Fig. 1 and Table 2. The time course of the response to amphetamine was found to be unaltered. The increases in motor activity are expressed as the scores obtained with amphetamine minus the mean control scores (Table 2). These increments are also expressed as percentages of the respective control motor activities (Table 2). After 100 µg of 6-OHDA there was a significant enhancement of the amphetamine effect at both 0·5 and 1·5 mg/kg. Because the control motor activity was unchanged, the percentage effect was also significantly increased. After doses of 2 × 250 µg of 6-OHDA, the amphetamine effect was not significantly different from that measured in the same animals before treatment.

These results show that amphetamine stimulates motor activity in the rat even after the substantial depletion of brain catecholamines induced by 6-OHDA. It has been suggested that catecholamines in the brain play an important part in the regulation of motor activity and in the stimulation of motor activity by (+)-amphetamine[5–7]. Our results suggest, however, that there is no simple relationship between brain catecholamine levels and the magnitude of the amphetamine response. For example, the amphetamine response was not significantly reduced after 2 × 250 µg of 6-OHDA, although brain NA and DA were reduced by 75–80 per cent. Spontaneous motor activity, however, was reduced in these animals. After 100 µg of 6-OHDA, spontaneous motor activity was not affected, but the amphetamine response was significantly enhanced. This dose caused a differential depletion of the two catecholamines, NA being more severely depleted than DA (Table 1). The enhanced

Table 1. EFFECTS OF 6-OHDA ON BRAIN NA AND DA

Dose 6-OHDA (µg/rat)	NA	DA
100	38·5 ± 3·2 per cent	65·0 ± 6·4 per cent
500 (2 × 250)	21·0 ± 3·9 per cent	26·5 ± 4·3 per cent
Control values (µg/g brain)	0·298 ± 0·009	0·584 ± 0·040

NA and DA contents of experimental animals are expressed as percentage of control values.

Values are means ± *S.E.* (twelve–sixteen animals) and are not corrected for losses during the isolation procedure.

Table 2. ACTIVITY MEASUREMENTS

Dose 6-OHDA (µg/rat)	Dose (+)-amphetamine (mg/kg)	Increment in activity (counts/10 min/rat)		Increment in activity as per cent of control activity	
		Pre 6-OHDA	Post 6-OHDA	Pre 6-OHDA	Post 6-OHDA
100	0·5	174 ± 24	396 ± 20†	115 ± 16·5	257 ± 20·4†
	1·5	358 ± 22	547 ± 31†	229 ± 13·8	367 ± 29·9†
500	0·5	204 ± 13	148 ± 27*	113 ± 7·4	124 ± 21·8
(2 × 250)	1·5	339 ± 23	272 ± 28*	187 ± 15·4	221 ± 24·0

Measurements were made 55 min after (+)-amphetamine, after subtraction of the respective control values for saline injected rats. Values given are means ± *S.E.* for sixteen animals.

* $0{\cdot}1 > P > 0{\cdot}05$. † $P < 0{\cdot}001$ (with respect to pre 6-OHDA value; *t* test).

amphetamine response may be related to this change in the ratio of the two catecholamines. It has been suggested that the dopaminergic system of the neostriatum may be directly involved in the hyperactivity produced by (+)-amphetamine[8] and that NA may have inhibitory effects on activity[9,10].

Animals with the severe depletion of brain NA and DA induced by 2 × 250 µg of 6-OHDA were difficult to distinguish from normal animals, when examined 7 or more days after treatment. During the first few hours after receiving the drug, the animals assumed a posture with hunched back and raised fur, resembling reserpine treated rats. Motor activity and rearing were much reduced for the first few days, and the animals were extremely sensitive to tactile stimuli, preferring to remain isolated from other rats in the home cage during this period. They were more irritable on handling than normal rats, and this persisted after the general appearance had returned to normal (one week after injection). Food intake and water intake decreased initially, but recovered within 72 h after drug treatment. It is possible that during recovery some functions of the brain catecholamines are taken over by residual catecholamine neurones or by other neural systems. The catecholamines remaining in the brain after 2 × 250 µg of 6-OHDA were depleted by treatment with reserpine (5 mg/kg, intraperitoneally), which produced a sedation in animals whose appearance had returned to normal.

Animals treated with 100 µg of 6-OHDA showed few obvious changes in behaviour, even during the first week after treatment. Mock fighting behaviour was observed in some of these animals after the administration of 1·5 mg/kg (+)-amphetamine; this resembled the behaviour reported in rats treated with L-DOPA and a peripheral DOPA decarboxylase inhibitor[11]. This "bizarre social behaviour" was not observed in normal animals or in those treated with 2 × 250 µg of 6-OHDA, and may also be related to the change in the ratio of NA and DA in the brain.

By using 6-OHDA it is possible to produce selective chemical lesions of catecholamine-containing neurones in the CNS, and such lesions seem to be virtually permanent. Furthermore, by adjusting the dose of 6-OHDA, a selective destruction of noradrenergic rather than dopaminergic nerve terminals may be achieved. Although brain catecholamines have been implicated in a wide variety of important behavioural and physiological functions, gross depletions of these amines by 6-OHDA do not seem to produce correspondingly large changes in behaviour.

We thank Drs H. Thoenen and J. Tranzer of F. Hoffmann La Roche, Basle, Switzerland, for generous supplies of 6-hydroxydopamine. K. D. E. is supported by a scholarship from the Medical Research Council.

K. D. EVETTS
N. J. URETSKY
L. L. IVERSEN
S. D. IVERSEN

Department of Experimental Psychology
and Department of Pharmacology,
University of Cambridge,
Cambridge.

Received January 5, 1970.

[1] Uretsky, N. J., and Iversen, L. L., *J. Neurochem.*, **17**, 269 (1970).
[2] Thoenen, H., and Tranzer, J. P., *Arch. Pharmak. Exp. Path.*, **261**, 271 (1968).
[3] Malmfors, T., and Sachs, C., *Europ. J. Pharmacol.*, **3**, 89 (1968).
[4] Burkard, W. P., Jalfre, M., and Blum, J., *Experientia*, **25**, 1295 (1969).
[5] Smith, C. B., *J. Pharm. Exp. Ther.*, **142**, 343 (1963).
[6] Stein, L., *Fed. Proc.*, **23**, 836 (1964).
[7] Weissman, A., Koe, B. K., and Tenen, S. S., *J. Pharm. Exp. Ther.*, **151**, 339 (1966).
[8] Munkvad, I., Pakkenberg, H., and Randrup, A., *Brain Behaviour and Evolution*, **1**, 89 (1968).
[9] Dewhurst, W. G., *Nature*, **218**, 1130 (1968).
[10] Spooner, C. E., and Winters, W. D., *Brain Res.*, **4**, 189 (1967).
[11] Lammers, A. J. J. C., and van Rossum, J. M., *Europ. J. Pharmacol.*, **5**, 103 (1968).

Aberrant mRNA in Parkinsonism: Support for Hypothesis from Studies with Chloramphenicol

THE observations that the normally high concentration of dopamine in the substantia nigra is reduced in Parkinsonism[1], that reserpine, which depletes catecholamines, can produce a Parkinsonian syndrome[2] and that both anticholinergic drugs and L-dopa partially relieve the symptoms of the disease[3,4], have led to the implication of an imbalance between dopaminergic and cholinergic activity in Parkinsonism. Our recent results, however, support previous reports that, apart from a neurotransmitter deficiency, a dysfunction of protein synthesis might be involved. According to this hypothesis, an unknown agent interferes with the normal function of the glial genome. It stimulates the synthesis of new messenger RNA (mRNA) which specifies proteins for growth and division. The normal inverse metabolic coupling between neurone and glia is disturbed during proliferation, and as a result similar changes to those in the proliferating glia may occur in the neurone, leading to its biochemical and functional de-differentiation. The neurone may no longer be capable of synthesizing certain functional proteins, including those needed for neurotransmitter synthesis, transport and storage. As a result, though not degenerate, it would fail to act as a signalling device. Our data in support of this hypothesis are (*a*) that reserpine, which induces a Parkinsonism-like syndrome in man[2], induces increased synthesis of DNA, RNA and proteins in animals, and intense glial proliferation[5]; (*b*) that an increase in RNA, inversely related to melanin content, occurs in the nigral neurones of Parkinsonian patients who died during the early stages of the disease (M. I., submitted for publication); (*c*) that an abnormal electrophoretic pattern of blood cell proteins was found in Parkinsonian patients (H. Pataryas and C. N. S., manuscript in preparation).

Previously it has been found that gliosis is constantly found in the substantia nigra affected by the disease, that demelanization of nigral neurone is commonly seen even when no loss of neurones is observed (MacAlpine, cited in ref. 6), and that demelanization of nigral cells occurs in humans when reserpine and phenothiazine derivatives are used therapeutically[7]. Moreover, in biopsy material of globus pallidus, a significant increase in aberrant mRNA content in the glia was found early in the development of the disease and a similar increase in RNA in the neurones was evident later[8]. Other studies have demonstrated that demelanization of cultured pigmented retinal epithelium cells occurs as a result of a dramatic increase in the rate of synthesis of new mRNA for the production of growth proteins for the rapidly dividing cells[9]. Further, it has been shown that cultured heart cells grown as monolayers dedifferentiate and stop beating[10]. Finally, cell hybridization experiments indicate that in hybrids of melanin producing cells with non-pigmented cells the non-pigmented cell genome may repress the expression of tyrosinase genes[11] and "such dedifferentiated pigment cells covertly maintain their potentiality to express the original melanotic function and can be made to re-express it"[9].

To test our hypothesis we first studied the effects of certain inhibitors of protein synthesis on patients with Parkinsonism, because some of these inhibitors, in addition to blocking proliferation, may also restore the "differentiated" state of the cell[9,10]. Chloramphenicol was chosen because it specifically inhibits protein synthesis resulting from the rapid turnover of mRNA in proliferating cells[12] and does not affect protein synthesis of differentiated mammalian cells[13], including neurones[14].

Patients included in the study were clinically diagnosed as suffering from Parkinson's disease; they understood that the treatment involved the risk of side effects, for the early detection of which frequent blood tests would be required. Age, sex, severity and course of the disease, possible aetiological factors and prevalence of particular neurological signs were random variables. Twenty-four subjects were selected; three were excluded on account of slight side effects, and of the remainder, eighteen Parkinson patients received chloramphenicol and three (controls) did not. The severity of major neurological signs such as rigidity, tremor and dyskinesia was noted before administering the drug. Speech impairment, abnormalities of posture and gait, extent of salivation, lachrymation and dysphagia were recorded. Standard functional tests included the time required for, and manner of, rising from a chair and a bed, handwriting, shuffling, dressing, undressing and walking a fixed distance (in the latter case the number of steps was also noted). Ergographic, EMG and H-reflex tracings were obtained. Haematological tests were performed three times a week. Blood pressure was measured regularly and ECGs and EEGs were taken.

Each patient was assigned to one of five groups of examiners, and was also periodically inspected by other examiners. One examiner knew all the patients and made his own evaluation, keeping independent records. Cinematographic records of eight patients were also made before, during and after the treatment. Patients who, on admission, had been receiving antiacetylcholine drugs continued to do so; others received only chloramphenicol. The dose of chloramphenicol was 1·5–2 g administered orally each day throughout the period of treatment (3–6 weeks). Patients knew that they were to be given a multiple stage treatment but were not told that this included innocuous pills before and/or after administration of chloramphenicol. Four patients received placebos as well for about a week before and a week after the administration of chloramphenicol.

The results were assessed semi-quantitatively. It was noted that a clinical change occurred in nearly all patients on active chloramphenicol treatment. The degree of response ranged from no improvement (one case) to complete restoration to normal (one case). The delay between administration of the drug and the appearance of the first signs of improvement ranged from 4 to 17 days, duration of delay apparently being unrelated to degree of clinical response. One patient, for example, experienced both the longest delay before observable improvement and the greatest benefit: all symptoms disappeared in four weeks. Improvement first appeared as a decrease in intensity in one or more of the neurological signs and/or as an increase in general mobility. Often the patient reported the change spontaneously. Dyskinesia and rigidity seemed to be affected earlier than tremors. In several cases, tremor disappeared completely, but considerable rigidity and dyskinesia persisted: this indicates that no correlation exists between the sequence and degree of any particular sign. Maximal improvement was reached 1·5–4 weeks after the onset of change. It persisted for a variable time (1–4 weeks after the termination of therapy). The decline followed a sequence which was the reverse of that noted in the course of improvement: tremor, the last sign affected by the drug, was the first to re-emerge upon the cessation of treatment. Some cases reverted to pretreatment level in less than two weeks; others still showed some benefit three months later. No clear relationship was noted between degree and duration of improvement following drug administration.

The results demonstrate that chloramphenicol, a specific inhibitor of protein synthesis in rapidly proliferating cells, modifies the chief symptoms of Parkinsonism. Viewed in conjunction with the concept of a close neurone–glia metabolic coupling[15], this supports the hypothesis that a protein synthesis abnormality in the nigral neurone, initiated by aberrant metabolism of its glia, is involved in the pathogenesis of this disease. The interval between the beginning of the therapy and the occurrence of observable clinical effects is consistent with the time required for the neurones to switch from the "dedifferentiated" to the "differentiated" state. The variability of clinical response is explicable on the assumption that the degree of clinical change depends on the percentage of "dedifferentiated" neurones which can be induced to revert to a "differentiated" state. Indeed, drugs like chloramphenicol may be more valuable in prevention than in cure.

Our results do not necessarily conflict with the view that dopamine deficiency is the primary cause of Parkinson's disease, because amine biosynthesis and function are dependent on protein containing carriers. The clinical effect of L-dopa administration may, however, not be attributable solely to the action of dopamine as a neurotransmitter. A direct effect of dopamine on nucleic acid synthesis has been demonstrated[16].

Our emphasis upon the beneficial effect of chloramphenicol on Parkinsonism is not meant to suggest a routine clinical use of the drug, but the results do indicate possible new approaches to a study of the pathogenesis and treatment of the disease.

C. N. STEFANIS

Neurobiology Research Laboratory,
Department of Neurology,
Athens National University,
Eginition Hospital.

M. ISSIDORIDES

Department of General Biology,
Athens National University,
Athens, Greece.

Received September 10, 1969.

[1] Hornykiewicz, O., *Wien. Klin. Wschr.*, **75**, 309 (1963).
[2] Steck, H., *Ann. Méd. Psychol.*,**112**, 737 (1954).
[3] Birkmayer, W., and Hornykiewicz, O., *Wien. Klin. Wschr.*, **73**, 787 (1961).
[4] Cotzias, G., Papavasiliou, P. S., and Gallene, R., *New England J. Med.*, **280**, 337 (1969).
[5] Mytilineou, C., *Biological Psychiat.*,**1**, 61 (1969).
[6] Friede, R., *Arch. Exp. Path. Pharmakol.*,**218**, 286 (1953).
[7] Forrest, F. M., Forrest, I. S., and Roizin, L., *Agressologie*, **4**, 259 (1963).
[8] Gomirato, G., and Hydén, H., *Brain*, **86**, 773 (1963).
[9] Whittaker, J. R., *J. Exp. Zool.*,**169**, 143 (1968).
[10] McCarl, R. L., and Shaler, R. C., *J. Cell Biol.*, **40**, 850 (1969).
[11] Davidson, R., Ephrussi, B., and Yamamoto, K., *J. Cell Physiol.*, **72**, 115 (1968).
[12] Weisberger, A. S., and Wolfe, S., *Fed. Proc.*,**23**, 976 (1964).
[13] Firkin, F. C., and Linnane, A. W., *Exp. Cell Res.*, **55**, 68 (1969).
[14] Rubin, A. L., and Stenzel, K. H., *Neurosciences Res. Prog. Bull.*, **3**, 25 (1965).
[15] Hydén, H., in *The Neuron* (edit. by Hydén, H.), 179 (Elsevier, Amsterdam, 1967).
[16] Seale, R. U., and Prasad, K. N., *Anat. Rec.*,**163**, 259 (1969).

Survival in Adult Mealworm Beetles (*Tenebrio molitor*) of Learning acquired at the Larval Stage

USING larvae of the beetle *Tenebrio molitor*, we tested the possibility that learning might survive through metamorphosis. This beetle shows a complete metamorphosis involving practically all parts of the body. Accurate information about the modifications of the epicuticle and its related structures in metamorphosis is available[1], but little is known about the modifications of the central nervous system.

The learning ability of the larva was compared with that of the adult of *Tenebrio* 27 years ago by Von Borell du Vernay, who found[2] no significant difference between the two stages. These findings were interpreted by him in accordance with the earlier observations of Bretschneider[3] on the "anatomical stability" of the mushroom bodies during the metamorphosis. Von Borell du Vernay suggested also that the "corpora pedunculata" could be

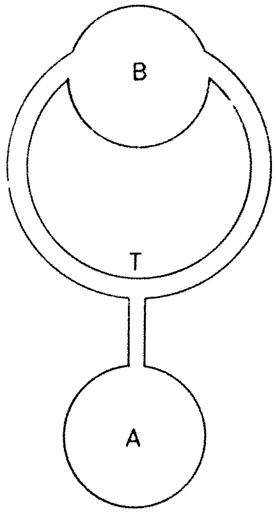


Fig. 1. The maze used for training the larvae and testing the adults. *A*, Starting point; *B*, goal; *T*, choice point. Total length of the path *AB*, 11 cm; width of the alleys, 4 mm for the larvae and 8 mm for the adults.

the anatomical site of memory. Vowles has recently shown that the "corpora pedunculata" in ants play a part in learning[4].

We trained larvae[5] and tested for persistence of the learned behaviour in the adults. A group of larvae, whose age homogeneity[6] was obtained by choosing animals with initial body weight in the range 8–15 mg, was split into two subgroups of one hundred each, the learning group L and the control group L_0. We used the simple technique of a two way maze (Fig. 1), the punishment being a bright light, the reward darkness. The larval and adult stages show a strong negative phototaxis. All animals passed through the maze once a day: the L group were punished immediately after a wrong choice, the L_0 group were not. Each animal lived in an individual box, and records were kept throughout its life. After each passage, the maze was changed so as to avoid clues deriving from previous use and was washed before being used again; bridges with photo-cells and thermistors assured the absence of light and temperature asymmetry between the two sides of the maze.

The learning group L was divided at random into two equal subgroups, the RL (right learning) and the LL (left learning) group: the RL animals were punished when the first three body segments had entered the left alley of the maze; the corresponding procedure was followed for the LL group. The number of choices made by the animals of the two groups, L, L_0, are given in Table 1.

The pupal stages were kept in the dark in their boxes, at room temperature, and at about 22° C and 50–60 per cent humidity. The duration of the pupal stage, in these conditions, averaged 17 days.

Table 1. RESULTS OBTAINED FOR THE FOUR GROUPS OF *Tenebrio*

		Observed number of choices towards the right	Observed number of choices towards the left	Total number of choices	Expected number of choices from chance	P-level
Larvae	RL	307	197	504	252	0·0005
	LL	194	277	471	235·5	0·007
Adults	RA	139	91	220	110	0·005
	LA	81	129	210	105	0·018
Control	L_0	304	309	613	306·5	> 0·95
	A_0	109	109	218	109	> 0·99

L, Larvae of the learning group; RL, larvae learning to go to the right; LL, larvae learning to go to the left.

A, Adults derived from larvae of the learning group; RA, adults which received training to go to the right in the larval stage; LA, adults which received training to go to the left in the larval stage.

L_0, A_0, Larvae and adults of the control group.

Adults of both groups were never trained; they were only tested. The last column gives the P-level obtained from the χ^2-test.

Thirty adults of the larval learning group and thirty of the control, selected at random at the end of metamorphosis, were passed daily without punishment through mazes similar to those that were used for the larvae, except that the width of the alley had been modified to maintain for the adults the same body-to-wall relationship. In this manner we obtained two samples of adults: adults A, the larvae of which had been trained, and adults A_0, which had not been trained. Table 1 shows the number of choices by each group towards right or left. The number of trials was less than with larvae, because the average life of the adult is shorter. The data clearly show that the adults A with previous experience of learning showed a significant tendency to perform as they had done as larva.

To show the absence of inherent tendencies to prefer one of the two directions in both life stages, we computed the correlation coefficient r between the choices towards right and left for each animal in the L_0 and A_0 groups. The r-value shows practically zero correlation ($r = 0·253$, $t = 1·420$, $0·10 < P < 0·20$).

There is therefore clear evidence that learning survives through metamorphosis. This agrees with the fact that at least some part of the brain remains relatively unchanged, for example, the corpora pedunculata and protocerebral bridge (Von Borell du Vernay[2]).

We thank Professor E. M. Eisenstein, State University of New York, and Professor J. Z. Young, University College London, for helpful discussions.

ANTONIO BORSELLINO
RUGGERO PIERANTONI
BARBARA SCHIETI-CAVAZZA

Istituto di Fisica dell'Università di Genova,
Laboratorio di Cibernetica e
Biofisica del CNR,
Camogli, Italy.

Received November 12, 1969.

[1] Wigglesworth, V. B., *The Physiology of Insect Metamorphosis* (Cambridge Univ. Press, 1954).

[2] Von Borell du Vernay, W., *Z. Vergl. Physiol.*, **30**, 84 (1942).

[3] Bretschneider, F., *Z. Naturw.*, **52**, 261 (1914).

[4] Vowles, D. M., in *Current Problems in Animal Behaviour* (edit. by Thorpe, W. H., and Zangwill, O. L.), 5 (Cambridge Univ. Press, 1966).

[5] Borsellino, A., Pierantoni, R., and Schieti-Cavazza, B., Atti Congr. Gruppo Naz. di Cibernetica del CNR, Pisa, 20–21 Aprile, 1967, 29–36.

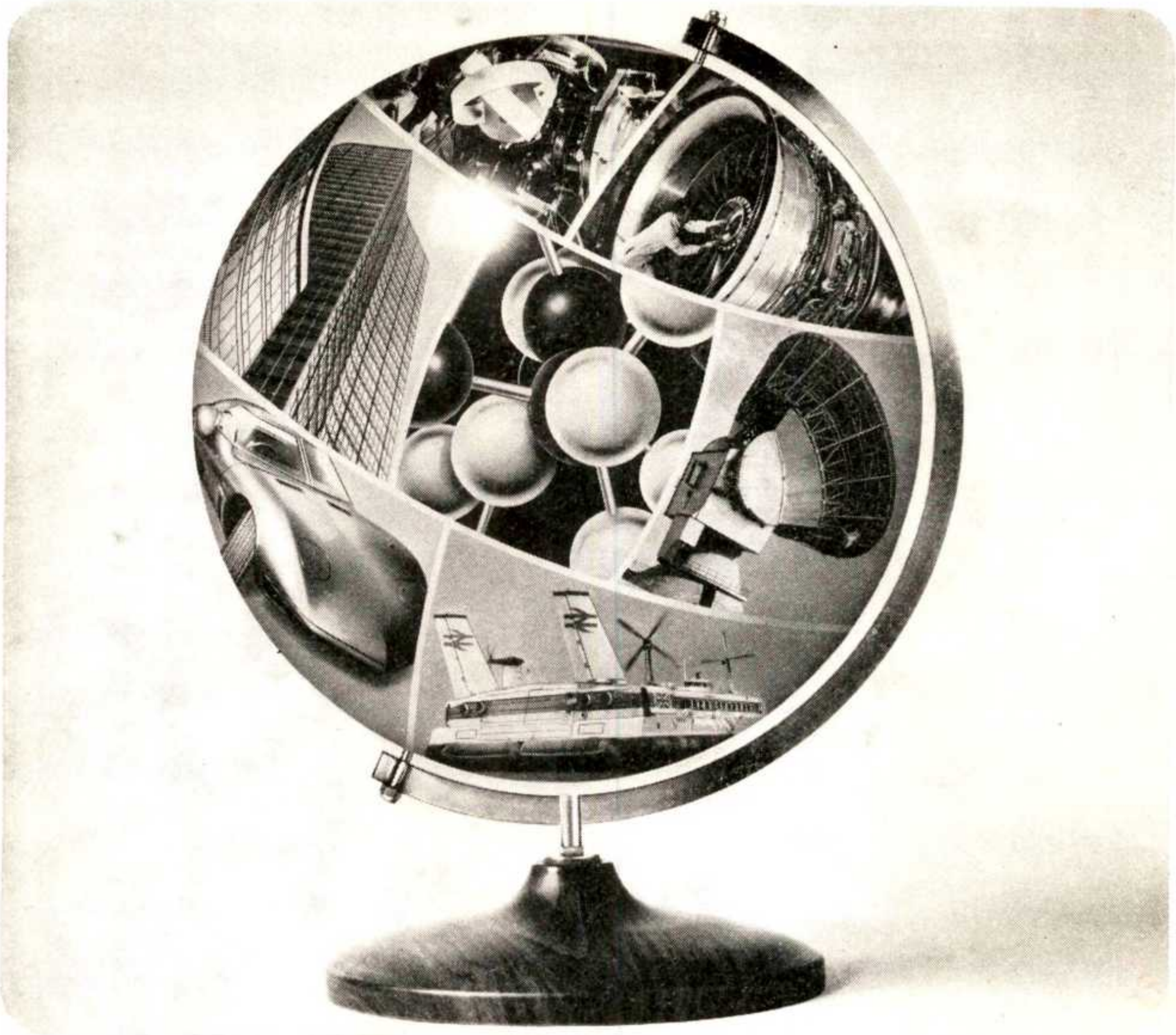
[6] Leclerq, J., *Bull. Soc. Roy. Sci. Belg.*, **6**, 296 (1956).

Gregarines: their Effect on the Growth of the Desert Locust (*Schistocerca gregaria*)

ALL stages of the desert locust *Schistocerca gregaria* are infected with a eugregarine *Gregarina garnhami*[1,2] (Fig. 1*a*). The morphology and life history of gregarines have been studied extensively, but little is known about the effects of gregarines on their invertebrate hosts.

The larvae of the mealworm *Tenebrio molitor* are normally unaffected[3] by heavy infestations of *G. polymorpha*, although pupal weight and length of larval life are influenced when other conditions are sub-optimal.

Lewis[4] has suggested that "a gregarine *Echinomera hispidia* may be responsible for the low number of *Lithobius forficatus* in damp woodlands" and Lipa[5] has concluded that serious damage is caused to the midgut epithelium of insects by gregarines. Except in cases of massive synchronous infection of sporozoites, the physical damage to epithelial cells is slight; eugregarines probably exert their greatest effect by acting as a physiological barrier between midgut cells and the digested food (Fig. 1*b*).



Tomorrow's world
depends on today's mathematics teachers.
If you are a maths graduate come and join them.

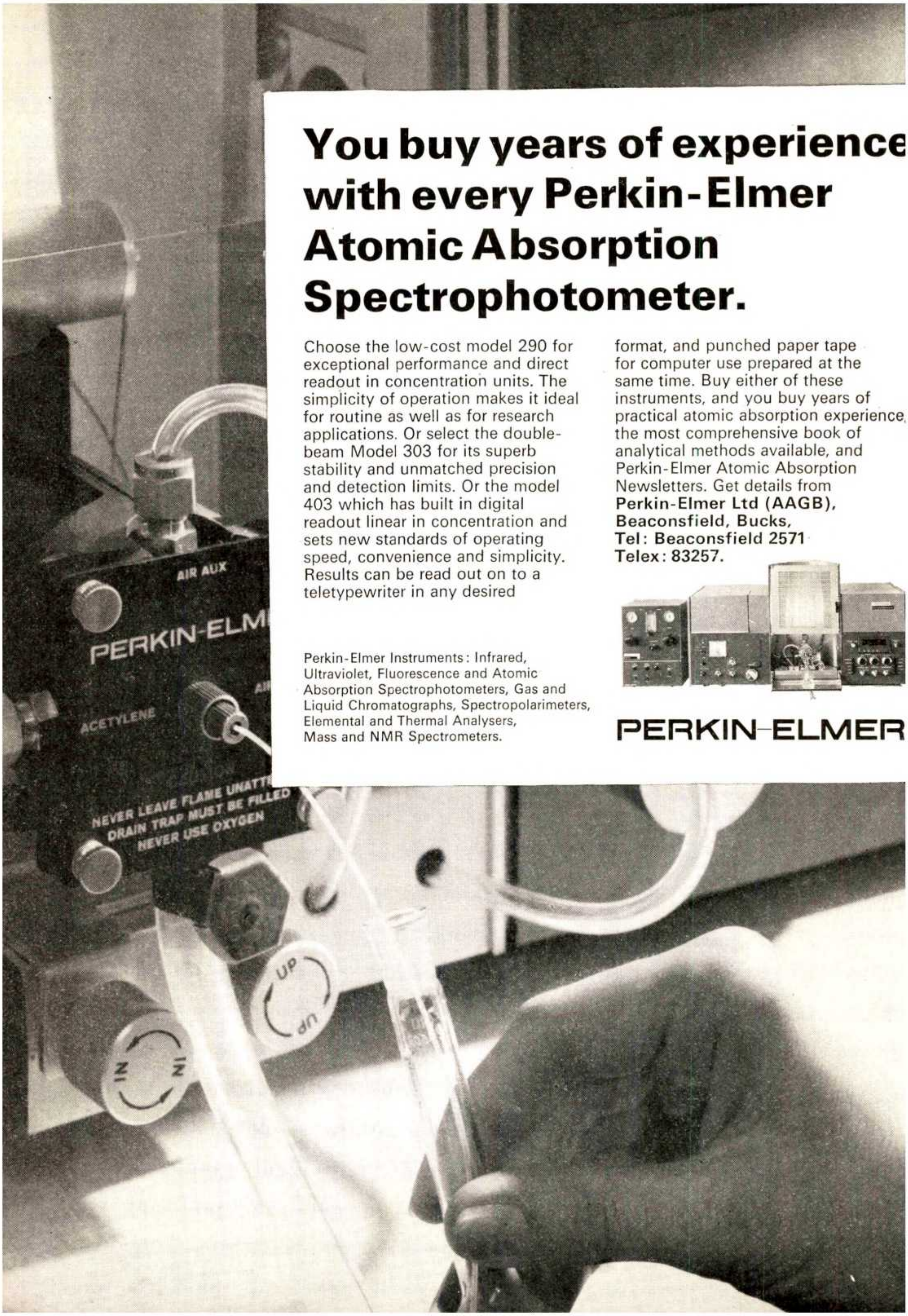
It's scarcely an exaggeration to say that the country's future depends on the quality of today's maths teachers. Technological advances depend on a sound understanding of mathematics. And that can only be gained from teachers dedicated to making their subject come alive. There's no room for boredom in modern maths lessons. And no chance of it in a modern teacher's world. Over 40% of maths and science graduates who started teaching seven years ago are already heads of departments. One in five graduates over 40 is a head teacher, while others put their teaching experience to good use in administration or inspection. Jobs for mathematics teachers are available in almost every part of the country. And there are long holidays.

Pay and Prospects. Starting salaries are competitive with those of other careers, ranging for graduates from £965 to £1,170. Looking forward, as head of a large department you could earn over £2,600; a deputy headship could take you above the £3,000 mark. Promotion, like responsibility, is seldom long in coming when you're a mathematics teacher. And if one is looking at long-term prospects one must look at the educational service as a whole: not all the jobs for which teaching experience is needed are in the schools. Want to find out more about teaching? Read our free booklet 'Careers in Education for Graduates'. Available from: The Department of Education and Science (Dept. N.2, Room 107), Curzon Street, London, W1Y 8AA.

Are you already in employment? Measure what you're doing now against teaching. Is it as interesting as you expected? Are the prospects as good? Are you in charge of your own daily activities in the way a teacher is? You can still come into teaching—you'll be *very* welcome.

Looking forward?

TEACH MATHS



You buy years of experience with every Perkin-Elmer Atomic Absorption Spectrophotometer.

Choose the low-cost model 290 for exceptional performance and direct readout in concentration units. The simplicity of operation makes it ideal for routine as well as for research applications. Or select the double-beam Model 303 for its superb stability and unmatched precision and detection limits. Or the model 403 which has built in digital readout linear in concentration and sets new standards of operating speed, convenience and simplicity. Results can be read out on to a teletypewriter in any desired format, and punched paper tape for computer use prepared at the same time. Buy either of these instruments, and you buy years of practical atomic absorption experience, the most comprehensive book of analytical methods available, and Perkin-Elmer Atomic Absorption Newsletters. Get details from

Perkin-Elmer Ltd (AAGB),
Beaconsfield, Bucks,
Tel: Beaconsfield 2571
Telex: 83257.

Perkin-Elmer Instruments: Infrared, Ultraviolet, Fluorescence and Atomic Absorption Spectrophotometers, Gas and Liquid Chromatographs, Spectropolarimeters, Elemental and Thermal Analysers, Mass and NMR Spectrometers.

The locusts used in this experiment came from a culture that has been maintained in the department for several years and originated from the Anti-Locust Research Centre, London.

To obtain hoppers that were free from gregarines, eggs were surface-sterilized by immersion in 70 per cent ethyl alcohol for 5 min, and left to hatch in sterile glass chambers.

Hoppers were grown in jars designed specially for maintaining parasite-free insects[6]. Five hoppers were placed in each jar, sharing a volume of approximately 1,000 cm³. When all the hoppers had moulted to the second instar (after 5–6 days) they were placed individually into 2 × 1 inch glass vials, randomized into two batches of 40 and designated *A* and *B*.

Batch *A* were offered barley shoots on which were placed gregarine sporocysts. Batch *B* were offered barley shoots free from sporocysts. After all the hoppers had eaten the barley they were returned to the jars, where they remained—four to each jar—until they had moulted to the fifth instar. They were then further subdivided—two per jar—until the end of the experiment. The hoppers were fed bundles of fresh barley shoots; a supply of moist fresh shoots was always available.

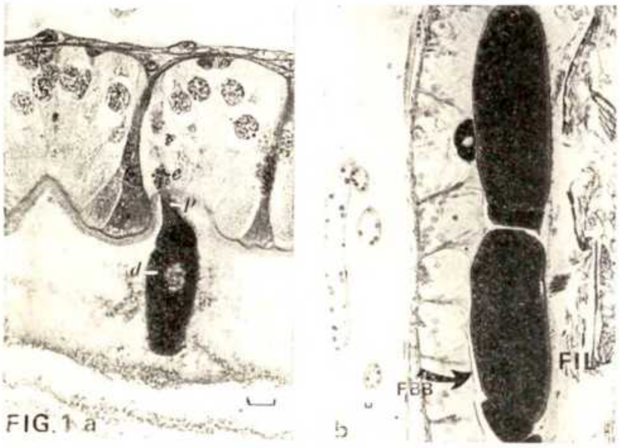


Fig. 1. *a*, L.S. of cephalont attached to midgut epithelium of *S. gregaria*: *e*, epimerite; *p*, protomerite; *d*, deutomerite. 8 μm section. P.A.S. celestine blue, Mayers haemalum and Orange G. Scale approximately 10 nm. *b*, L.S. of two sporonts in syzygy illustrating how such associations can act as a barrier between food in lumen (FIL), and free brush border (FBB) of epithelial cells. Just one such association could cover the free surface of some 500 intestinal cells. Stain as in *a*. Scale approximately 10 μm.

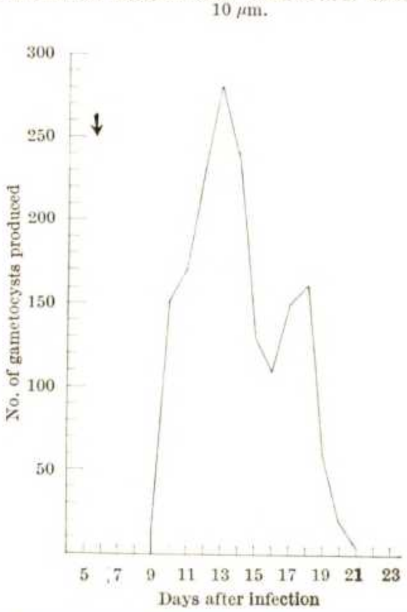


Fig. 2. The number of gametocysts produced by all infected locusts over the 23 days after exposure to sporocysts. The arrow shows when the first associations are found free in the intestine.

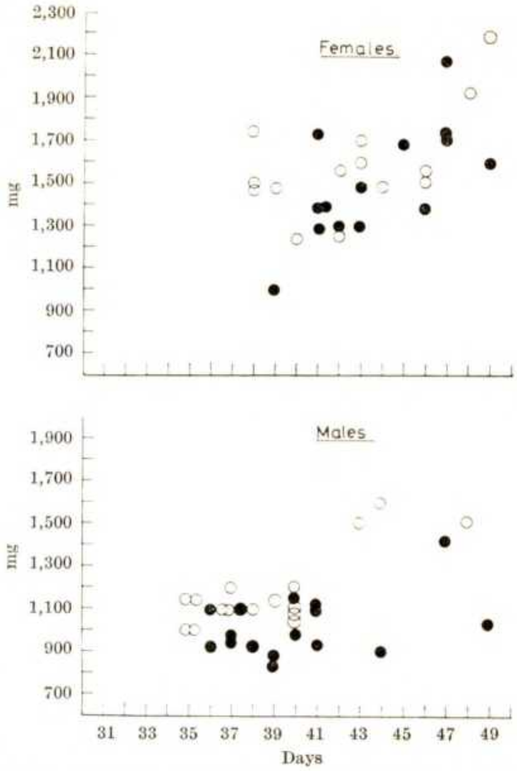


Fig. 3. Scattergrams of weights of fledglings against length of time from hatching to fledging for males I and NI bottom, and females I and NI above. ○, Not infected; ●, infected.

The jars were arranged in a circle around a 60 W electric light bulb, and the light period was controlled at 10 hours. The temperature inside the jars was 30° ± 2° C when the light was on, and 26° ± 1° C when it was off.

The degree of infection was checked in the live animals by examining the faecal pellets on consecutive days after infection; daily gametocyst production is shown in Fig. 2, and reaches a peak around 12–14 days after infection. Five infected hoppers were killed to check the degree of infection, which was found to be moderate (100–200 gregarines per locust). The weights of all the locusts were taken within a few hours of the final moult.

To study any changes taking place in infected gut cells, techniques similar to those used previously[2] were used.

Sixty locusts developed completely and soon after the final moult an analysis was carried out on the weight, and on the total length of nymphal life (Tables 1–3). There were two significant differences between sexes, the males being lighter in weight and having a shorter nymphal life than the females. Infection had no effect on the

Table 1. ANALYSIS AFTER FINAL MOULT

		Length of nymphal life Mean time in days		Mean weights (mg)	
		Infected	Non-infected	Infected	Non-infected
Males	(32)	40	39	1,026	1,181
Females	(28)	43	42	1,507	1,587

Table 2. ANALYSIS OF VARIANCE OF TIME FROM HATCHING TO FINAL MOULT (see Fig. 3)

	N	*SS*	*MS*	*VR*	*P*
Bet. sexes	1	194·7860	194·7860	14·9469	⩽0·001 V. sig.
Bet. treatments	1	20·4167	20·4167	1·5667	N. sig.
Treat. × sex	1	0·0075	0·0075	<<<1	N. sig.
Replicates	56	729·7898	13·0319		
Total	59	935			

Table 3. ANALYSIS OF VARIANCE OF FINAL WEIGHTS OF FLEDGLINGS

	N	*SS*	*MS*	*VR*	*P*
Bet. sexes	1	2·973215	2·973215	122·5128	⩽0·001
Bet. treatments	1	0·226935	0·226935	9·35097	⩽0·001
Treat. × sex	1	0·0242686	0·0242686	0·5374	N. sig.
Replicates	56	2·5741714			
Total	59	5·9784			

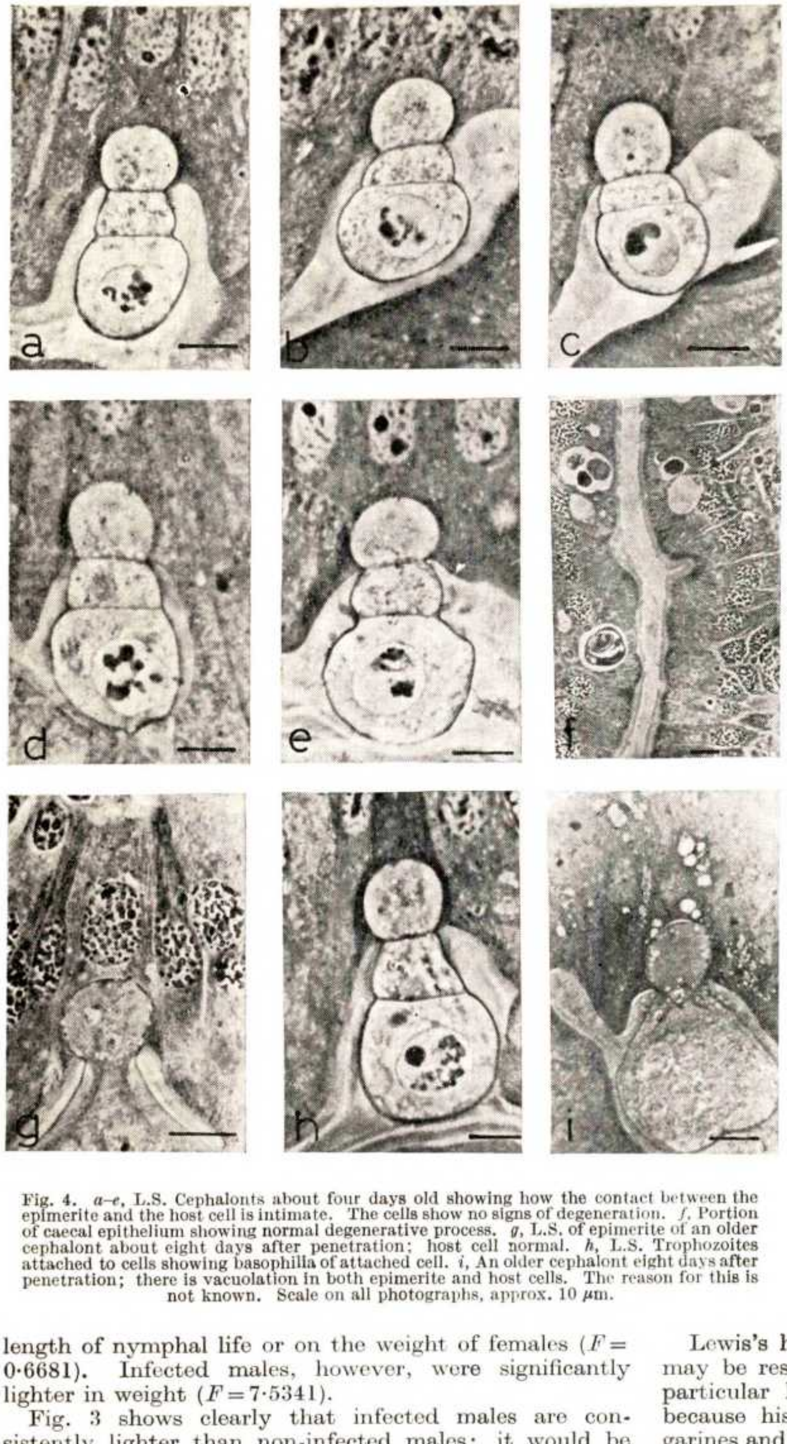


Fig. 4. *a–e*, L.S. Cephalonts about four days old showing how the contact between the epimerite and the host cell is intimate. The cells show no signs of degeneration. *f*, Portion of caecal epithelium showing normal degenerative process. *g*, L.S. of epimerite of an older cephalont about eight days after penetration; host cell normal. *h*, L.S. Trophozoites attached to cells showing basophilia of attached cell. *i*, An older cephalont eight days after penetration; there is vacuolation in both epimerite and host cells. The reason for this is not known. Scale on all photographs, approx. 10 µm.

length of nymphal life or on the weight of females ($F=0\cdot6681$). Infected males, however, were significantly lighter in weight ($F=7\cdot5341$).

Fig. 3 shows clearly that infected males are consistently lighter than non-infected males; it would be interesting to compare the effects of gregarines on both sexes in conditions less favourable to the host—for example, when food was in short supply.

Fig. 4 (*a–i*) shows a number of gregarines attached to individual midgut cells of *Schistocerca*. Cells that have gregarines attached to them show signs of displacement at their free ends, but it is not clear whether the displacement causes temporary or permanent damage; only in a small number of cells can degenerative effects be observed.

The following data were recorded from sections taken from a late instar hopper five days after infection with sporozoites and one day before a moult: (1) Cells without gregarines attached showing degeneration: many hundreds (Fig. 4(*f*)). (2) Cells with gregarines attached not showing damage: 45 (Fig. 4 (*a–e*)). (3) Cells with gregarines attached showing damage (basophilia): 2 (Fig. 4 (*h*)).

Gregarines remain attached to host cells for relatively short periods of time (5–8 days); they then become detached and form associations that remain in the midgut and continue to grow. Fig. 2 shows the daily production of gametocysts. Detached pairs in association can be found after 5–6 days (arrow, Fig. 2).

The gregarines shown in Fig. 4 (*a–e*) had been attached for 4–6 days. Fig. 4 (*g*) is the epimerite of a large gregarine eight days after infection. In the desert locust, 20 per cent of the midgut cells are replaced at each moult[3]. Biddeman, quoted by Wigglesworth[7], states that there is complete replacement of the midgut epithelium every 48 hours in *Tenebrio molitor*. It would therefore seem that even when eugregarine infections are heavy there will not be sufficient time for significant damage to develop.

Although the locusts were grown in optimal conditions with little competition for food or roosting sites, only moderately heavy infections of gregarines had a significant effect on the weight of the fledgling males. The degree of infection was certainly not as heavy as that usually encountered in routine laboratory stocks of locusts, or in an experiment where more than 10,000 gregarines per locust did not seem to affect moulting or feeding.

The locust thus differs from the mealworm *Tenebrio molitor* in which massive infestations of *Gregarina polymorpha* have no significant effect on larvae grown in optimal conditions.

Lipa's photographs[5], which purport to show damage caused by gregarines, reveal such severe processing damage that determination of gregarine damage is impossible. Because the parasite's survival depends on the survival of the cell to which it is attached, it is unlikely to destroy its essential anchor. Although attached gregarines may impair the functioning of epithelial cells directly, it is more likely that gregarines exert their effect on the physiology of digestion and absorption by presenting a barrier between the free brush border (Fig. 1*b*, FBB) of the intestinal cells and food in the lumen (Fig. 1*b*, FIL).

Lewis's hypothesis[4] that a gregarine (*Echinomera* sp.) may be responsible for the absence of *Lithobius* from a particular habitat does not seem tenable, particularly because his centipedes contained low numbers of gregarines and the differences between numbers of gregarines in both populations were very small. It would seem that some other factor must be responsible for its scarcity from certain habitats.

OWEN G. HARRY

Department of Zoology and
Comparative Physiology,
University of Birmingham.

Received August 4; revised November 24, 1969.

[1] Canning, E. U., *J. Protozool.*, **3**, 50 (1956).
[2] Harry, O. G., *J. Protozool.*, **12** (2), 296 (1965).
[3] Harry, O. G., *J. Protozool.*, **14** (4), 539 (1967).
[4] Lewis, J. G. E., *J. Zool.*, **151**, 163 (1968).
[5] Lipa, J. L., *Acta Protozoologica*, **5**, 92 (1968).
[6] Harry, O. G., *Bull. Ent. Res.*, Part 4, **58** (1969).
[7] Wigglesworth, V. B., *The Principles of Insect Physiology* (Methuen, London, 1959).

Effect of Cycloheximide and Actinomycin *D* on the Behaviour of the Headless Cockroach

AVAILABLE evidence suggests that although actinomycin *D* and cycloheximide inhibit long term memory in mice and goldfish, these antibiotics have little or no effect on the primary acquisition of a new task[1,2]. It was therefore very interesting that Brown and Noble[3] should report that cycloheximide inhibited the ability of a headless cockroach to avoid an electric shock by keeping its leg raised[4]. This behavioural inhibition seems to be correlated with the extent of inhibition of protein synthesis[5]. There are many possible explanations, but Brown and Noble reported no change in sensitivity to the shock stimulus when cycloheximide was given, so that this factor can be ruled out. The activity of the leg, however, was greater after cycloheximide had been given. This suggested that if this drug caused increased activity, it would interfere with the achievement of a criterion (two shocks or less in 3 min) that depended on a relatively quiescent state. We report here the results of an attempt to ascertain whether cycloheximide affects learning performance by the increasing activity of the leg. As well as cycloheximide, we have investigated the effects of actinomycin *D*.

Individuals of *Periplaneta americana* were anaesthetized with carbon dioxide, and the dorsal surfaces were attached to the underside of glass rods, 3/8 inch in diameter and 8 inches long, with warm sticky wax (L. D. Caulk Co., Milford, Delaware). All legs, except the right prothoracic, were covered by a piece of gauze, the ends of which were embedded in Dentina bite wax (Browning's Dental Supply Co., Hull, England) attached to the opposite side of the glass rod. Cockroaches were decapitated and wounds were sealed with 'Vaseline', which was covered 1 h later with flexible collodion (USP, Fisher Scientific Co.) to prevent leakage. The anal area was also sealed with warm sticky wax. The free prothoracic leg of each cockroach was wired with silver wire, 0·004 inch in diameter, according to the method of Eisenstein and Cohen[6], so that the leg received a shock because it completed an electrical circuit when lowered into a saline solution. The direct current voltage in the circuit was controlled between 15 and 20 V, and 10 to 40 μA was registered on the meter. Cockroaches were trained to a criterion of two shocks or less per minute for 3 consecutive minutes, after which the trained leg was primarily in a flexed position.

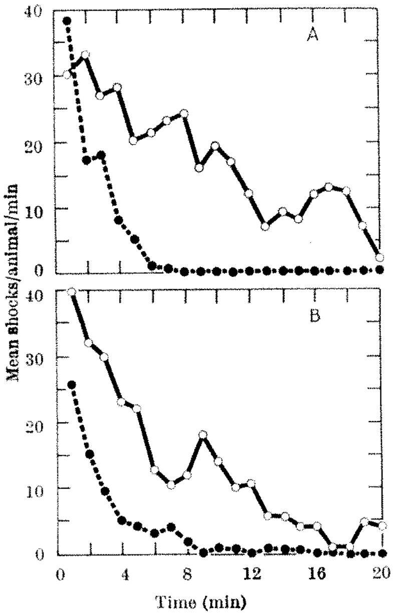


Fig. 1. Effect of cycloheximide and actinomycin *D* administered before training. *A*, Eight cockroaches received two 5 μl. injections of insect saline while eight others received a solution of 0·9 mg/ml. of cycloheximide in insect saline. After 5 min, training commenced (○—○, cycloheximide; ● - - - ●, saline control). *B*, Eight cockroaches received 5 μl. of 1 mg/ml. of actinomycin *D* in insect saline, while eight others received a solution of 0·8 mg/ml. of mannitol in insect saline. After 5 min, training commenced (○—○, actinomycin *D*; ● - - - ●, mannitol saline control).

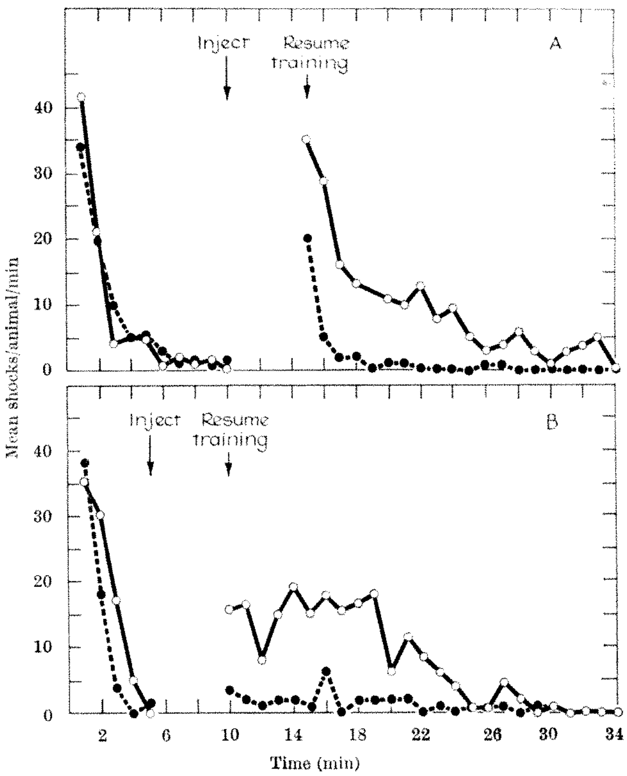


Fig. 2. The effect of cycloheximide and actinomycin *D* administered after training. *A*, After 10 min of training, eight cockroaches received cycloheximide while eight others received saline injections as described in Fig. 1*A*. Training resumed after 5 min (○—○, cycloheximide; ● - - - ●, saline control). *B*, After 5 min of training, eight cockroaches received actinomycin *D* while eight others received mannitol saline injections as described in Fig. 1*B*. Training resumed after 5 min (○—○, actinomycin *D*; ● - - - ●, mannitol saline control).

Drugs were administered in insect saline (0·16 M NaCl; 0·0018 M $CaCl_2$ $2H_2O$; 0·002 M KH_2PO_4; 0·0018 M K_2HPO_4) (ref. 7) containing either 1 mg/ml. of actinomycin *D* (Nutritional Biochemical Corp.) or 0·94 mg/ml. of cycloheximide (Sigma). Controls for the actinomycin *D* experiments were injected with a solution containing 0·8 mg/ml. of mannitol in insect saline while the controls for the cycloheximide experiments received insect saline. Injections were made through the ventral surface of the prothorax using a 10 μl. Hamilton syringe. All solutions were coded and all experiments were conducted blind.

Typical consequences of injection (Fig. 1*A* and *B*) were similar with both drugs. Animals injected with either drug were more active and received more shocks than control animals at all points. The effects of cycloheximide agree with the data of Brown and Noble[3,5]. Clearly the animals receiving cycloheximide or actinomycin *D* experienced delay in acquiring the avoidance behaviour which minimizes the number of shocks (that is, a raised leg) and throughout the training procedure they received more shocks.

To test whether the drugs affected the primary acquisition of the task or the activity of the leg, these were administered after the cockroaches had achieved criterion. The learning curves are shown in Fig. 2*A* and *B*. After training, the animals were injected with either the drug or the control solution and 5 min later training was resumed. Fig. 2 shows that both cycloheximide and actinomycin *D* interfere with the performance of the trained legs. This was accompanied by increased leg activity that led to increased shocks. The number of shocks decreased rapidly in the group that did not receive drugs, while the treated cockroaches experienced a much slower decline in the number of shocks per minute.

These results confirm and extend those of Brown and Noble[3,5], who showed that cycloheximide, a potent inhibitor of protein synthesis, brought about a decrease in the acquisition of avoidance behaviour by this cockroach preparation. We have also shown that actinomycin *D*, a potent inhibitor of RNA synthesis, has similar effects. The mechanism of action of the drugs is not clear, however. It is possible that the synthesis of RNA and protein is essential for primary acquisition of a new task, but it is also possible and more probable that the interference with learning performance is secondary to the learning process itself, and occurs because these drugs increase leg activity. This activity interferes with the quiet state essential to enable the leg to remain raised so as to avoid shocks. Eisenstein[8] has also suggested this possibility. This interpretation is strengthened by the fact that a trained leg is also prevented from performing this task by these two drugs. While it is possible that handling during injection caused amnesia so that the preparation had to relearn the task—a process which could be dependent on the synthesis of RNA and protein—it seems more likely that the drugs affected performance by stimulating leg activity. The mechanism by which leg activity could be increased is not known.

We thank Dr E. M. Eisenstein for help and advice. This work was supported in part by a research grant from the US Public Health Service. E. G. was supported by a research career development award from the Division of General Medical Science, US National Institutes of Health. A. H. was supported by a genetics training grant from the US National Institutes of Health.

E. GLASSMAN
A. HENDERSON*
M. CORDLE
H. M. MOON†
J. E. WILSON

Department of Biochemistry and the
Neurobiology Program,
University of North Carolina.

Received October 28, 1969.

* Present address: Columbia University Medical Center, New York City.

† Present address: Laboratory of Molecular Biology, National Institute of Arthritis and Metabolic Diseases, Building 2, National Institutes of Health, Bethesda, Maryland 20014.

[1] Cohen, H. D., and Braondes, S., *Proc. US Nat. Acad. Sci.*, **58**, 157 (1967).
[2] Agranoff, B. W., Davis, R. E., and Brink, J. J., *Brain Res.*, **1**, 303 (1966).
[3] Brown, B. M., and Noble, E. P., *Brain Res.*, **6**, 363 (1967).
[4] Horridge, G. A., *Proc. Roy. Soc.*, B, **157**, 33 (1962).
[5] Brown, B. M., and Noble, E. P., *Biochem. Pharmacol.*, **17**, 2371 (1968).
[6] Eisenstein, E. M., and Cohen, M. J., *Animal Behaviour*, **13**, 104 (1965).
[7] Yamasaki, T., and Narahashi, T., *J. Insect Physiol.*, **4**, 1 (1960).
[8] Eisenstein, E. M., *Brain Res.*, **11**, 471 (1968).

Lytic Enzyme produced *in vitro* by Insect Cells: Lysozyme or Chitinase?

THE mechanism of immunity in insects is very poorly understood: no immunological reaction of the antigen–antibody type is known[1]. Bactericidal or bacteriolytic[2,3] properties of insect tissues, body fluids or even secretions have been reported, but in the absence of precise biochemical data the nature of the substance responsible has not been established.

During the *in vitro* cultivation of insect cells, we noticed that the cells produce a factor which destroys several kinds of microorganisms. We have tried to characterize this substance produced *in vitro*. The insect cell lines we have used were previously developed by one of us[4] from the embryos of two cockroaches: *Periplaneta americana* L. and *Blabera fusca* B. An attempt was made to grow the symbiotic bacteria of *Periplaneta* in these cell cultures[5]. Some of the bacteria were lysed rapidly on association with the cells. Furthermore, on several occasions, these same cells were able to resist different infections by bacteria or fungi.

In studying this lytic activity, therefore, we chose an EP$_a$ cell strain (from *Periplaneta americana*) because of its high resistance to infection. The cultures were grown at 30° C in Roux bottles containing 100 ml. of medium S19 (ref. 6) with a *p*H indicator and without any antibiotic. The initial inoculum was about 10^9 cells. When the *p*H had fallen to 6·5, the medium was collected, filtered through 'Millipore' membranes to remove the cells and dialysed against a *p*H 6·5 phosphate buffer.

Looking for the bacteriolytic substance in the metabolized medium, we tried to establish and then to measure a possible lysozyme-like activity. We used the standard turbidimetric assay against a suspension of killed cells of *Micrococcus lysodeikticus*[7]. The final culture medium, unlike the fresh medium, was significantly bacteriolytic; its enzymatic activity was equivalent to that of a 10–20 µg/ml. solution of hen egg-white lysozyme. This activity disappeared when the cells were ground in the medium. Thus the lysis of *Micrococcus lysodeikticus* may be accounted for by the production and release of an enzyme in the supernatant of EP$_a$ cell cultures. This enzyme has thus the same behaviour as hen egg-white lysozyme (EC 3.2.1.17) in similar conditions, and hydrolyses the mucopolysaccharide found in bacterial cell walls, a polymer of alternating residues of N-acetylglucosamine and N-acetylmuramic acid. A very similar peptidoglycan has been found in the walls of the symbiotic bacteria of cockroaches[8], which would explain their rapid destruction in the medium of EP$_a$ cell cultures.

We also tried similarly to correlate the lysis of yeasts and fungi with the occurrence of a specific enzymatic activity. It is well known that their cell walls are composed chiefly of chitin, a polymer of N-acetylglucosamine[9]. Chitin, however, is usually not a good substrate for enzymatic determinations and it is more convenient to use low molecular polymers of N-acetylglucosamine[10]. We found a highly significant activity against such substrates in the medium metabolized by EP$_a$ cells. For example, an enzymatic preparation which (measured against *Micrococcus lysodeikticus*) corresponds to a 1 µg/ml. solution of hen egg-white lysozyme is able to hydrolyse 300 µg of chitopentaose in less than 4 h. To obtain a similar result with hen egg-white lysozyme, it is necessary, in the same experimental conditions, to use an enzyme concentration which is twenty times as high.

We conclude that the lytic enzyme produced *in vitro* by our insect cells is more like a chitinase (EC 3.2.1.14) than a true lysozyme, for it is a poor muramidase and an excellent chitinase with *M. lysodeikticus* cells and chitopentaose as substrates. With these same two substrates and in similar experimental conditions, an exactly inverse situation was found for lysozymes of vertebrates[11].

J. C. LANDUREAU

Laboratoire de Biologie Animale,
Faculté des Sciences,
12 rue Cuvier, Paris 5.

P. JOLLÈS

Laboratoire de Chimie Biologique,
Faculté des Sciences,
96 Boulevard Raspail,
Paris 6.

Received June 12; revised September 15, 1969.

[1] Steinhauss, E. A., *Insect Pathology* (Academic Press, New York, 1963).

[2] Malke, H., *Z. Allg. Mikrobiol.*, **5**, 42 (1965).

[3] Mohrig, W., and Messner, B., *Biol. Zentralblatt*, **87**, 439 (1968).

[4] Landureau, J. C., *CR Acad. Sci.*, **262**, 1484 (1966).

[5] Landureau, J. C., *Exp. Cell Res.*, **50**, 323 (1968).

[6] Landureau, J. C., and Jollès, P., *Exp. Cell. Res.*, **54**, 391 (1969).

[7] Jollès, P., Charlemagne, D., Petit, J.-F., Maire, A.-C., and Jollès, J., *Bull. Soc. Chim. Biol.*, **47**, 2241 (1965).

[8] Daniel, R. S., and Brooks, H. A., *Experientia*, **23**, 499 (1967).

[9] Bacon, J. S., and Davidson, E. D., *Biochem. J.*, **101**, 36 (1966).

[10] Rupley, J. A., *Biochim. Biophys. Acta*, **83**, 245 (1964).

[11] Jollès, P., *Angew. Chem. Int. Ed. Engl.*, **8**, 227 (1969).

Plant Pigments identified in the Common Blue Butterfly

FLAVONOIDS are rare in the animal kingdom, but Ford[1], using a simple colour test, suggested that they were present in a number of butterflies. A difficulty in characterizing the pigments is the fact that a large number of specimens, of the order of hundreds, is required, and it is not always possible to obtain one species in such large numbers. Morris and Thomson[2], using some 400 specimens, identified tricin and a glycoside of this flavone as well as a glycoside of another flavone, orientin, from the wings of the marble white butterflies *Melanargia galathea*. They found[3] that the small heath butterflies *Coenonympha pamphilus* contained tricin both free and as glycoside but not orientin. The flavonoids seem to be obtained by the insects through the caterpillars feeding on a particular food host. Carotenoids, on the other hand, are well known in animals, and Goodwin[4] has suggested that insects accumulate their dietary carotenoids as such or that they may be altered to form other carotenoids not found in the insects' food. A number of specimens of the common blue butterfly *Polyommatus icarus* Rott. were collected both in France and in England with a view to characterizing the flavonoids and the carotenoids and to comparing these with their food plants. One hundred specimens of *P. icarus* from Box Hill, Surrey, were feeding on rest harrow *Ononis repens* and those from St Martial, France (800 specimens), on a species of *Anthyllis*; both plants are members of the Leguminosae.

Flavonoids were extracted by conventional methods[5], refluxing with 70 per cent methanol for 2 h. The extract was evaporated *in vacuo* at about 40° and the residue dissolved in a small volume of 70 per cent methanol. This was then streaked on Whatman No. 1 paper and run in various solvents (Table 1). The characteristic colours under ultraviolet light with and without ammonia fumes were noted. R_f values and absorption spectra were compared with those given by Harborne[5]. Not all the bands that were obtained after chromatography in 30 per cent acetic acid were identified, because some were found in too small amounts. The compounds that were tentatively identified in the French butterflies were the free flavonol 3-O-methyl-kaempferol and the flavonol glycoside quercetin 3,4′-diglucoside (Table 1). A flavone glycoside and an isoflavone with properties shown in Table 1 were also obtained, but it was not possible to identify them further.

Table 1. FLAVONOIDS IN THE FRENCH COMMON BLUE

Band	Colour in ultraviolet $-NH_3$	Colour in ultraviolet $+NH_3$	R_f values in: 30 per cent acetic	R_f values in: 'Forestal'	R_f values in: Phenol PhOH	$\lambda_{\max}$ in ethanol	Compound
I	Blue	Blue	0·13	0·90	—	~265, 272, ~279, 320	Isoflavone*
II	Brown	Green	0·49	0·74	0·85	267, ~295, 347	3-O-Methyl-kaempferol
III	Brown–yellow	Bright yellow	0·43	0·67	0·27	257, ~267, 295, ~355	Quercetin 3,4′-diglucoside
IV	—	Green	0·66	0·70	—	272, ~279, 330	Flavone glycoside*

~ Indicates a shoulder. * Unidentified compounds.

Table 2. FLAVONOIDS AND CAROTENOIDS OF *P. icarus* AND THOSE OF *O. repens*

	Imagines English	Imagines French	*O. repens*
Carotenoids			
Phytoene	+	+	+
α-Carotene	+	−	+
β-Carotene	−	+	+
γ-Carotene	+	−	+
Lutein	+	+	+
Lutein epoxide	−	+	−
Neoxanthin	−	+	+
Auroxanthin	−	−	+
Flavoxanthin	+	+	+
Flavonoids			
Isoflavone	−	+	+
3-O-Methyl-kaempferol	−	+	−
Quercetin 3,4′-diglucoside	−	+	−
Flavone glycoside	−	+	−

Carotenoids were extracted in methanol and partitioned with diethyl ether. The ethereal extracts were reduced *in vacuo* at about 40°, taken up in a small volume of *n*-hexane, separated by column chromatography and identified by our usual methods[6]. The results for carotenoids and flavonoids in the French and English butterflies of *P. icarus* and those of *O. repens* are given in Table 2. We could not obtain the food plant of the French species.

Phytoene was found in insects of both countries as well as in the food plant *O. repens*. As far as we know, this is the first record of this colourless C_{40} polyene in insects even though it is found quite abundantly in a great number of plants. Contrary to what was found in certain other insects, such as *Schistocerca gregaria* and *Locusta migratoria migratoroides* R. and F.[7], the carotenoids of the food plants of *P. icarus* are unchanged in the imagines even though not all the carotenoids present in *O. repens* were identified in the imagines. As for the flavonoids, the unknown isoflavone was obtained in the French butterflies and in *O. repens* but not in the English ones. Flavonoids were not identified at all in the English butterflies, but small amounts were present because only 100 specimens were used. Isoflavones are found mainly in the Leguminosae, and the presence of one such compound in the butterflies shows that the compounds are obtained by the insects from the particular food plant. The flavonols quercetin and kaempferol were identified in the French butterflies, though not found in *O. repens*. These compounds are well known in closely related members of the Leguminosae[8] and it is quite likely that they are also found in *Anthyllis*. The larvae of *M. galathea*, however, are known to feed on certain grasses that contain tricin and orientin but lack quercetin. Harborne[5] has explained the absence of orientin in *C. pamphilus* in suggesting that, because orientin contains a catechol nucleus, it may be more readily metabolized by insects. This does not, however, explain its presence in *M. galathea* and, further, in our investigation we identified quercetin, which contains a catechol nucleus. Orientin might have been present in small amounts and not detected in *C. pamphilus*, as was the case with the English imagines in our investigation in which no flavonoids were identified, because the sample was smaller.

L. R. G. V. acknowledges a grant from the Central Research Fund of the University of London.

JOHN FELTWELL
L. R. G. VALADON

Departments of Zoology and Botany,
Royal Holloway College (University of London),
Englefield Green, Surrey.

Received September 26; revised December 2, 1969.

[1] Ford, E. B., *Roy. Entomol. Soc.*, **19A**, 92 (1944).

[2] Morris, S. J., and Thomson, R. H., *J. Insect Physiol.*, **9**, 391 (1963).

[3] Morris, S. J., and Thomson, R. H., *J. Insect Physiol.*, **10**, 377 (1964).

[4] Goodwin, T. W., *The Comparative Biochemistry of the Carotenoids* (Chapman and Hall, London, 1952).

[5] Harborne, J. B., *Comparative Biochemistry of the Flavonoids* (Academic Press, London, 1967).

[6] Valadon, L. R. G., and Mummery, R. S., *Biochem. J.*, **106**, 479 (1968); *Phytochemistry*, **6**, 983 (1967).

[7] Goodwin, T. W., and Srisukh, S., *Nature*, **161**, 525 (1948); *Biochem. J.*, **45**, 263.

[8] Harborne, J. B., *Phytochemistry*, **8**, 1449 (1969).

Suspected Insecticide Resistance Mechanism in the Peach–Potato Aphid

POPULATIONS of the peach–potato aphid, *Myzus persicae* (Sulzer), that have survived exposure to organophosphorus insecticides are sometimes received for identification in the British Museum (Natural History). Microscopical examination reveals that resistant populations have better developed lateral abdominal tubercles on segments 2–4 than susceptible populations. The resistance of *Myzus persicae* to demeton-methyl is not stable, a twenty-five-fold resistance being acquired during seven clonal generations exposed to it and lost during thirty generations not exposed[1]. Experiments being done at Rothamsted Experimental Station (C. J. B., in preparation) made it possible to study the morphology of aphids from susceptible and resistant aphid populations and to compare them with each other and with susceptible members of a previously resistant population.

Large lateral abdominal tubercles are usually present on abdominal segments 2–4, and sometimes 1 and 5, of the spring migrants of *Myzus persicae* that develop on peach trees. These tubercles are usually much less well developed or may not occur on the summer forms that develop on herbs. The tubercles can be difficult to detect in the wingless forms because both the tubercles and the surrounding rugose cuticle are transparent. The tubercles are evident in the winged females as the transparent tubercles are placed on the otherwise dark lateral sclerites. Winged females only were considered, but there is no reason to think that they differ from the wingless females in this character, except for the ease of observing the tubercle. Occasionally, a spinule with an enlarged base, resembling a small but pigmented tubercle or a pale ill-defined area, occurs in the position on the lateral sclerite where the tubercle occurs when present. The traces are regarded as incipient or rudimentary tubercles and have each been scored as half a tubercle in Table 1.

Table 1 summarizes the data from fourteen samples of *Myzus persicae*. The first two samples are spring migrants from peach in which each specimen usually has from four to six large tubercles on lateral abdominal sclerites 2–4. Sample 2 is the combined data from six separate collections in the area within the space of a few days. Samples 3–7 are from populations known to be susceptible to organophosphorus insecticides and the specimens seldom bear more than one, often small, lateral abdominal tubercle, the means for the populations ranging from 0·00 to 0·61 lateral tubercles per specimen. No lateral tubercles were observed in population *S* at Rothamsted in 1967 (samples 4 and 5). Sample 6 is the combined data for three small samples taken at about weekly intervals while population *S* was infested by an internal hymenopterous parasite. The three successive samples had means of 0·38 (three tubercles on eight specimens), 0·64 (nine in fourteen) and 0·71 (five in seven) tubercles per specimen. No internal parasites were observed in any of the specimens of sample 6, but some of the aphids may have occluded parasite eggs. Sample 7 was taken after the parasites had been eradicated and lateral abdominal tubercles were again absent. Samples 8–10 are of the corresponding insecticide resistant populations. Samples 13 and 14 are also from populations known to resist organophosphorus insecticides. The specimens from the resistant populations usually bear at least one lateral abdominal tubercle and the means for the populations range between 0·75 and 2·2 tubercles per specimen. Samples 11 and 12 are from the same population as 9 and 10 but were taken about two years after it had lost its resistance, and had means of only 0·04–0·07 tubercles per specimen.

The seventh and eighth abdominal tergites may also bear tubercles resembling those on the lateral sclerites, but placed dorsally. These tubercles have been called "spinal tubercles"[2], and are often present in aphid genera that deform the leaves of Rosaceae[2]. The tenth and eleventh columns of Table 1 summarize the occurrence of spinal tubercles. Spinal tubercles are much better developed in the spring forms (samples 1 and 2) which develop on peach than in the summer forms (samples 3 to 14). They are not clearly correlated with insecticide resistance, for instance, if there is any difference Russell's[3,4] susceptible population *NC* has more spinal tubercles than his resistant population *RR*. The known resistant populations of *Myzus persicae* summer forms have means of 0·50 to 0·92 spinal tubercles per specimen, and the known susceptible populations have a mean between 0·00 and 0·80 tubercles per specimen. The Rothamsted population *R* did not lose its spinal tubercles when it lost its resistance, and the successive means of 0·50, 0·58 and 0·49 make it improbable that the loss of resistance is from contamination of the culture with specimens from population *S*.

The function of the lateral abdominal tubercles is not established, but there is circumstantial evidence that they are concerned, with wax excretion. The lateral abdominal tubercles of the fundatrices of *Dysaphis pyri* are very large and have "distinct granular facies which suggest the pore plate of a wax gland group"[6]. The wax, or triglyceride[5] secreting organs characteristic of aphids, the siphunculi (=cornicles), are considered to be modified lateral abdominal tubercles. The siphunculi are relatively longer in the more resistant populations as indicated by the last column of the table. Resistant populations have

Table 1. DATA FROM ALATE PARTHENOGENETIC FEMALES OF *Myzus persicae*

Sample number	Host plant	Locality	Date	Collector	Designation of population	Number of individuals	Abdominal tubercles				Mean of siphunculi: antennal segment III length ratio
							Lateral sclerites 2–4		Tergites 7 and 8		
							Total number	Mean	Total number	Mean	
1	Peach	Woburn Abbey, Beds	May 1964	J. P. Doncaster	—	6	30	5·00	20	3·33	—
2	Peach	Ferrara, Italy	May 1966	F. Baranyovits	—	54	266	4·93	137	2·54	—
3	Chinese cabbage	Cambridge	May 1965	G. E. Russell	*NC*	15	6	0·40	12	0·80	0·74
4	Turnip	Rothamsted	July 1967	C. J. Banks	*S*	11	0	0·00	2	0·18	0·67
5	Turnip	Rothamsted	November 1967	C. J. Banks	*S*	6	0	0·00	0	0·00	0·65
6	Turnip	Rothamsted	October 1969	C. J. Banks	*S*, with parasites	28	17	0·61	10	0·36	0·71
7	Turnip	Rothamsted	November 1969	C. J. Banks	*S*	12	0	0·00	1	0·08	0·68
8	Chinese cabbage	Cambridge	May 1965	G. E. Russell	*RR*	10	9	0·90	6	0·60	0·76
9	Turnip	Rothamsted	July 1967	C. J. Banks	*R*	12	11	0·92	6	0·50	0·76
10	Turnip	Rothamsted	November 1967	C. J. Banks	*R*	12	9	0·75	7	0·58	0·74
11	Turnip	Rothamsted	October 1969	C. J. Banks	*R*, no longer resistant	36	2·5	0·07	17·5	0·49	0·66
12	Turnip	Rothamsted	November 1969	C. J. Banks	*R*, no longer resistant	12	0·5	0·04	3	0·25	0·67
13	Cucumber	Venlo, Netherlands	May 1967	D. Hille Ris Lambers	Very resistant	5	11	2·20	4	0·80	0·74
14	Capsicum	Poeldyk, Netherlands	September 1969	F. Baranyovits	Resistant	13	18	1·38	12	0·92	0·76

the siphunculi : antennal segment III ratio with means from 0·74–0·76 and susceptible populations from 0·66–0·74. The mean for population *R* dropped to 0·66 when it lost its resistance. Comparable figures are not given for the spring migrants because their siphunculi are cylindrical and not clavate like those of the summer forms. It is possible that the mechanism of insecticide resistance in the populations which we studied is to excrete the insecticide in association with wax or lipid, through the lateral abdominal tubercles or the siphunculi. The mechanism may have evolved for the disposal of waste products arising from the destruction of the eggs of parasites or for secreting a substance repellent to parasites. If the lateral tubercles are involved in insecticide resistance, the records of considerable resistance on peach trees in the spring[7,8] are not surprising because the spring forms *Myzus persicae* on peach usually have well developed tubercles.

In addition to material reared at Rothamsted, Dr F. Baranyovits, Mr J. P. Doncaster, Dr D. Hille Ris Lambers and Dr G. E. Russell have kindly provided specimens for study.

V. F. EASTOP

Department of Entomology,
British Museum (Natural History),
Cromwell Road, London.

C. J. BANKS

Department of Insecticides and Fungicides,
Rothamsted Experimental Station,
Harpenden, Hertfordshire.

Received November 24; revised December 3, 1969.

[1] Dunn, J. A., and Kempton, D. P., *Entomol. Exp. Appl.*, **9**, 67 (1966).
[2] Hille Ris Lambers, D., *Temminckia*, **4**, 76 (1939).
[3] Russell, G. E., *Bull. Entomol. Res.*, **56**, 191 (1965).
[4] Eastop, V. F., and Russell, G. E., *Nature*, **215**, 514 (1967).
[5] Strong, F. E., *Ann. Entomol. Soc. Amer.*, **60**, 668 (1967).
[6] Stroyan, H. L. G., *The British Species of Sappaphis Matsumura* (HMSO, London, 1957).
[7] Berville, P., Bezut, M., Leclant, F., and Prout, M., *Phytoma*, **203**, 1 (1968).
[8] Leclant F., *Pomol. Fr.*, **10**, 355 (1968).

Vegetative Hybridity in *Phytophthora infestans*

Phytophthora infestans, the fungus that causes blight in potatoes, probably originated in Mexico[1], where two sexually compatible mating groups (A_1 and A_2) occur with equal frequency and oospores, resulting from normal sexual reproduction, are produced[2]. Elsewhere, by contrast, only one mating type (A_1) of the species has been recognized, so that in this situation the sexual stage of the fungus is not considered to have a role in the development of blight[3]. *P. infestans* is variable and highly adaptable to its different host genotypes, and much potato breeding experience shows that, even outside Mexico, the fungus adapts remarkably rapidly to varieties that bear *R*-genes from the wild species, *Solanum demissum*[4]. Indeed, it now seems clear that no *R*-gene resistance can be expected to last for more than a few years, for up to eleven specificities occur frequently in the population of *P. infestans* in Britain[5].

The question is: how do new and sometimes very complex races originate where the fungus is functionally asexual ? It is usually assumed that the accumulation of mutations is responsible, but this view raises obvious difficulties, though mutations, both natural and induced[6,7], do undoubtedly occur. The adaptability of the fungus suggests the presence of some sort of system of genetic variability. Such a system has now been found and is described here. Briefly, I show that mixtures of races with different specificities, say *a* and *b*, grown together on a susceptible host, yield new races pathogenic to R_aR_b hosts which are themselves immune to the original *a* and *b* races.

I use the conventional nomenclature whereby a specificity in the fungus is assigned a number that corresponds to the *R*-gene whose hypersensitive reaction it is capable of overcoming. Race 1, 2 thus attacks potatoes that are R_1 or R_2 or R_1R_2 but not clones that carry R_3 or R_4 and so on (which are hypersensitive to that race). Races are identified by a set of differential host clones of known *R*-gene constitution. For example, the race 1, 2, 3, 4, 7, 10 could be identified by virulence towards three clones $R_1R_2R_3R_4$ and R_7 and R_{10} and by the evocation of hypersensitive responses on clones carrying R_5, R_6, R_8 and R_9.

Briefly, the main result was that race 1, 2, 3, 4, 7 was obtained by mixing race 4 with race 1, 2, 3, 7 and race 1, 2, 3, 4, 7, 10 was derived from a mixture of race 3, 4, 10 and race 1, 2, 3, 7. The races 4 and 3, 4, 10 (both originally from cv. 'Majestic') were maintained on an R_4 host while the two isolates of race 1, 2, 3, 7 came from and were maintained on cv. 'Pentland Dell' ($R_1R_2R_3$). Suspensions of sporangia (in cold distilled water) were used in the combinations indicated to inoculate a series of test leaves, namely three tester clones, possessing the genes R_4, $R_1R_2R_3$ and $R_1R_2R_3R_4$ respectively, and 'Pentland Dell'. Large single drops of the mixtures (races 4 + 1, 2, 3, 7 or races 3, 4, 10 + 1, 2, 3, 7) were applied to the bases of the leaves and infection of all but the $R_1R_2R_3R_4$ leaves was successful. When the lesions were large enough, the inoculated half of each leaf was discarded and a sporangial suspension was prepared from the remainder. With each test series, half of the suspension from the R_4 host was added to that from the $R_1R_2R_3$ host and the remainder to that from 'Pentland Dell'. These mixtures were then applied to the series of tester leaves. In contrast to the first cycle of inoculations, growth occurred on all the leaves and transfers from each leaf to $R_1R_2R_3R_4$ leaves proved positive, though sometimes weak. After two subsequent transfers through $R_1R_2R_3R_4$ leaves, the races present were confirmed on the complete differential host series. Isolates derived from the mixture of races 4 and 1, 2, 3, 7 proved to be race 1, 2, 3, 4, 7 and those from races 3, 4, 10 and 1, 2, 3, 7 were race 1, 2, 3, 4, 7, 10. The four original isolates and the new races showed no alteration in racial constitution while undergoing four series of transfers on the differential hosts.

It is therefore established that mixtures of races grown together on a susceptible host combine genetically to produce new races that have the sum of the parental specificities. The mechanism is obscure but, because the zoospores of *P. infestans* are uninucleate[8], nuclear fusion is implied. This sort of genetic combination contrasts with the coexistence of races in mixtures without combination[5,6], and its frequency is still unknown. My several other experiments have sometimes succeeded and sometimes failed. For example, race 1, 2 has been produced from mixing race 1 with race 2 and race 2, 3, 4 from mixing the same race 2 with race 3, 4. In simultaneous trials, however, mixtures of the race 1 with race 2, 4 or race 3, 4 and mixtures of the race 2, 4 with race 3, 4 showed no combination. So far, then, the evidence in favour of vegetative hybridity in *P. infestans* is strong; there is no evidence yet of genetic recombination.

From the plant pathological viewpoint, these results are significant because they show that any susceptible potato variety that harbours two or more complex races may generate still higher levels of complexity. The extraordinary complexity of blight races in Britain revealed in a recent survey[5] is thus more readily understood than would be the case if the accumulation of mutations alone were responsible.

It may seem surprising that this "hybridization" behaviour has not been found previously. The reason seems to be that, in mixture studies (of which there have been several[7,9]), investigators have always tested the products on the "parental" differentials and not on the more complex differentials required to detect hybrids. McKee, however, has observed[9] that infection rates by zoospores derived from mixtures were higher than expected, a result that is at least compatible with the occurrence of hybridity.

JEAN F. MALCOLMSON

Scottish Plant Breeding Station,
Pentlandfield,
Roslin,
Midlothian.

Received August 27; revised December 4, 1969.

[1] Niederhauser, J. S., *Proc. Int. Congress Plant Path.*, 138 (1968).
[2] Gallegly, M. E., and Galindo, J., *Phytopathology*, **48**, 274 (1958).
[3] Smooth, J. J., Gough, F. J., Lamey, H. A., Eichenmuller, J. J., and Gallegly, M. E., *Phytopathology*, **48**, 165 (1958).
[4] Simmonds, N. W., *Rept. Scot. Plant Breed. Stat., 1968–69*, 18 (1969).
[5] Malcolmson, J. F., *Trans. Brit. Mycol. Soc.*, **53**, 417 (1969).
[6] Gallegly, M. E., and Eichenmuller, J. J., *Amer. Potato J.*, **36**, 45 (1959).
[7] Wilde, P., *Arch. Mikrobiol.*, **40**, 163 (1961).
[8] Marks, G. E., *Chromosoma* (Berl.), **16**, 681 (1965).
[9] McKee, R. K., *Trans. Brit. Mycol. Soc.*, **47**, 365 (1964).

GENERAL

Generation of Hypotheses and Theories

THE view that induction is an operation of valid inference of the same kind as deduction, expounded notably by John Stuart Mill, has been rightly rejected by modern scientists and philosophers. Instead, the growth of scientific understanding is looked on as a hypothetico-deductive activity. According to this view, hypotheses or theories are created, subsuming far more than the known facts they are intended to explain; they are tested for internal logical consistency and compatibility with the known facts; and implications are deduced from them for test by further observation or experiment. If such a test is adequately carried out and gives a negative result, the theory is scrapped or, alternatively, modified so as to be compatible with the new facts. Thus theories (if they have an adequate degree of generality) cannot be absolutely verified, though they can be absolutely falsified. Supporting evidence can corroborate them—strengthen our reasons for believing that they are true—but not verify them.

This much would be accepted, I think, by most working scientists. But protagonists of the hypothetico-deductive view of scientific method, in reacting against Mill's account of induction, have taken an extreme position which is untenable and also inhibits them from consideration and study of what is the central and most interesting aspect of induction, namely, the generation of the hypotheses and theories themselves. Popper[1], for example, writes "... there is no such thing as a logical method of having new ideas, or a logical reconstruction of this process. My view may be expressed by saying that every discovery contains an 'irrational element' ...". Hempel[2] writes: "There are, then, no generally applicable 'rules of induction', by which hypotheses or theories can be mechanically derived or inferred from empirical data. The transition from data to theory requires creative imagination." And Medawar[3] says "although one can put oneself in the right frame of mind for having ideas and can abet the process, the process itself is outside logic and cannot be made the subject of logical rules".

Even if it were true that no completely rational account could at present be given of any inductive operation, it would be both unjustified and conducive to the blocking of enquiry to assert as a matter of principle that there could be no such account. To do so is, indeed, to evade the key issue in the "logic of discovery". But in fact, not only in science but also in everyday thinking, rules are constantly employed in hypothesis formation. A child throws a stone and sees it sink in the pool—he makes the (deductively invalid) inference that all stones if dropped on water will sink. He has used a rule of abstraction and generalization which, formulated in the language of first-order predicate logic, reads: from $P(a)$ infer $(x)P(x)$; that is, from the fact "the object a has the property P" generate the hypothesis "all objects have the property P". Not only is this simple rule all-pervasive in science and thought, but its power can be judged from the fact[4] that, applied to relations instead of properties, and implemented as the sole inductive principle in a computer program, it has generated a remarkably close approximation to the axioms of group theory on the basis of only ten elementary facts about two simple mathematical groups.

This example is a clue to where one might fruitfully look for the discovery of rules of induction, for abstraction plus generalization is just the inverse of a standard rule (instantiation) of deductive inference, namely: from $(x)P(x)$ infer $P(a)$. And in fact, in spite of accepting Popper's dogmatism, Medawar seems to be dimly aware of this possibility, for he says[3] that "nowadays the tendency is to use 'experimentation' to stand for the acts used in testing a hypothesis, leaving 'induction' as a vague word to signify all the various ways of travelling upstream of the flow of deductive inference".

The process of abstraction, applied in the language of higher-order logic, is enormously powerful in the generation of new concepts. For example, suppose in some domain of phenomena involving a 2-place relation denoted by R, there is available the fact

$$R(a, b) \ \& \ R\,(b, c) \supset R\,(a, c),$$

where a, b, c denote particular objects in the domain. One might then apply two operational rules in succession: first, abstraction plus generalization as before, giving

$$(x)\ (y)\ (z)\ [R(x, y) \ \& \ R\,(y, z) \supset R\,(x, z)];$$

then, asserting the material equivalence of this to $T(R)$, where T is a gratuitously introduced new second-order one-place predicate letter, giving

$$T(R) \equiv (x)\ (y)\ (z)\ [R(x, y) \ \& \ R\,(y, z) \supset R\,(x, z)],$$

R being now a free predicate variable. One has thereby formally created and defined the abstract and most useful concept of transitivity, here denoted by T. This has been done in effect by two kinds of abstraction, first on the individual constants a, b, c, then on the relational constant R.

It is interesing that Pierce (see ref. 5) had long ago suggested that the instinctive structure of human intelligence imposed severe constraints on the admissible hypotheses human beings could make about their experience and that only because of this was the attainment of knowledge by them possible at all. If there are such negative rules, it would be surprising if there were not positive ones too.

BERNARD MELTZER

Metamathematics Unit,
University of Edinburgh,
9 Hope Park Square,
Meadow Lane,
Edinburgh, EH8 9NW.

Received January 15, 1970.

[1] Popper, K. R., *The Logic of Scientific Discovery*, 32 (Basic Books, 1959).
[2] Hempel, C. G., *Philosophy of Natural Science*, 15 (Prentice-Hall, 1966).
[3] Medawar, P. B., *The Art of the Soluble*, 147, 134 (Methuen, 1967).
[4] Meltzer, B., in *Machine Intelligence 5* (Edinburgh University Press, in the press).
[5] Chomsky, N., *Language and Mind*, 78 (Harcourt, Brace and World, 1968).

Book Reviews

ECONOMIC REASONING

A Theory of Economic History

By J. Hicks. Pp. vii+181. (Clarendon Press: Oxford; Oxford University Press: London, October 1969.) 25*s* boards; 10*s* paper.

UNLIKE the work of his peers, Nicholas Kaldor and Joan Robinson, Sir John Hicks's work has been marked less by sheer originality than by elegance. From wage theory to growth theory, from the theory of demand to public finance, Hicks has produced work which is less a breakthrough than a consolidation of earlier insights. This is a traditional style: Mill and Marshall—and, until the *General Theory*, Keynes—sought to establish their claims to orthodoxy rather than originality, to intellectual power rather than dash.

Yet, unexpectedly, the doyen of English academic economists has produced a book of originality and power which may prove to be not only his greatest contribution to scholarship but the book which establishes his rightful place in intellectual life. Distinguished economists—Marshall, Keynes, Dobb—have often made excursions into economic history, to illuminate their theories. Some economic historians, like Cunningham, Clapham and Hobsbawm, have sought to use theory to explain their history. Marx has been of course the exemplar of the marriage of economic theory and history. It is hardly an exaggeration to say that there is some of Marx's intellectual strength and adventure in Hicks's latest book, though, if this slightly absurd comparison were to be drawn, it could be said that what it lacks is implicit in its essay-form, the deep grasp of detail.

The problem is a simple one. What is the economic process, historically? According to Hicks, man did not start in primitive simplicity, but in societies dominated by custom, and subject to command by a king or an army chieftain.

From this simple but well-argued point, he goes on to the development of taxation and of a bureaucracy to ensure the payment of taxation. Public finance, therefore, takes precedence over trade. "Their central economic nexus is revenue", he writes of early societies. The market grows up alongside the religious festivals or other gatherings, first with general traders who increasingly specialize. The mercantile economy develops within the protective embrace of the command and customary economies, but it becomes itself a source of conflict between different societies. Hicks sees the city state as the peculiarity which divides Asian history from European history; and it is the relation of the merchants to the cities that he sees to be crucial, because the merchants can help to evolve legal forms that are especially suitable to their own form of trading, and so allow all-round advantages to grow from wider trade.

Beyond this, the development of money, law and credit evolved from city states which coalesced into a semi-mercantilized state. In his discussion of money, Hicks achieves his first act of sheer intellectual brilliance—he uses contemporary monetary theory with a power that illuminates the origins of money in a way that earlier attempts (notably D. H. Robertson's by no means negligible contribution in *Money*) did not.

The fact that he does so in simple non-technical language, and largely omits what he thinks to be incorrect (without refuting it), may mask his achievement. Similarly with his exposition of modern capital theory (pages 148–154), which is a miracle of compression.

This book, like Joan Robinson's *Economic Philosophy*, has much to give the specialist. It also has much to give the non-specialist who wishes to gain an insight into economic reasoning.

JOHN VAIZEY

RISE OF THE SPINNING MACHINE

The Textile Industry

An Account of the Early Inventions of Spinning, Weaving and Knitting Machines. By W. English. (Industrial Archaeology, Vol. 4.) Pp. xiv+242+32 plates. (Longmans: London, December 1969.) 55*s*.

THIS book is one of a series on industrial archaeology prepared under the general editorship of Mr L. T. C. Rolt. It is an account of some of the early developments in spinning, weaving and knitting machines and is a useful addition to the already considerable literature on the subject. The author has successfully presented a great deal of detailed technical information in a readable way and has linked this with interesting observations on the inventors of the early machines and on the times in which they lived.

The fascinating story of invention for the textile industry is told clearly and is well illustrated, so that those who are not familiar with textile machines can readily appreciate the significance of each new development. An extremely wide range of topics is considered. There are, for example, chapters on hand spinning wheels, early carding machines, the spinning machines of Arkwright and of Paul, Hargreaves's spinning jenny, Roberts's automatic mule, Kay's fly shuttle, Cartwright's loom, Lee's stocking frame, and developments in pattern weaving. There are also essays on the rise of the woollen and worsted industries and of the flax industries, and the book includes two chapters on the factory system. In other chapters, more recent progress in spinning, weaving and knitting machines is briefly considered.

On reading the book it is impossible not to be impressed by the great tradition of the textile industry. Men of widely different backgrounds contributed to its development. Kay, Hargreaves, Arkwright and Crompton had practical knowledge of the industry and were highly skilled engineers. Dr Edmund Cartwright, however, one of the outstanding inventors, knew nothing about textile processing when he became interested in the problem of weaving. He was educated at New College, Oxford, and was awarded the degree of Doctor of Divinity in 1806. At the age of 41 when he was rector of Goadby Marwood, he happened to discuss with some Manchester businessmen the possibility of constructing a loom which could be driven by water or steam power. The general opinion of manufacturers of the day was that the construction of such a loom was almost impossible. Although he knew nothing about the subject, "having never at the time seen a person weave", Cartwright decided to disprove the argument. In this he was successful and in due course he patented satisfactory machines.

In an industry which has so successfully exploited human ingenuity, it is inconceivable that it should now become stagnant and unprogressive. The contemporary textile industry is still a fruitful field for the inventor, who is now generally a sophisticated engineer or an applied scientist, and as the author of this book says in his concluding paragraph, "the future of the textile industry appears as a vision of more new raw materials, of spinning without spindles, weaving without shuttles and knitting without needles".

Mr English has presented his material well. It is well documented, there is a good bibliography and the book will give great pleasure to all who are interested in the origins of today's highly industrialized society.

C. S. WHEWELL

TOWN BLIGHT

Urban Decay

An Analysis and a Policy. By Franklin Medhurst and J. Parry Lewis, with a chapter by Elizabeth Gittus. Pp. xvi + 174. (Macmillan: London, October 1969.) 65*s*.

THE problem of urban decay is undoubtedly one of the most urgent facing planners today. It is estimated that in 1961 between 2·5 and 3·5 million houses in England and Wales were in need of some kind of renewal, and that by 1981 another million houses will have been added to these. In spite of the apparent success of some local renewal projects, planners are still basically undecided about the best way of handling the existing problem, and seem to have little idea about what ought to be done to make sure that urban decay is less of a problem in the future. The main reason for this seems to be that not enough is known about the conditions of decay, nor about the complex processes involved. Hence it is unfortunate that the authors of this slim volume cannot be considered to have made more than a superficial contribution to solving the problem.

The empirical analyses reported in the book are unimpressive and somewhat disappointing. They add little to our knowledge of the conditions of decay and, regrettably, do not yield any new insights into the complex processes at work. This may be because the studies seem to have been ill-conceived, in part because of the rather naive level of quantitative analysis that was used. This is particularly true of the attempts made to index the level of physical and environmental decay, and although the analysis of change in part of Manchester's twilight area is interesting, the details which are provided could have been put to better use. Instead, these details are used to suggest hypotheses which seem to form the basis of on-going research into the broader aspects of urban change presented at length in chapter five. It is a great pity that the discussion is in terms of what might be done rather than what has been done.

The book is, nevertheless, not without its good parts, and for me these include the short but excellent review of the sociological aspects of urban decay contributed by Elizabeth Gittus and the attempts made by the authors in the last chapter to formulate a policy towards decay. Here it is sensibly argued that greater effort should be made to repair decaying property rather than to demolish it, and a number of suggestions are made whereby the costs of repair could be reduced and the benefits to property owners increased. The authors stress, moreover, that any policy towards decay must be contained in a larger urban policy set within the broader framework of policies for regional development. BARRY J. GARNER

SOPHISTICATED AERIALS

Radiotelescopes

By W. N. Christiansen and J. A. Högbom. (Cambridge Monographs on Physics.) Pp. x + 231. (Cambridge University Press: London, September 1969.) 90*s*; $14.50.

TWENTY-FIVE years ago a radio telescope was just an aerial, but today there are many sophisticated and elegant systems which bear little resemblance to Grote Reber's original telescope and which can extract and use the information in the incoming waves more efficiently than the classical parabolic reflector. The authors of this book, Christiansen and Högbom, have for many years been associated with the design of unusual radio telescope systems. Christiansen's grating telescope was used in very early studies of the radio sun and, when it was later multiplied with an orthogonal grating, the language of the art was enriched by the colourful "chris-cross". One would expect a book of quality from these authors and, indeed, one is not disappointed. It is aimed at the graduate or near-graduate level and will satisfy a demand for a reliable and almost complete reference work on the subject.

It is almost complete. One would not fault it because it could not include all the tricks of the trade. Nevertheless, one could not recommend it as the only reference work on radio telescopes to a class of budding radio astronomers. It would have to be garnished with a subsidiary work on aerials and practical tips. In the index and in the text, one looks in vain for Yagis, helices and all sorts of little facts and details that build up to the sophisticated systems that appear in the two hundred and twenty well printed pages. They are not to be found. How does one obtain circular polarization ? How does one methodically taper an extended aerial without tears and sweat, and what steps should be taken to maintain coaxial feeders constant on extended phased systems ? Chapter 6 tells one the facts about feeder attenuation that may be gleaned directly from the manufacturers' literature; it tells one that the beam may be steered by varying the phase, but it omits the associated problems of phase stability, though these problems were first tackled and solved by radio astronomers. Finally, a minor point of presentation: it would have been much easier to navigate from the "contents" sheet to the relevant pages if the page numbers had appeared in the contents.

These minor criticisms only represent the greed of the reviewer who would have liked to have had a little more for his money, but who would nevertheless strongly recommend this little book to all radio astronomers, whether budding or fully fledged. It should also form part of the library not only of every department of astronomy, physics and electronics, but also all other sciences where these principles may yet find application.

R. C. JENNISON

MODERN METEOROLOGY

Essentials of Meteorology

By D. H. McIntosh and A. S. Thom. (The Wykeham Science Series for Schools and Universities.) Pp. xv + 238. (Wykeham: London and Winchester, 1969.) 20*s*.

THE teaching of meteorology in schools is usually undertaken by the geography master, with the result that students retain an impression of a collection of maps and statistics with little physical content. Some meteorologists believe that this leads to difficulties in attracting suitable workers to the field. Accordingly, they will welcome this admirable book by two staff members of the University of Edinburgh for its thoroughly scientific treatment of present meteorological knowledge at a level attainable in the upper forms of schools or early years of university.

The reader is introduced in considerable detail to almost all topics of interest to modern meteorology, a range too great to list here. Broadly, however, basic physical laws, such as Newton's laws of motion and the gas laws, are cast into a form applicable to the elements. The reader will learn how atmospheric scientists picture weather phenomena from dust devils to cyclones and how they are observed, how the energy of these motions is replenished from the Sun and lost again to space, how their behaviour may be forecast and why such forecasts are expected to improve.

There are already available several texts which are intended as introductions to meteorology for enthusiastic sixth formers. The distinctive feature of this book is the unflinching use of mathematics, permitting brief and precise arguments and affording a more accurate and comprehensive feeling for the subject. In places, the student is confronted with partial derivatives, vectors and laplacians. He will not be familiar with such notation, and, although its meaning is explained as it is introduced, some difficulty must be anticipated. (For this reason the misprint in the first use of vector notation on page 91 is regrettable.) Mathematical complexity reaches its height in the account of computer-based forecasting where key new ideas are introduced almost line by line with baffling brevity. No links are actually missing, however, and the tenacious will be rewarded. Let reluctant mathematicians be undeterred by that warning; they will find much which is interesting and succinctly explained. Some chapters, for example those on rain processes and on methods of observation, require no mathematics at all, and others require very little. Physics teachers may extract from these a fund of illustrations, useful to the lower school.

The style is rather disjointed and I was distracted by the number of footnotes and references to sections later in the book, but the attractive finish, the wealth and authority of the information and the reasonable price more than compensate.

R. S. Harwood

SOUTHERN AFRICAN GEOLOGY

Geological History of Southern Africa

By S. H. Haughton. Pp. 535 + 16 plates. (The Geological Society of South Africa: Johannesburg, 1969.) R10.50.

Southern Africa is of exceptional geological interest, as well as of immense economic importance on account of its mineral resources. For an account of the geology of this region one formerly referred to the *Geology of South Africa* by A. L. du Toit, which is one of the best books on regional geology ever written. Dr Haughton revised the third edition, which was published in 1954, but, since then, so much new work has been carried out that a completely new book became necessary. Nobody is better qualified to evaluate the formidable mass of geological data relating to Southern Africa than Haughton, who has been in the forefront of South African geology for more than half a century. He has written a careful, judicially balanced and authoritative account, which will serve as a standard reference on the geology of southern Africa for many years.

One inevitably compares the new book with du Toit's masterpiece. Haughton is less successful. His arrangement, partly chronological, partly geographical, is confusing. Regional correlation tables, with ages, would have helped the reader, and more maps and diagrams are needed (some of those used are poor). Too much attention is paid to stratigraphic nomenclature and too little to fundamental problems. One looks in a new book on the geology of southern Africa for an up to date account of some of the geological wonders of that region. There are the very old rocks of the Barberton mountainland which are being taken as a model for the earliest stages in the formation of continental crust; the great dyke of Rhodesia and the Bushveld complex of the Transvaal are two enormous bodies of basic igneous rock in which the separation of layers of crystals has produced remarkable mineral concentrations. Around Kimberley and elsewhere there are pipes which contain not only diamonds but also samples carried up from the upper mantle. The Vredefort dome, surrounded by a radial array of shatter cones, has been interpreted as a huge meteorite impact structure. There are fascinating problems connected with the concentration of gold and uranium in the Witwatersrand conglomerate beds. The Dwyka glaciation of southern Africa in the Carboniferous period was for long the most important evidence for continental drift. The Karroo vertebrates on which Haughton is an authority, and the australopithecines from the cave deposits in the Transvaal, are of outstanding interest. Haughton, in this book, does not succeed in leading the reader to appreciate the interest and significance of these and other such phenomena, nor does he provide a clear view of the present state of knowledge regarding them. Much of this new knowledge depends on geophysical and to some extent geochemical work to which Haughton pays too little attention. For example, the palaeomagnetic evidence which tells us about the latitude and orientation of the southern African continent through geological time is almost ignored. Radiometric age determinations are treated rather as though they were an innovation of still doubtful value.

The coloured geological map of southern Africa on a scale of 1 : 5,000,000 is much clearer than the corresponding map in du Toit's book, besides containing a great deal of new detail. The price of the book is low; it is a good buy.

R. M. Shackleton

PLASMA THEORY AND OBSERVATION

Plasma Waves in Space and in the Laboratory

Vol. 1. Edited by J. O. Thomas and B. J. Landmark. (Proceedings of NATO Advanced Study Institute, Røros, Norway, 17–26 April 1968.) Pp. 487. (Edinburgh University Press: Edinburgh, September 1969.) 160*s*.

This volume, the first of two reporting on the Røros school, is composed of more than twenty papers, of a basic introductory or review nature (rather than current research, scheduled for volume two). The topics range widely to cover theory of probes, observations in the laboratory, in the ionosphere and in interplanetary space and theory of plasma resonances and VLF emissions—that is, "space" plasmas in the local, accessible and non-astrophysical sense. In a short review, it is impossible to comment on the papers individually: however, common to many of the contributions is an emphasis on squaring theory with observation. This is meeting with increasing success as is particularly evident in Crawford's discussion of wave propagation in stable laboratory plasmas, and in studies with ionospheric probes (Balmain and Otsiutik). The importance, for the advancement of plasma science, of space plasma as an experimental medium is brought up in a very readable introductory paper on ionospheric resonances (Thomas and Andrews)—free from boundaries, the plasma has relative uniform density, is fully ionized and virtually collisionless. With the development of resonance techniques and probe systems (cf. Storey, Aubry and Meyer on their quadripole probe), sophisticated experiments on natural plasmas are becoming a reality.

The blurb on the dust-jacket claims a role as an up to date textbook, but apart from very few sections—notably the illuminating "Rowlands" theory of plasma waves—the book reports completed work rather than useful approaches. Problems of non-linear waves and turbulence are hardly touched on. Moreover, in trying to be a textbook in excluding both abstracts and conference discussion, the book loses potential usefulness. Comments on the significance and validity of a piece of work—that important and unique contribution of a conference—are lost.

On the other hand, I would congratulate the editors and publishers in producing an attractive, high quality book. There are few printing errors, except in the paper on pitch-angle instabilities, where misprints and poor format are annoying. An error of more substance occurs in Frederick and Scarf's paper on cyclotron modes, where the substitution of β_+ for β_- in equation (14) ignores one region of validity of the classical Bernstein modes. Plasma physicists of diverse interests will indeed find this volume both interesting and useful in cross-fertilizing our fast-diverging studies of plasmas, but its importance as a student text can be only minor.

M. K. Wallis

LIE GROUPS

Lectures on Lie Groups

By J. Frank Adams. (Mathematics Lecture Note Series.) Pp. xii + 182. (W. A. Benjamin: New York and Amsterdam, 1969.) n.p.

THE theory of Lie groups occupies a central position in modern mathematics. This theory is of interest to workers in such fields as algebraic topology, differential geometry, algebra and functional analysis, in addition to its applications in theoretical physics. Although some good texts on the subject are available, the wide area from which its ideas are drawn make it more difficult to learn than more homogeneous branches of mathematics. This new text by Professor Adams in the Benjamin "Lecture Note Series" will be very useful for mathematicians and research students wishing to acquaint themselves with the subject.

This book is not an exhaustive account, but aims to prove the main results on the representations of compact Lie groups, introducing those concepts which are necessary for this purpose. It is based on a course of lectures addressed primarily to algebraic topologists, but the book will also be useful to workers in other fields. It will be particularly useful as a textbook for a graduate course on Lie groups.

The author's style is concise, and this conciseness gives a pleasing clarity to the text. In some places, however, conciseness is carried to an extreme—particularly in chapter one where not all the definitions are given as clearly as one would wish.

After introducing the basic definitions, the author discusses 1-parameter subgroups and the exponential map. Properties of representation spaces are then discussed, including relations between real, complex and quaternionic representations, and the Peter–Weyl theorem is proved. Maximal tori in compact groups are then considered, and the usual concepts of roots, weights, the Weyl group, the Dynkin diagram and the Stiefel diagram are introduced. The method for finding the fundamental group $\pi_1(G)$ from the Stiefel diagram is described. The connexion between the irreducible complex representations and the weights in the fundamental chamber is proved, and the structure of the representation ring of a compact connected simply connected group is derived. The proofs are complete except for some of the results in the early chapters, where references are given instead. There are frequent examples in the text for the classical unitary, symplectic and orthogonal groups.

R. W. CARTER

SYNTHETIC ANTIBIOTICS

Synthesis of Penicillin, Cephalosporin C and Analogs

By Maghar S. Manhas and Ajay K. Bose. (New Directions in Organic Chemistry: A Series of Monographs based on Lectures and Seminars at Stevens Institute of Technology.) Pp. xii + 118. (Dekker: New York, October 1969.) $8.75.

Two discoveries made at the turn of the last decade have led during the past ten years to the introduction of a wide range of β-lactam antibiotics into clinical medicine. The discovery by the Beecham Group that 6-aminopenicillanic acid could be obtained by fermentation enabled thousands of semi-synthetic penicillins to be prepared. The second discovery made by Abraham and Newton was that cephalosporin C was a β-lactam antibiotic which was not destroyed by penicillinases and was effective over a much wider antibacterial spectrum than the penicillins. In this book, Dr Manhas and Professor Bose review developments in this area, which have chiefly occurred during the past ten to fifteen years.

After a short introductory chapter, the use of physical methods is discussed. It is surprising, however, that while all the chief methods are presented, including recent applications of the nuclear Overhauser effect, the authors fail to mention the very interesting ultraviolet absorption spectra of the cephalosporins.

Chapters 3 and 4 constitute the major part of the book and are concerned with the synthesis of the penicillins and cephalosporins and their analogues respectively. These chapters provide a useful summary of the literature although the authors have perhaps given undue space to their own synthetic contributions. One of the most interesting features of cephalosporin chemistry has, however, been neglected. This concerns the unusual facility with which the allylic acetoxy-group is substituted by nitrogen and sulphur nucleophiles in aqueous medium. This type of substitution, first noticed by Abraham and Newton, has been extensively studied by the Glaxo group.

The mode of antibacterial action of the penicillins is now very largely understood at the molecular level, largely because of the work of Strominger and his collaborators. It is surprising that this work is mentioned only very briefly and the conclusions regarding the essential molecular features for antibacterial activity are ignored. These conclusions allow a rational approach for future synthetic endeavours which the authors anticipate.

The text contains very few errors. Structural formulae are clearly presented and liberally used. The occasional omissions and mistakes in formulae are generally irritating rather than misleading. The literature is surveyed to July 1969.

G. LOWE

LABORATORY TECHNIQUES

Electrophoresis of Proteins in Polyacrylamide and Starch Cells

By A. H. Gordon. Pp. 149. Hfl.15; 35*s*.

An Introduction to Gel Chromatography

By L. Fischer. Pp. 246. Hfl.25; 58*s*.

Immunochemical Techniques for the Identification and Estimation of Macromolecules

By J. Clausen. Pp. 175. Hfl.15; 35*s*.

(Laboratory Techniques in Biochemistry and Molecular Biology, edited by T. S. Work and E. Work.) (North-Holland: Amsterdam and London, 1969.)

THESE books are paperback editions of the three parts which make up volume one of a series "Laboratory Techniques in Biochemistry and Molecular Biology" edited by T. S. Work and E. Work. Thus the research worker can acquire an individual text on the technique of most interest to him rather than purchase the complete volume which presumably will be principally destined for departmental libraries. All three volumes set out to give expert practical advice and are intended for readers with little or no previous experience of the techniques discussed. This aim is satisfactorily achieved by all three authors, who adopt a similar approach: a brief outline of the basic principles followed by a detailed account of the methods, apparatus and practical applications.

The first book is devoted to gel electrophoresis using both polyacrylamide and starch supports. The author describes lucidly the advantages and disadvantages of both materials as applied to various problems. He discusses clearly the preparation of the gels, the choice of a suitable apparatus and the detection and recovery of separated material, and he provides a very useful appendix of buffer solutions.

The second book is an introduction to gel chromatography, and the author describes the preparation of the gel, the choice of a suitable column and the various elution techniques. The many practical applications mainly involve the use of 'Sephadex', but this is probably not due to any loyalties of the author to his employer, Pharmacia Fine Chemicals AB, the manufacturers of 'Sephadex', but is merely a reflexion of the domination of the market by this product. Although any reference work on biochemical techniques must contain a chapter on gel chromatography, this book, which is the most expensive of the trio, must be considered a poor investment because it contains little more information than can be obtained from the technical literature provided free by the manufacturers of these materials.

The third book describes the uses of immunochemistry for the identification of various macromolecules, for example, polysaccharides, proteins and lipids. The author follows the pattern set by the other two books and concentrates upon the practical applications of the technique and, in particular, pointing out to the reader the possible errors and misinterpretations which can arise. It is pleasantly free from the bewildering terminology which many authors in this field use with reckless abandon and which may have previously discouraged potential users of this valuable technique.

All three books are printed on good quality paper; they are easy to read and should stand up reasonably well to the constant handling a laboratory manual will inevitably receive. The title on the spine of my volume on immunochemistry contained a glaring error—'macromoleculse'.

N. SHAW

CONSTITUENTS OF LICHENS

Chemical and Botanical Guide to Lichen Products

By Chicita F. Culberson. Pp. xi+628. (University of North Carolina Press: Chapel Hill, November 1969.) $12.50.

THIS is a good book which will probably be the standard text on the subject for some time to come. Lichens produce a wide range of compounds which have been variously called lichen acids, lichen substances or lichen products. They include depsides, depsidones, dibenzofurans, usnic acids, chromones, xanthones, anthraquinones, carotenes, sterols, terpenes, terphenylquinones and pulvinic acid derivatives. Many of these compounds are known only from lichens, particularly those which are acetate-polymalonate derived aromatic phenols joined by esterification, oxidative coupling or both.

Two quite different groups of scientists are interested in these compounds. First, and for obvious reasons, there are organic chemists. Second, there are lichen taxonomists. It is over a century since Nylander described the "colour tests" which became an integral part of the identification and classification of lichens. In recent decades, the development of simple microchemical methods for the identification of specific compounds has had a major impact on lichen taxonomy. Indeed, in the taxonomy of no other group of plants has chemistry been used so successfully as in lichens.

This book is good because it caters so well for both the chemist and the lichen taxonomist. Dr Culberson herself is an organic chemist with an eminently well deserved reputation in the chemistry of lichen products. She has been closely associated in her work with her husband, a lichenologist who is the leading expert in the world in the chemical taxonomy of lichens. Hence, the lichen nomenclature in this book is modern and reliable, with the synonymy thoroughly checked.

The essential core of the book consists of two major reference sections. The first lists more than 300 chemical constituents of lichens, with physical data, chemical structures and annotated references. The second lists the occurrence of these compounds in approximately 2,000 species, forms and varieties of lichens. There is also an excellent chapter on the biogenesis of lichen products and their relationship to the products of free-living fungi. In dealing with the chemical constituents, the author has not restricted herself to secondary products or lichen acids, but she also includes carbohydrates, amino-acids, vitamins and growth factors.

Some lichen taxonomists might have appreciated an introductory guide to the actual methods of using microchemical techniques, although copious references on them are given. Likewise, the chemist might have been given some guide as to what compounds might be expected in previously unstudied lichens, especially where primary metabolites are concerned.

Perhaps the most important comment I can make, however, is an earnest wish that the author and publishers will be able to produce new editions of this book when advances in research make this necessary.

D. C. SMITH

GROWING VASCULAR CELLS

Tissue Cultures

By O. J. Pollak. (Monographs on Atherosclerosis, Vol. 1.) Pp. xii+143. (Karger: Basle and New York, 1969.) SFr./DM 41; 82*s*.

A MONOGRAPH on tissue culture might seem to be an unusual start for a series devoted to atherosclerosis. Workers in the field will, however, appreciate the need for a fundamental study of the prime reactor in the atherosclerotic process, namely, the vascular endothelial cell.

The book is comprehensive and has a full bibliography. It emphasizes and summarizes the many difficult problems encountered when carrying out and interpreting experiments with growing vascular cells. Not the least of these problems is the precise identity of the cell that emerges in the course of tissue culture; a problem that is still not entirely solved. A more intensive cytochemical study of such cells might be of value in establishing cell identity; the author discusses and emphasizes cytochemical techniques as applied to tissue culture.

Much of the work that is described is concerned with aortic endothelial cells. It does not necessarily follow that they resemble the lining cells of coronary arteries that have not been so fully studied. This important point is made, but perhaps not forcibly enough.

The end of the book is better than the beginning. Towards the end there is more discussion and critical comment. The beginning is more of a list of results and names of authors with little or no commentary on the quality or significance of the reported works.

It is sometimes difficult to understand the meaning of sentences largely because of the American use of English, but generally speaking the subject is clearly and concisely expressed.

This book provides a rare, valuable summary of modern work on arterial cell culture and is an important addition to the writings about atherogenesis. G. A. GRESHAM

Obituaries

Dr G. D. Greville

GUY D. GREVILLE, who died on December 13, 1969, at the age of 62, had been head of the Department of Biochemistry at the Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge, where he succeeded Sir Rudolph Peters in 1959. After graduating with a double first in chemistry at Cambridge in 1929, he worked for some years in the biochemical section of the Middlesex Hospital Medical School, where he collaborated with F. Dickens and E. C. Dodds. In 1937 he was appointed research biochemist in charge of the biochemical laboratories at Runwell Hospital, Essex, and in 1944 joined the Ministry of Supply team, headed by Dr M. Dixon, working at Cambridge on the actions of war gases on enzymes. When the team was disbanded, he was appointed to a lectureship in the Department of Biochemistry at the University of Cambridge and continued there until the move to Babraham in 1959.

Greville's main contributions were in the field of mitochondrial metabolism and the action of "uncoupling" drugs. His first biochemical studies were carried out in collaboration with F. Dickens and dealt with sugar conversions and breakdown in embryonic, tumour and adult normal tissues. In 1933 he began, with E. C. Dodds, an investigation of the metabolic stimulation produced by dinitrophenol, and that led on to a study of "uncoupling" drugs that formed the subject of his PhD thesis and became an interest that continued throughout the rest of his life. In the early 1950s improved methods made possible the preparation of mitochondria and more direct investigation of their metabolic activities. In collaboration with A. L. Lehninger, Greville showed that both D and L-β-hydroxybutyrate undergo oxidation in mitochondria although the L-isomer was thought to be the "naturally occurring" form. The initial paths of oxidation of the two forms were found to be different, but it seemed that the D-form also occurred naturally and played a part in ketone body and fatty acid metabolism.

In 1955 Greville and D. M. Needham showed that dinitrophenol accelerates the ATPase activity of both myosin and mitochondria and, from 1956, Greville began a collaboration with J. B. Chappell on mitochondrial swelling. They established that the swelling induced by a variety of agents, for example phosphate, thyroxine and calcium ions, was dependent on respiration and a supply of energy. After moving to Babraham in 1959, Greville continued to work on the biochemistry and morphology of mitochondria. Important studies were made of the influence of the composition of the suspending medium on the properties of these organelles and the mechanism by which they accumulate divalent metal ions. In collaboration with E. A. Munn, he isolated a new protein, for which the name calliphorin has been proposed, from mitochondria of the flight muscles of *Calliphora erythracephala*. Calliphorin has been purified to a single component and, together with related proteins, seems to be an important storage substance fairly widely distributed among the insect orders.

Everyone who has known or worked with Greville pays tribute to the immense care and patience he exercised in his experimental work and the meticulous repetition used to ensure the accuracy of his observations. He spent a large part of his time helping other people with their research work, but was so self-effacing that many of these contributions went unacknowledged. His long experience and critical attitude made him an invaluable source of information and in recent years he wrote many noteworthy reviews on mitochondrial biochemistry. An endeavour to which he recently devoted much time and care has been his co-editorship with P. N. Campbell of *Essays in Biochemistry* on behalf of the Biochemical Society. Authors contributing essays have been somewhat staggered to find that the Editor had checked every reference in lists frequently running to several hundred. At the time of his death, Greville was actively working on the sixth volume of this immensely successful series which is widely read for both pleasure and profit by advanced students and established workers in biochemistry.

Greville worked in a field in which there has been much controversy and moved among workers between whom deep and sometimes bitter disagreements had arisen. His balanced viewpoint here was highly regarded throughout the scientific world. It is characteristic of his modesty and humour that when asked to scrutinize Mitchell's chemiosmotic hypothesis of respiratory chain and photosynthetic phosphorylation he introduced this as follows: "The present writer when asked for a bird's eye appraisal of the Mitchell hypothesis felt that the wrong phylum had been selected, and he offers instead a worm's eye scrutiny. His chief qualifications are that he is completely uncommitted and likely to be psychologically satisfactory, since he is on excellent terms with those of the protagonists whom he happens to know."

Dr I. A. Galloway

IAN GALLOWAY, director of the Animal Virus Research Institute at Pirbright in Surrey from 1939 to 1963, died suddenly on December 22, 1969. He had been the leading figure in foot and mouth disease research in Britain for many years and was responsible for the major development of the laboratory at Pirbright and for the research programme which has given it its high international reputation in the field of foot and mouth disease.

Galloway was born in Edinburgh in 1900 and educated at George Heriot's School and the Royal (Dick) School of Veterinary Studies, obtaining his membership of the Royal College of Veterinary Surgeons in 1922 and his BSc the following year. His early training in research was at the Institut Pasteur, where he entered the service of Professor Levaditi. Galloway returned to England in 1926 to work at the National Institute for Medical Research at Hampstead on behalf of the Foot and Mouth Disease Research Committee. He was at Hampstead until 1939, when it was decided to appoint a scientific superintendent to the research station operated by the committee at Pirbright. Galloway remained at Pirbright for twenty-four years, during which he transformed the research station from a small field unit into a well equipped institute for virological research.

Galloway's contribution must be seen against the enormous changes in virological research which occurred during his career. In the early 1920s much effort was devoted to the search for experimental animals to avoid expensive experimentation in the natural hosts. Galloway's first publication recorded the pathogenicity of foot and mouth disease virus for the rabbit, a host which, although susceptible, was never destined to supplant the guinea-pig in this field. In Paris Galloway had become interested in the problem of the size and physical characteristics of the virus; at Hampstead he and W. A. J. Elford began studies of virus size based on ultrafiltration analysis and later on ultracentrifugation. Their findings, which gave values of 8–12 nm by filtration and 20 nm by centrifugation, were not widely different from those determined twenty or more years later by more refined techniques.

The facilities at Pirbright in 1939 were a challenge to Galloway, allowing him to scale up his work and make use of the farm animal instead of the guinea-pig. He insisted that, if results of value were to be obtained (for example) in vaccination experiments, adequate numbers of cattle should be employed to give statistically significant results and, stimulated by him, methods for accurate assay of the virus and for the estimation of immunogenic potency of vaccines were developed during the 1940s. Comparison of vaccines of different strains within one general immunological type brought to light hitherto unsuspected differences. Papers from the institute published in 1949 showed the importance of subtype differences in immunization programmes, and this work has influenced the practical application of foot and mouth disease vaccines all over the world.

By his personal contacts overseas, Galloway stimulated an interest in dealing with foot and mouth disease in underdeveloped countries, first of all by surveying the strains of virus that were present. This work led to the discovery of three new types in Africa in the late 1940s and a further type in Asia in 1955. The survey work established by Galloway became so obviously valuable that its continuance was guaranteed by the establishment at

Pirbright of the World Reference Laboratory for Foot and Mouth Disease in 1957.

Under his direction in the 1950s the institute developed many new techniques, notably in tissue culture, and Galloway was able to encourage the development of more advanced work in the biochemistry and biophysics of the virus which he himself had been associated with twenty years earlier, with Elford.

Galloway took a leading part in much international discussion on the control of foot and mouth disease. He advised the US Government in 1946–47 on the campaign against the outbreak in Mexico and was responsible for founding the Technical Group of the European Commission for the Control of Foot and Mouth Disease. Through these international activities it can be fairly claimed that he helped greatly towards the cooperation which has achieved a reduction of the disease in many of the most seriously affected areas.

He is survived by his wife, one son and two daughters.

Correspondence

Sternglass's Assumptions

SIR,—The letter under the above title by P. L. Taylor (*Nature*, **225**, 881; 1970) is an example of arguing in a circle. He proves that infant mortality is an exponential function of time by making the assumption that the overall fractional rate of change is constant !

As we have stated in our paper (*Nature*, **224**, 1257; 1969), there is no justification for such an assumption. Even if we postulated an exponential dependence for each individual cause of death, it would not be true for the total mortality, since the different causes of infant mortality are reduced at different rates. Indeed, there is at least one cause, due to genetic defects, which is either irreducible or can only be reduced extremely slowly. Thus a plot of the logarithm of total mortality against time would give a curve with a gradually decreasing slope and not a straight line.

Yours faithfully,

PATRICIA J. LINDOP
J. ROTBLAT

Department of Physics,
The Medical College of St Bartholomew's Hospital,
Charterhouse Square, London EC1.

Information Explosion

SIR,—I hope that the scientific community will heed Professor Webb's warning (*Nature*, **225**, 132; 1970) that we need new ways of presenting information if we are to survive the crisis brought about by the growth of the published literature.

Our colossal indifference to this basic problem stems perhaps from our belief that we can solve it by using computers. Yet this approach is obviously hopeless. Machines can retrieve information for the reader, but they cannot assimilate it for him.

If we examine the literature, we see that it is designed to save publishing costs and not to aid the reader in rapidly and easily comprehending complex information. What fundamentally we need to do then is (1) recognize that the cost of publication is an integral part of the cost of research and be prepared to pay adequate page costs and (2) redesign the literature for rapid reading and easy assimilation.

Although this program will raise the cost of publication by about 10 per cent, this increased cost will be offset by the manifold greater economic returns resulting from the increased usefulness of the information being published.

Yours faithfully,

F. BRUCE SANFORD

US Bureau of Commercial Fisheries,
Division of Publications,
Seattle, Washington 98101, USA.

Keep Off the Grass

SIR,—As mentioned in your leading article[1] of February 7, 1970, more research is needed on cannabis, the effects of which have often been compared with those of alcohol. But a present obstacle against undertaking these highly desirable researches is the great difference between the problems of excessive alcohol consumption and those of the illegal consumption of marihuana, hashish or other cannabis products. The difference is that results obtained from biochemical, pharmacological or behavioural studies of alcohol can be correlated with the amount of alcohol circulating in the individuals under test (a good number of reliable methods of assay are available), whereas none of the results, even those of recent date[2-4], can be linked with the tetrahydrocannabinols (THC), either as such or metabolized and present in the experimental subjects. This present lack of a quantitatively reliable assay of the amount of active principle or principles of cannabis, absorbed and maybe circulating in the blood or perhaps in other body fluids, makes an accurate assessment of the pharmacological or behavioural data almost meaningless, especially if such results are compared with the effects of alcohol or other drugs or obtained with control groups. May I express a strong hope that this problem of an analytical method, obviously for micro-quantities, will be solved soon, because without it there is little chance of establishing the true nature (benign or dangerous) of cannabis ?

THC may be transformed into the materials which, in fact, affect the central nervous system, and there are in any case wide individual variations in the subjective and objective effects. Moreover, illegally acquired marihuana cigarettes (as is often ignored) are usually low in their contents of active constituents, and the long-term effects of cannabis on Western man are unknown. All these uncertainties add to the great difficulty of giving unbiased judgment about the kind of harm (temporary or permanent) that abuse of the drug would bring about. This leaves the presence of tobacco, mixed with hemp particles in the pot cigarettes, outside any consideration of damage to health.

One further point merits emphasis: if one takes "harm" as measure of the degree of hardness of a drug, and not the number of habituates or addicts, then the amphetamine and barbiturate groups are surely harder than cannabis. But that alone is no recommendation to free cannabis from its legal shackles. On the contrary, without entering a large list of reasons for this viewpoint, one can only agree with the last sentence of the leader of *The Times*[5], just as one has to agree with your leader, when it places education, information and public discussions at the top of priorities in the struggle against the problem of drug taking, and at the same level as legislation.

Yours faithfully,

F. BERGEL

Magnolia Cottage,
Bel Royal,
Jersey, Channel Islands.

---

[1] *Nature*, **225**, 485 (1970).

[2] Weil, A. T., Zinberg, N. E., and Nelsen, J. M., *Science*, **162**, 1234 (1968).

[3] Weil, A. T., and Zinberg, N. E., *Nature*, **222**, 434 (1969).

[4] Crancer, jun., A., *et al.*, *Science*, **164**, 851 (1969).

[5] Leader, and Fowler, N., *The Times*, February 7 (1970).

SI Units

SIR,—Much erudite discussion has been initiated by the adoption of SI units by some journals, notably those of the Royal Society. There are plenty of scientists well versed in the strange problems of electromagnetic units and dimensions, but may I as a sometime sailor raise a small squeal of protest at recent horrors perpetrated in the name of SI by *Phil. Trans. Roy. Soc.* on my innocent nautical units.

A ship, everyone knows—even the French—travels at so many knots. An ocean current likewise, since it is usually combined with a ship's speed, is most frequently quoted in knots. There is nothing inherently inferior in knots (of celestial origin), certainly not in comparison with the abomination $m\ s^{-1}$ with which I find my proof besmirched. Ask any sailor from Ordinary Seaman to Admiral of the Fleet to proceed at $2\cdot8\ m\ s^{-1}$ when you mean $5\frac{1}{2}$ knots and the response, though the request come from the Royal Society its very self, is likely to be nautical and brief.

To add insult to injury, the same proof describes the vessel I use as "94 Mg (tonnes) gross". This absurdity is what our SI fiends believe a gross tonnage of 94 is. Perhaps one day some of these megagrammage proponents would care to see how their precious unit is calculated—by taking a tape and measuring up (*inter alia*) the living accommodation on board.

There comes a stage when the SI-ing has to stop. Somebody in the cellars of Carlton House Terrace probably spends all his time taking the scientists' lingua franca and nano-ing and tesla-ing it. When he runs out of papers (and the present policy will soon ensure that), he might start on the classics. Prince Hal's injunction to Falstaff "not to come near my person by ten mile" is ripe for conversion and decimalization.

Yours faithfully,

DAVID DAVIES

47 Cross Street,
Belmont,
Massachusetts 02178.

University News

Dr John Stewart Pate has been appointed to a personal chair in plant physiology in the Department of Botany, **Queen's University of Belfast.**

Dr T. J. Biscow has been appointed to the second chair in physiology, **University of Bristol.**

Dr Joseph E. Murray has been appointed professor of surgery at the Peter Bent Brigham Hospital and the Children's Hospital Medical Center in the Faculty of Medicine, **Harvard University.**

Dr A. M. James, Queen Elizabeth College, London, has been appointed to the chair of physical chemistry, tenable at **Bedford College, London.**

Dr J. K. Sutherland, University of London, has been appointed professor of organic chemistry in the **University of Manchester,** in succession to Professor A. J. Birch.

A research council has been established at the **University of Moncton,** New Brunswick, to plan and coordinate research activities. **Dr Brian T. Newbold** has been appointed president of the council and full time director of research, and the other members of the council are **Dr Auréa Cormier,** School of Home Economics, **Dr Léonard LeBlanc,** Faculty of Science, **Father Jules Léger,** Department of History, **Dr Gaston Allaire,** Department of Music, **Dr Pierre Dion,** Psycho-Social Centre, and **Dr Emmanuel Sajous,** Department of Economics.

Mr Gerald Dix has been appointed to the chair of planning and the directorship of the Institute of Planning Studies in the **University of Nottingham.**

Dr George Eason has been appointed to the new chair of mathematics for applied scientists in the Department of Mathematics, **University of Strathclyde.**

Appointments

Dr Gordon I. Hobday has been appointed managing director of **Boots Pure Drug Company Limited,** in succession to Mr Kenneth Williamson.

Dr F. T. Hamblin, at present chairman and managing director of ICI Deutschland GmbH and of ICI (Switzerland) AG, has been appointed to the new post of director of the **British Plastics Federation.**

Announcements

The **Grand Prix Scientifique de la Ville de Paris** has been awarded to **Professor Lucien Malavard.**

D. P. Rose, University of Bradford, has been awarded the **Sir Walter Puckey Prize** in production engineering, administered by the Institution of Production Engineers.

Dr George H. Mitchell has been awarded the Clough memorial medal of the **Edinburgh Geological Society,** for his contributions to the knowledge of the geology of the Lake District, the West Riding of Yorkshire and the south of Scotland.

The **US National Science Foundation** will provide travel grants for about eighty scientists from the United States to attend the NATO advance study institutes to be held in Europe this summer. Applications should be made to the director of the appropriate institute.

The **Biochemical Society** has made the following awards: **the CIBA medal** to Professor T. W. Goodwin, Liverpool, for his work on the biosynthesis of carotenoids and plant sterols; **the Colworth medal** to Dr George Radda, Oxford, for his study of the role of flavins in biological systems.

The **Van den Berghs nutrition awards**—prizes of £500, £250 and £100—will be presented to the writers of the three best papers on the theme "Getting the Most out of Food". Entrants should be resident in the United Kingdom, and papers should be written in English and should not exceed 5,000 words. Further information can be obtained from Van den Berghs Ltd, Kildare House, Dorset Rise, London EC4.

The **Zoological Society of London** has made the following awards: **the Stanford Raffles Award,** to Mr Rex A. Jubb, Grahamstown, South Africa; the **Scientific Medal,** to Professor R. McN. Alexander, University of Leeds, Dr R. V. Short, University of Cambridge, and Professor T. R. E. Southwood, Imperial College, London; **the Thomas Henry Huxley Award,** to Dr Gillian D. Sewell, King's College, London.

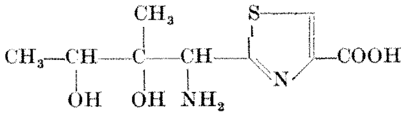
The clean air awards, to be made by the National Society for Clean Air, will now be known as the **Arnold Marsh Clean Air Awards** in memory of Arnold Marsh, director of the society during 1928–68. Contributions to the cause of clean air by individuals, industry and local government authorities are eligible for the awards, which will be made in October. Further details can be obtained from the National Society for Clean Air, Field House, Breams Buildings, London EC4.

The council of the **Institute of Physics and the Physical Society** has made the following awards: **Guthrie medal and prize,** to **Professor A. B. Pippard,** University of Cambridge; **Rutherford medal and prize,** to **Professor S. Devons,** Columbia University; **Glazebrook medal**

and prize, to **Dr E. Eastwood,** English Electric Company; **Maxwell medal and prize,** to **Dr R. J. Eden,** University of Cambridge; **Charles Vernon Boys prize,** to **Dr A. Hewish,** University of Cambridge; **A. B. Wood medal and prize,** to **Dr B. S. McCartney,** National Institute of Oceanography; **Simon memorial prize,** to **Professor W. Meissner,** Bavarian Academy of Sciences.

The council of the **Geological Society of London** has made the following awards: the **Wollaston Medal** to Professor P. Kuenen, Dutch Geological Institute; the **Murchison Medal** to Professor R. M. Shackleton, University of Leeds; the **Lyell Medal** to Professor F. H. Stewart, University of Edinburgh; the proceeds of the **Wollaston Fund** to Dr D. H. Matthews, University of Cambridge; the proceeds of the **Murchison Fund** to Dr A. C. Bishop, British Museum (Natural History); moieties of the **Lyell Fund** to Professor B. M. Funnell, School of Environmental Science, Norwich, Dr R. Goldring, University of Reading, and Mr R. McQuillin, Institute of Geological Sciences.

ERRATUM. The following corrections should be made to the article "The Structure of Thiostrepton" (*Nature*, **225**, 233; 1970): In Table 1, page 234, all minus (−) signs should be replaced by the letter L and plus (+) signs by the letter D; similar corrections should be made in Fig. 2, page 234, and on line 33 of page 235. In Table 1, the chemical formula of thiostreptine is



In ref. 7, the name of the first author is Cross, D. F. W.

ERRATUM. The author of the article "Effects of Histamine on the Toad Spinal Cord" (*Nature*, **225**, 196; 1970) is A. K. Tebēcis.

ERRATUM. In the article "Activity of Various Fractions of Bradykinin Potentiating Factor against Angiotensin I Converting Enzyme" by S. H. Ferreira, L. J. Green, V. A. Alabaster, Y. S. Bakhle and J. R. Vane (*Nature*, **225**, 379; 1970), the abbreviation pGa on line 12 should read PCA (pyrrolidone carboxylic acid).

CORRIGENDUM. The following corrections should be made to the article "Rotational Properties of Neutron Star Models" by J. M. Cohen and A. G. W. Cameron (*Nature*, **224**, 566; 1969). The term Ω^2 inside the square brackets in equation (2) should be deleted. The entries in column 5 of Table 1 should be identical with those in column 4, and the entries in columns 3 and 4 of Table 2 should be replaced by $1{\cdot}805 \times 10^4$ and $4{\cdot}6 \times 10^{-7}$ times the entries in column 4 of Table 1, respectively. The conclusions of the paper are not affected. We thank K. Thorne and J. Hartle for raising questions concerning the validity of equation (2). The discrepancy was due to a copying error.

International Meetings

March 12–13, **High-Resolution Analyses,** Oak Ridge, Tennessee (Charles D. Scott, ORNL, PO Box X, Oak Ridge, Tennessee 37830, USA).

March 13–16, **US National Science Teachers Association Convention,** Cincinnati (NSTA, 1530 Kingshighway Boulevard, St Louis, Missouri 63110, USA).

March 22–25, **American Wildlife and Natural Resources,** Chicago (C. R. Gutermuth, 709 Wire Building, Washington DC 20005, USA).

March 23, **The Wildlife Society Annual Meeting,** Chicago (Fred G. Evenden, Executive Director, Suite S-176, 3900 Wisconsin Avenue NW, Washington DC 20016, USA.)

March 23–24, **503 Meeting of the Biochemical Society,** London (The Biochemical Society, 7 Warwick Court, Holborn, London WC1).

March 23–25, **Biomathematics and Computer Science in the Life Sciences,** Houston (Office of the Dean, University of Texas Graduate School of Biomedical Sciences at Houston, Division of Continuing Education, PO Box 20367, Houston 77025, USA).

March 24–26, **Solid Wastes Management,** Houston (H. Nugent Myrick, University of Houston, Cullen College of Engineering, Department of Civil Engineering, Environmental Science and Engineering Program, 3801 Cullen Boulevard, Houston, Texas 77004, USA).

March 25–27, **Fundamental and Practical Aspects of Pest Management,** Raleigh (Pest Management Conference, Department of Entomology, North Carolina State University, Raleigh, North Carolina, USA).

March 31–April 3, **Great Lakes Research,** Buffalo (Robert A. Sweeney, Great Lakes Laboratory, State University College, 5 Porter Avenue, Buffalo, NY, USA).

April 2–3, **Electrochemistry,** Guildford (Dr D. S. Allam, Department of Chemical Physics, University of Surrey, Guildford, Surrey).

April 3, **Collagen Club Meeting,** Bristol (Dr A. J. Bailey, ARC Meat Research Institute, Langford, Bristol).

April 5–9, **International Association of Gerontology, European Biological Section,** Leeds (IAGEBS Secretariat, Department of Medicine, The General Infirmary, Leeds LS1, 3EX, Yorkshire).

April 7–9, **Impact of Air Pollution on Vegetation,** Toronto (D. A. Thomas, Air Management Branch, Ontario Department of Energy and Resources Management 1 St Clair Avenue West, Toronto, Ontario, Canada).

April 7–10, **Thermophysical Properties of Solids at High Temperatures,** Salford (The Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

April 8–9, **Extrapolation to Man,** Bradford (Professor G. D. H. Leach, University of Bradford, Yorkshire).

April 8–10, **The Biochemistry of the Terpenoids,** Liverpool (Dr J. B. Harborne, Botany Department, The University, Reading RG1 5AQ).

April 10–12, **Chemical Aspects of Tribology,** Reading (Education Officer, Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London SW1).

April 10–12, **Institute of Information Scientists Conference,** Reading (Conference Office, Institute of Information Scientists, 5–7 Russia Row, Cheapside, London EC2).

April 12–17, **Annual Meeting of the Federation of American Societies for Experimental Biology,** Atlantic City (Mrs T. C. Heatwole, 5110 West Franklin Street, Richmond, Virginia 23226 USA).

April 13–14, **Polymer Structure and Properties,** Manchester (Registrar, University of Manchester Institute of Science and Technology, Sackville Street, Manchester M60 1QD).

April 13–14, **Combustion Emission: Effects, Measurement and Control,** Leeds (Dr G. Dixon-Lewis, Houldsworth School of Applied Science, The University, Leeds, 2).

April 13–17, **Automotive Drive-Line,** Jersey (J. E. Lonsdale, Automobile Division, Institution of Mechanical Engineers, 1 Birdcage Walk, London, SW1).

April 14–16, **Computer Graphics,** Uxbridge (Mrs R. Gregory, Department of Computer Science, Brunel University, Uxbridge, Middlesex).

April 14–17, **Automatic Test Systems,** Birmingham (The Conference Registrar, Institution of Electronic and Radio Engineers, 8–9 Bedford Square, London, WC1).

April 17–19, **Discoveries in Biological Psychiatry,** Baltimore (Symposium Secretary, Taylor Manor Hospital, Ellicott City, Maryland 21043, USA).

April 21–22, **Organic Photochemistry,** Manchester (Dr M. G. Barlow, Department of Chemistry, University of Manchester Institute of Science and Technology, Sackville Street, Manchester 1).

April 22–24, **Space Congress,** Cocoa Beach (Timothy H. Hanrahan, The Aerospace Corporation, PO Box 4007, Patrick AFB, Florida 32925, USA).

April 27–May 8, **Measurement, Analysis and Control of Noise,** Sheffield (K. H. Sutherland, Industrial Liaison Officer, Department of Mechanical Engineering, University of Sheffield, Mappin Street, Sheffield S1 3JD).

May 4–7, **London Engineering Congress 1970,** London (LECO '70 Press Office, Council of Engineering Institutions, 2 Little Smith Street, London SW1).

May 11–15, **Random Vibration and Random Signal Analysis,** Sheffield (K. H. Sutherland, Industrial Liaison Officer, Department of Mechanical Engineering, University of Sheffield, Mappin Street, Sheffield, S1 3JD).

May 18–19, **Space Applications of Solid State Luminescent Phenomena,** University Park, Pennsylvania (Professor J. N. Weber, Materials Research Laboratory, University Park, Pennsylvania 16802, USA).

May 22–29, **Tenth International Cancer Congress,** Houston (Tenth International Cancer Congress, PO Box 20465, Astrodome Station, Houston, Texas 77025, USA).

May 25–26, **Cholinergic Ligand Interactions,** Buffalo (Dr D. J. Triggle, School of Pharmacy, State University of New York at Buffalo, New York 14214, USA).

June 1–6, **IX International Mineral Processing Congress,** Prague (The Executive Committee, Ore Research Institute, Modřanská 23, Praha 4, Czechoslovakia).

June 2–July 14, **Ecology of Fishes ; Structure and Function of Marine Animals; Marine Microbiology** (Summer Courses), Port Aransas (The Director, The University of Texas Marine Science Institute, Port Aransas, Texas 78373, USA).

June 8–19, **Applied Optics** (Summer School), London (Registrar, Imperial College, London SW7).

June 15–18, **Tissue Culture Association Annual Meeting,** Washington DC (Mr V. P. Perry, American Foundation for Biological Research, 11125 Rockville Pike, Rockville, Maryland 20853, USA).

June 22–July 3, **The Physics of Quantum Electronics** (Summer Course), Prescott, Arizona (S. F. Jacobs, Optical Sciences Center, University of Arizona, Tucson, Arizona 85721, USA).

June 29–July 11, **Foundations of Quantum Mechanics** (Summer Course), Varenna (Professor F. Selleri, Istituto di Fisica dell' Università, Via Amendola 173, Bari, Italy).

June 29–July 10, **Health Physics** (Summer School), London (Dr H. D. Evans, Nuclear Technology Laboratories, Department of Chemical Engineering and Chemical Technology, Imperial College, London SW7).

July 6–17, **Design and Analysis of Scientific Experiments** (Summer Course), Cambridge, Massachusetts (Director of the Summer Session, Room E19–356, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA).

July 13–25, **Mantle and Core in Planetary Physics** (Summer Course), Varenna (Professor M. Caputo, Istituto di Fisica dell' Università, Via Irnerio 46, 40126 Bologna, Italy).

July 14–16, **Special Ceramics,** Stoke-on-Trent (Mr P. Popper, British Ceramic Research Association, Queens Road, Penkhull, Stoke-on-Trent ST4 7LQ).

July 15–August 25, **Marine Chemistry; Marine Geology; Estuarine Ecology** (Summer Courses), Port Aransas (The Director, The University of Texas Marine Science Institute, Port Aransas, Texas 78373, USA).

July 19–24, **Cosmic Rays,** La Paz (Sixth Interamerican Seminar on Cosmic Rays, Laboratorio de Fisica Cósmica, Universidad Mayor de San Andrés, La Paz, Bolivia, South America).

July 20–24, **Coastal Geodesy,** Munich (Professor R. Sigl, Institut für Astronomische und Physikalische Geodäsie, 8 München 2, Arcisstrasse 21, Germany).

July 27–August 8, **Critical Phenomena** (Summer Course), Varenna (Professor F. P. Ricci, Istituto di Fisica dell' Università, Piazzale delle Scienze 5, 00185 Roma, Italy).

September 2–4, **Man–Computer Interaction,** Teddington (Conference Department, Institution of Electrical Engineers, Savoy Place, London WC2).

September 7–9, **Computational Physics,** London (Meetings Officer, Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

September 8–12, **Molecular Structure and Spectroscopy,** Columbus (Professor K. Narahari Rao, Molecular Spectroscopy Symposium, 174 West 18th Avenue, Columbus, Ohio 43210, USA).

September 15–18, **Solid State Devices,** Exeter (Meetings Officer, Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

September 16–17, **International Power Sources Symposium,** Brighton (D. H. Collins, Joint Services Electrical Power Sources Committee, POB 136, 26 Wellesley Road, Croydon, Surrey).

September 19–22, **Third Asian and Australasian Congress of Anaesthesiology,** Canberra (Professor D. Joseph, University of Sydney, Sydney 2006, Australia).

September 20–24, **Molecular Spectroscopy,** Brighton (Administrative Secretary, Institute of Petroleum, 61 New Cavendish Street, London W1).

September 20–25, **Congress of the International Society for Burn Injuries,** Prague (A. B. Wallace, Royal College of Surgeons, 18 Nicolson Street, Edinburgh).

September 21–23, **Temperature Measurement,** Warwick (Mr F. R. Bareham, Alcan Research and Development Limited, Southam Road, Banbury, Oxfordshire).

September 21–24, **Human Factors in Telephony,** London (F. E. Williams, PO Research Department, Dollis Hill, London SW2).

September 21–24, **Electronic Engineering in Ocean Technology,** Swansea (Conference Registrar, IERE, 9 Bedford Square, London WC1).

September 21–25, **Seventh International Congress of Plant Protection,** Paris (Société Français de Phytiatrie et de Phytopharmacie, 57 Boulevard Lannes, 75 Paris XVI, France).

September 21–25, **Congress of the International Society for Rock Mechanics,** Belgrade (Congress Secretariat, c/o Institute Za Vodoprivredu Jaroslav Cenri, Bulevar Vojvode Misica 43, 43 Belgrade, Yugoslavia).

September 22–24, **The Vitreous State,** Bristol (Secretary, The Faraday Society, 6 Gray's Inn Square, Gray's Inn, London WC1).

September 23–25, **High Energy Physics,** Durham (Institute of Physics and the Physical Society, 47 Belgrave Square, London SW1).

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Wanted: In or near Mill Hill, London. Furnished 2 or 3-bedroom house or flat for New Zealand post-doctoral fellow, wife and two children. From June 8, 1970, for 1 year. Please contact A. J. Cunningham, Wallaceville Animal Research Centre, Private Bag, Wellington, New Zealand.

Wanted: Furnished accommodation for chemist, wife and two daughters for mid-July to mid-August, with easy access to Queen Elizabeth College, London. Reasonable terms. Please contact Professor L. Hough, Department of Chemistry, Queen Elizabeth College, London W8.

Wanted: Furnished centrally heated house or flat in vicinity of Glasgow for doctor, wife and three children, from September 1970–June 1971. Please contact Dr John J. Furth, Department of Pathology, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania 19104, USA.

Wanted: Furnished accommodation for biochemist, wife and two sons aged 5 and 3 for one year beginning October 1970. Prefer direct public transportation to King's College, London. Please contact Dr D. E. Olins, Biology Division, Oak Ridge National Laboratory, PO Box Y, Oak Ridge, Tennessee 37830, USA.

Vacant: Large furnished home. Please contact Dr D. E. Olins, Biology Division, Oak Ridge National Laboratory, PO Box Y, Oak Ridge, Tennessee 37830, USA.

Wanted: Furnished house or flat with 2 bedrooms for American professor, wife and two boys, aged 9 and 11, near University of Oxford, for 8 months to 1 year beginning August 18, 1970. Please contact Dr Morton M. Weber, chairman, Department of Microbiology, St Louis University School of Medicine, 1402 S. Grand Blvd., St Louis, Missouri 63104, USA.

Wanted: Exchange of modern, well furnished, spacious, 3-bedroom house (and possible use of car) of research physician in Mount Vernon, New York, 15 min from Bronx and 40 min from central Manhattan, for well heated, 1-bedroom, or larger, flat or house close to Sir William Dunn School of Pathology, Oxford. September 1970 through April or May 1971. We have no children nor will we accept family with small children. Rental of either accommodation would also be considered. Also require small flat in Oxford for single female research associate. Please contact Dr Harold P. Klinger, Department of Genetics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA.

Vacant: House in Cambridge for 12 months from mid-August 1970. Very comfortably furnished, 3 large bedrooms, living room, dining room, breakfast room, kitchen, bathroom and W.C., garage with room above, garden. All necessary appliances, including dishwasher, linen, dishes, etc. On bus route, ten minutes to centre of town. Part time domestic help available. Please contact Dr P. Sykes, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW.

British Diary

Monday, March 9

Management by Objectives (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Myrtos: an Early Bronze Age Settlement in Crete (5.45 p.m.) Dr P. Warren, University of London, at the Institute of Archaeology, 31–34 Gordon Square, London WC1.

Training—a Systems Approach (5.30 p.m.) Captain G. Huggett, Institution of Electrical Engineers, at Savoy Place, London WC2.

Tuesday, March 10

Advanced Passenger Train (7 p.m.) Dr S. Jones, Institution of Electrical Engineers; and the Institution of Mechanical Engineers, at Culham Laboratory, Harwell, Didcot, Berks.

Comparative Form and Function of some Intertidal New Zealand Ophiuroids, Dr R. J. Pentreath; **The Development of Branchiopod Crustacea,** Dr D. T. Anderson; Film (5 p.m.) Zoological Society of London, at the Zoological Gardens, Regent's Park, London NW1.

Deformation and Fracture of Soft Materials (2): Mechanics of Foamed Elastic Materials (4.30 p.m.) Professor A. N. Gent, University of London, at Queen Mary College, Mile End Road, London E1.

Lord Hailey: His Contribution to Africa (5.15 p.m.) Sir Frederick Pedler, Royal Society of Arts, Commonwealth Section; and the Royal African Society, at the Royal Society of Arts, John Adam Street, London WC2.

Management Effectiveness for Engineers (6 p.m.) Mr H. Makepeace, Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Some Bronze Age, Iron Age and Early Medieval Settlements in the Netherlands (5.45 p.m.) Professor H. T. Waterbolk, University of London, at the Institute of Archaeology, 31–34 Gordon Square, London WC1.

Steam Engines (6 p.m.) Mr R. M. Palmer, Institution of Mechanical Engineers at 1 Birdcage Walk, London SW1.

Wednesday, March 11

A Geochronological Study of the Lewisian of the Laxford Area, North-west Scotland, Dr R. St J. Lambers and Dr J. G. Holland; **The Sgurr Beag Slide—a Major Tectonic Break within the Moinian of the Western Highlands of Scotland,** Mr P. W. G. Tanner (5 p.m.) Geological Society, at Burlington House, Piccadilly, London W1.

Catalytic Analysis (6 p.m.) Mr A. Townshend, Society for Analytical Chemistry, Midlands Section, at the University of Birmingham.

Electron Spin Resonance in Biological Systems (two-day symposium) at the School of Pharmacy, Regional College of Technology, Byrom Street, Liverpool.

Electronic Production in the 1970's (7.30 p.m.) Mr P. Newell, Institution of Electronic and Radio Engineers, at the Bull's Eye, Basildon, Essex.

Electronics in Cars (5.30 p.m.) Mr L. G. Cripps, Institution of Electrical Engineers, at Savoy Place, London WC2.

Food Legislation (6 p.m.) Mr E. Whittle, Mr R. Blanchfield and Mr R. Knight, Society of Chemical Industry, Food Group; The Association of Public Analysts; and the Institute of Food Science and Technology, at the Middlesex Hospital Medical School, London W1.

History of the Communication of Scientific and Technological Ideas—Summary and General Discussion (1 p.m.) Mr. F. Greenaway, Royal Institution, History of Science Discussion Group, at 21 Albemarle Street, London W1.

Immunological Aspects of Malignancy (2 p.m.) Dr G. Hamilton Fairley, University of London, at the Royal Postgraduate Medical School, Du Cane Road, London W12.

Polymer Processing (9.30 a.m. symposium) Institution of Chemical Engineers, at Salford University.

Problems in Colour Measurement (2 p.m.) Dr D. Patterson; **Colour Measuring Instruments** (3.30 p.m.) Colour Group (Great Britain), Northern Section, at the University of Leeds.

Standards and the Introduction of Metrication (7.30 p.m. brains trust) Mr R. W. Jones and Mr P. C. Young, Plastics Institute, at the Polygon Hotel, Southampton.

The Sand and Gravel Industry (3 p.m.) Natural Environment Research Council, at the Institute of Geological Sciences, Exhibition Road, London SW7.

The Spread of Research at The City University (1.10 p.m.) Professor Richard Cox, City University, at the Graduate Business Centre, Gresham College, Basinghall Street, London EC2.

Welded Process Plant (two-day conference) Institution of Mechanical Engineers; and the Welding Institute, at 1 Birdcage Walk, London SW1.

Thursday, March 12

Applications of Oxygen Sensors in Biological and Other Systems (6.30 p.m.) Society for Analytical Chemistry, at the Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2.

Control of Biochemical Pathways (two-day symposium) at Luton College of Technology, Park Square, Luton. Chairman: Professor P. J. Randle.

Materials in Space Flight (7.15 p.m.) Mr N. H. Langton, Institution of Chemical Engineers, at the University of Strathclyde, Glasgow.

Moon Rock (1.15 p.m.) Dr J. Bastin, University of London, at Queen Elizabeth College, 61–67 Campden Hill Road, London W8.

New Developments in Electron Microscopy with special emphasis on their Application in Biology (9.45 a.m., two-day meeting) Royal Society, at 6 Carlton House Terrace, London SW1.

Recent Progress in the Study of Fast Reactions (symposium, 2 p.m.) Chemical Society, at the Royal Institution, Albemarle Street, London W1.

Rough Surfaces as an Aid to Improving Heat Transfer (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

The Future of Agricultural Research (2.30 p.m.) Sir Gordon Cox, KBE, FRS, East Malling Research Station, at Withersdane, Wyme College, Ashford, Kent. (Amos Memorial Lecture).

The Harmony of Growth (5.30 p.m.) Dr Elsie Widdowson, University of London, at Senate House, London WC1.

Friday, March 13

Automation of the Design and Production of a Large Digital Computer (5.30 p.m.) Mr C. D. March, Mr G. Haley and Mr D. Adshead, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Photogeneration of Quinones in Solution of Singlet Oxygen Molecules (1 p.m.) Professor D. R. Kearns, Royal Institution, Photochemistry Discussion Group, at 21 Albemarle Street, London W1.

Thermoset Acrylics—What Next and Where? (6.30 p.m.) Mr P. V. Robinson, Oil and Colour Chemists' Association, at the Manchester Literary and Philosophical Society, 36 George Street, Manchester 1.

Understanding the Solar System (9 p.m.) Professor T. Gold, FRS, Royal Institution, at 21 Albemarle Street, London W1.

Wheatstone and the Generator (5.30 p.m.) Mr B. P. Bowers, Institution of Electrical Engineers, at Savoy Place, London WC2.

Saturday, March 14

Pigments and Extenders (10 a.m.) Oil and Colour Chemists' Association, Scottish—Student Group, at St Enoch Hotel, St Enoch Square, Glasgow.

Wild Life for the Future (3.30 p.m.) Mr George Howat, Inner London Education Authority, at the Horniman Museum, London Road, Forest Hill, London SE23.

Monday, March 16

Nutritional Ideas in the Early Nineteenth Century (5.30 p.m.) Miss A. J. O'Hara May, British Society for the History of Science, at the Science Museum, Exhibition Road, London SW7.

Sonar (5.30 p.m.) Mr T. N. Reynolds, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Choice of Industrial Drives up to 100 hp (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Parliamentary and Scientific Committee (5.30 p.m.) Mr Roger Gresham Cooke, MP, Royal Institution, Library Circle, at 21 Albemarle Street, London W1.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 265, No. 1163 (11 December 1969): The Numerical Solution of Certain Integral Equations with Non-Integrable Kernels Arising in the Theory of Crack Propagation and Elastic Wave Diffraction. By R. Burridge. Pp. 353–381. 17*s* ; $2.20. Vol. 265, No. 1164 (11 December 1969): Volcanic Geology of Mount Suswa, Kenya. By R. W. Johnson. Pp. 383–412. 28*s*; $3.65. (London: The Royal Society, 1969.) [1112

British Museum. Periodical Publications in the National Reference Library of Science and Invention. Part 1: List of Non-Slavonic Titles in the Bayswater Division. Pp. 289. (London: British Museum, 1969.) 25*s*. [1212

PEP Broadsheet 516: Aycliffe to Cumbernauls: a Study of Seven New Towns and their Regions. By Ray Thomas. Pp. vii + 801–962. (London: Political and Economic Planning, 1969.) 20*s*. [1212

Imperial College, Royal School of Mines. 1966–1969 Research Report. Pp. iii + 88 + 8 plates. (London: Imperial College, Royal School of Mines, 1969.) [1212

Department of Education and Science—National Advisory Council on Education for Industry and Commerce. Report of the Committee on Technician Courses and Examinations. Pp. viii + 127. (London: HM Stationery Office, 1969.) 12*s* 6*d*. [1212

The Theatre in the Living Room. By Shaun Sutton. (BBC Lunch-time Lectures Eighth Series—I.) Pp. 15. (London: BBC, 1969.) [1512

ARC Institute for Research on Animal Diseases—Report 1968. Pp. 42. (Compton, Berkshire: Institute for Research on Animal Diseases, 1969.) 6*s* 6*d*. [1512

The Proposal for a European Company. By Dennis Thompson. (European Series, No. 13.) Pp. 73. (London: Chatham House; and Political and Economic Planning, 1969.) 7*s* 6*d*. [1512

Bulletin of Special Courses in Higher Technology, Management Studies and Commerce, 1969/70. Part 2: Spring and Summer Terms. Pp. 114. (London: London and Home Counties Regional Advisory Council for Technological Education, 1969.) 10*s*. [1512

The Design of Physics Buildings. (Proceedings of the IPPS/RIBA Conference at the University of Lancaster, 2–4 January 1969.) Pp. 52. (London: RIBA Publications, Ltd, 1969.) 60*s*. [1512

John Innes Institute. Fifty-ninth Annual Report, 1968. Pp. 51. (Norwich: John Innes Institute, 1969.) [1612

Bulletin of the British Museum (Natural History). Geology. Vol. 18, No. 6: Lower Llandovery (Silurian) Trilobites from Keisley, Westmorland. By J. T. Temple. Pp. 197–230 + 6 plates. 42*s*. Appendix to Supplement No. 3: Appendix to "Studies on Mesozoic and Cainozoic Dinoflagellate Cysts". By R. J. Davey, C. Downie, W. A. S. Sarjeant and G. L. Williams. Pp. 24. 16*s*. (London: British Museum (Natural History), 1969.) [1612

University of Hull. Annual Report 1968/1969. Pp. 153. (Hull: The University, 1969.) [1712

Second Report of the Countryside Commission for the year ended September 30, 1969. Pp. viii + 75 + 11 photographs. (London: HM Stationery Office, 1969.) 10*s* (50*p*) net. [1812

Other Countries

US Atomic Energy Commission. The Nuclear Industry, 1969. Pp. xiii + 338. (Washington, DC: Government Printing Office, 1969.) $2.50. [312

Chemico-Biological Interactions, Vol. 1, No. 1, October 1969. Pp. 1–124. Subscription rate: Dfl. 90.00 plus Dfl. 5.40 for postage. (Amsterdam: Elsevier Publishing Company, 1969.) [412

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin 169: Late Upper Devonian (Famennian) Rhynchonellid Brachiopods from Western Canada. By Paul Sartenaer. Pp. 269. $8. Paper 68–71: Botanical Investigations at Three Known Mineral Deposits. By Lily Usik. Pp. v + 32. $1.50. 3 Geological Maps. (Ottawa: Queen's Printer, 1969.) [512

OECD. Reviews of National Science Policy—Canada. Pp. 453. (Paris: Organization for Economic Co-operation and Development, 1969.) 54 francs; 82*s*; $11.80; 40 DM; Sw. fr. 43.20. [512

Museum of Comparative Zoology, Harvard University. Breviora. No. 332 (24 October 1969): The Brazilian Triassic Cynodont Reptiles *Belesodon* and *Chiniquodon*. Pp. 16. No. 333 (24 October 1969): The Chenares (Argentina) Triassic Reptile Fauna. V: A New Chiniquodontid Cynodont, *Probelesodon lewisi*—Cynodont Ancestry. By Alfred Sherwood Romer. Pp. 24. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1969.) [1112

Science Council of Canada. Report No. 6: A Policy for Scientific and Technical Information Dissemination. Pp. 35. (Ottawa: Queen's Printer, 1969.) $0.75. [1212

Rubber Research Institute of Malaya—Annual Report 1968. Pp. 140. (Kuala Lumpur: Rubber Research Institute of Malaya, 1969.) [1512

Science Council of Canada. Report No. 5: University Research and the Federal Government. Pp. 28. (Ottawa: Queen's Printer, 1969.) $0.75. [1512

National Research Council of Canada. Annual Report on Support of University Research 1968–1969. (N.R.C. No. 10784.) Pp. viii + 406. (Ottawa: National Research Council of Canada, 1969.) $1. [1512

Publications de l'Institut National pour l'Étude Agronomique du Congo. Série Scientifique No. 111: Sur les Relations qui Existent entre Quelques Facteurs Climatiques, le Développement et la Productivité du Cotonnier en Région (Am) N de Köppen. Par J. Demol. Pp. 48. (Bruxelles: Institute National pour l'Étude Agronomique du Congo, 1969.) 125 francs. [1512

Australia. Annual Report of the Museum of Applied Arts and Sciences, Sydney, for the year 1968. Pp. 24. (Sydney: Museum of Applied Arts and Sciences, 1969.) [1512

Israel Academy of Sciences and Humanities. Proceedings, Section of Sciences, No. 15: The Recent History of the Benzine Formula. By Ernst D. Bergmann. Pp. 12. (Jerusalem: Israel Academy of Sciences and Humanities, 1969.) IL. 1; $0.50. [1512

Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 135: Die Anfälligkeit Verschiedener Gramineen gegen *Cerosporella herpotrichoides* Fron im Hinblick auf due Fruchtfolgezusammenhänge bei der Halmbruchkrankheit des Weizens. Von Dr Peter Hartz. Pp. 38. (Berlin-Dahlem: Biologischen Bundesanstalt für Land- und Forstwirstschaft, 1969.) 12 DM. [1512

Commonwealth Scientific and Industrial Research Organization. Australia. Biennial Report of the Division of Physics, 1967–69. Pp. 54. (Sydney: CSIRO, 1969.) [1612

HOW TO BUY NATURE

Volumes start in January, April, July and October, but subscriptions may begin with any issue.

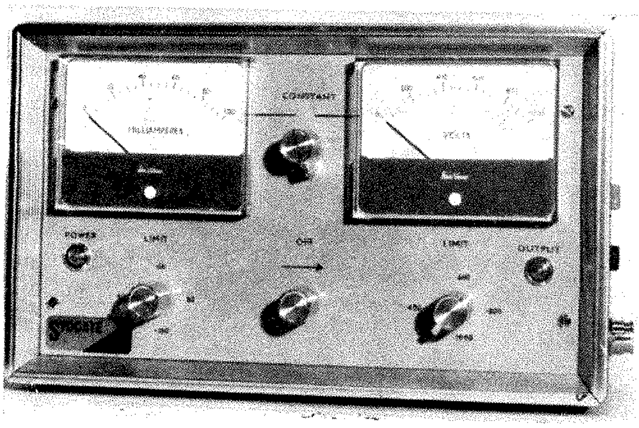
The direct postal price per subscription for one copy of NATURE each week is:

12 MONTHS (52 issues and 4 indexes)

Great Britain and Eire................................**£14.0.0** each
United States of America........(by air freight)............ **$48** each
Canada..........................(by air freight).......... **$52** each
Elsewhere overseas by surface mail....................**£14.0.0** each
(Charge for delivery by air mail on application)
Shorter periods *pro rata*. (Minimum three months.)

Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
4 LITTLE ESSEX STREET, LONDON WC2
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2
Subscription Department
MACMILLAN (JOURNALS) LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 5431
Advertisements only should be addressed to
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephones 01-242 6264/01-405 4743
Telegrams: Textualist London WC2
Registered as a newspaper at the Post Office
Copyright © Macmillan (Journals) Limited, March 7, 1970

ELECTROPHORESIS POWER SUPPLIES



We now manufacture the largest range of power supplies, covering the range from 0.300 to 0.2500 volts with:

Constant current, constant voltage
Eight models in standard range
Specials made to order
Continuously adjustable
Two current ranges 100 and 200 MA
Adjustable limits on voltage and current

OTHER PRODUCTS

- Digital stop watches and timers
- Data conversion equipment

STOGATE TECHNICAL DEVELOPMENTS LTD.

Industrial Estate, Daux Road, Billingshurst,
Sussex Tel: Billingshurst 2750

STOGATE

'MICHROME'

STAINS & REAGENTS FOR BIOLOGY

Acridine orange, Aquamount, Fluormount, Lipid crimson, Matamount, May-Grünwald, Leishman, Giemsa Toluidine blue, Cresyl fast violet, Papanicolaou stains, Thioflavine S, Brilliant cresyl blue, Nile blue, Pyronin Y, Trifalgic acid, Rhodanile blue, Haplo, etc.

CATALOGUE AVAILABLE ON REQUEST

DYES FOR USE IN ALL INDUSTRIES

RESEARCH & PRODUCTION CHEMISTS: if you are seeking dyes with specific chemical and physical properties suitable for particular manufacturing and/or testing processes you are invited to contact us for supplies.

We hold stocks of an enormous variety of dyes, ready for immediate despatch, including:

Cyanine, Dicyanine, Fast Bordeaux O, Aurantia, Crystal violet, BPC-Scarlet R, BPC, Diazonium salts, Neutral violet, Lacmoid, Nile blue, Janus green, Brilliant green BPC, Rosolic acid, Methyl green, p-Ethoxy-chrysoidin, Methyl red, Nitrazine yellow, Pinacryptol green, etc.

INDUSTRIAL RESEARCH ESTABLISHMENTS

are invited to make use of our professional services as consultants on the selection and use of dyes as research and production tools.

EDWARD GURR LTD

Michrome Laboratories,
42 Upper Richmond Road West, London, S.W.14
Cables: Micromlabs London SW14. Telephone: 01-876 82289

QUALITY — RELIABILITY — SERVICE
PROMPT DELIVERY

new·4·u
50 BIOCHEMICALS

●●●●●●●●●●●●●●●●●●

ENZYMES
Alcohol Dehydrogenase (*Lyophilised*)
α -Amylase
Esterase
ß-Fructosidase (*Invertase*)
Gluconate-Kinase
α -Glucosidase (*Maltase*)
Guanosine-5-monophosphate Kinase
3-HBDH *increased activity*
Inorganic Pyrophosphatase
L-Asparaginase
NAD-Pyrophosphorylase
Nucleoside-monophosphate Kinase
Phosphodiesterase *from Beef heart*
Phosphorylase a
Phosphorylase b
Polynucleotide Phosphorylase
Sorbitol Dehydrogenase
Trypsin on carrier molecule

CoENZYMES
NAD *LDH inhibitor free*
NADH *Low background fluorescence*
NADP *improved grade*
Acetyl Coenzyme A

DEOXY NUCLEOTIDES
Deoxy-adenosine-5′-monophosphate
Deoxy-cytidine-5′-monophosphoric acid
Deoxy-guanosine-5′-monophosphate
Deoxy-thymidine-5′-monophosphate

NUCLEOTIDES
Adenosine-2′: 5′-diphosphate
Adenosine-3′, 5′diphosphate
Adenosine-5′-phosphosulphate
Cytidine-5′-diphospho-glucose
Guanosine-5′-triphosphate · *Sodium salt*

CYCLIC NUCLEOTIDES
Cytidine-3′, 5′-monophosphate
Deoxy-thymidine-3′, 5′-monophosphate
Guanosine-3′, 5′-monophosphate
Inosine-3′, 5′-monophosphoric acid
N^6-Monobutyryl-adenosine 3′, 5′-monophosphate
2′-O-Butyryl-inosine-3′, 5′-monophosphate
Uridine-3′, 5′-monophosphate

SUGAR PHOSPHATES & SUBSTRATES
5-Amino-4-imidazole-carboxamide-ribotide
D-Erythrose-4-phosphate
L-γ-Glutamyl-p-nitranilide
N-Acetyl-S-acetacetyl-cysteamine
Orotidine
S-Adenosyl-L-methionine

MOLECULAR BIOLOGICALS
Poly A
Poly C
Poly I
Poly U
DNA *High molecular weight*
$tRNA^{phe}$

●●●●●●●●●●●●●●●●●●

For full analytical details write to
Boehringer Corporation (London) Ltd.,
Bilton House, Uxbridge Road, London, W.5.
If you live in Britain or the Commonwealth
or
Boehringer Mannheim G.m.b.H.,
68, Mannheim 31, P.O.B. 51, West Germany
if you live outside the Commonwealth

mannheim boehringer

CSIRO

RESEARCH SCIENTIST

AUSTRALIA

WHEAT RESEARCH UNIT

GENERAL The Wheat Research Unit of the Commonwealth Scientific and Industrial Research Organization is housed in new laboratories in a wing of the Bread Research Institute of Australia with which it works in close association. It is on a site, eight miles from the centre of Sydney, accommodating several CSIRO Divisions. The Unit is engaged in fundamental studies of the wheat grain including the biochemical aspects of quality and reactions which occur during processing. The Unit is particularly well equipped to undertake protein fractionation and has good range of general equipment and excellent library facilities.

DUTIES The appointee will join a team investigating the physical, chemical and biochemical properties of the proteins, non-starchy polysaccharides, lipids and other components of the wheat grain. Initially he may be required to collaborate in fractionation and purification of his research materials. His previous training and experience will be considered in determining how he will participate in the overall research programme.

QUALIFICATIONS Applicants should have a Ph.D. degree in some field of physical chemistry or biochemistry or postgraduate research experience of equivalent standard and duration, supported by satisfactory evidence of research ability. Some experience in the fractionation and characterization of natural products or in the measurement and interpretation of oxidation reduction phenomena would be an advantage.

SALARY Depending upon qualifications and experience, the appointment will be made within the salary range of Research Scientist, $A6,139–$A7,789 p.a., or Senior Research Scientist, $A8,105–$A9,448 p.a. Salary rates for women are $A428 p.a. less than the corresponding rates for men. Promotion within CSIRO to a higher classification is determined by merit.

CONDITIONS The appointment, which carries with it Commonwealth Superannuation Fund or Provident Account privileges will be conditional upon passing a medical examination. An initial probationary period of twelve months may be specified. Fares paid for appointee and his dependent family. Further details supplied on application.

Applications (quoting Appointment No.: 651/32), and stating full name, place, date and year of birth, nationality, marital status, present employment, details of qualifications and experience, together with the names of not more than four persons acquainted with the applicant's academic and professional standing, should reach:

Mr. R. F. Turnbull,
Chief Scientific Liaison Officer,
Australian Scientific Liaison Office,
64 Kingsway,
LONDON, W.C.2.

by the **3rd April, 1970.**

Applications in U.S.A. and Canada should be sent to The Scientific Attaché, Australian Embassy, 1601 Massachusetts Ave. NW, WASHINGTON DC 20036.

(949)

UNIVERSITY COLLEGE HOSPITAL

GOWER STREET, LONDON, W.C.1

SENIOR BIOCHEMIST required from May 1, 1970, for interesting active Metabolic Research Unit. Excellent opportunity for own research project leading to postgraduate degree. Previous experience in clinical biochemistry is essential. Candidates wishing to see the Department should write to Professor C. E. Dent, F.R.S.

Application forms are obtainable from the Assistant Secretary to be returned by March 31, 1970. (977)

MEDICAL RESEARCH COUNCIL

(CLINICAL RESEARCH CENTRE)

Division of Immunology (Head of Division, Dr. G. L. Asherson) seeks chemist or biochemist with experience of polysaccharide chemistry to study the role of bacterial residues in human disease.

Salaries and conditions similar to those of Universities.

Further details from and applications to Personnel, Clinical Research Centre, 164 Tottenham Court Road, London WIP 9LH. (856)

SOUTH-EASTERN REGIONAL HOSPITAL BOARD

SCOTLAND

REGIONAL TRAINING SCHEME FOR BASIC-GRADE BIOCHEMIST

Applications are invited for the post of Basic-grade Biochemist in the training scheme operated jointly by the Departments of Clinical Chemistry at the Royal Infirmary of Edinburgh and at the Western General Hospital, Edinburgh. The successful candidate will be appointed to this position for one year in the first instance, renewable for a further two years.

Applicants should have graduated in Biochemistry or Chemistry or one of the related biological sciences, and the course of training will prepare him/her for the Mastership in Clinical Biochemistry.

Written applications, giving full personal particulars, details of training and experience, and the names of two referees, should be submitted to the Secretary, South-eastern Regional Hospital Board, Scotland, 11 Drumsheugh Gardens, Edinburgh EH3 7QQ, by March 31, 1970, from whom further details about the Training Scheme may be obtained. (919)

UNITED SHEFFIELD HOSPITALS

THE CENTRE FOR HUMAN GENETICS
SHEFFIELD

NON-MEDICAL SCIENTIFIC OFFICER

required for a period of 21 months to take part in research in the culture of amniotic fluid cells. Experience in Cytogenetics desirable but not essential.

Salary: 1st or 2nd Class Honours degree: £1,134 rising by 6 increments to £1,423. Other appropriate degree: £1,008 rising by 9 increments to £1,423.

Applications, stating age, qualifications, present and previous appointments, if any (with dates), together with the names and addresses of two referees: QUOTING REFERENCE SO2(CHG), to Chief Administrative Officer, United Sheffield Hospitals, 10 Beech Hill Road, Sheffield S10 2RZ, by March 14, 1970. (948)

UNIVERSITY LECTURESHIP OR A UNIVERSITY DEMONSTRATORSHIP IN THE DEPARTMENT OF GENETICS

The Appointments Committee of the Faculty of Biology "A" hope soon to be in a position to appoint a University Lecturer or a University Demonstrator in the Department of Genetics. Candidates should be able to teach mathematical genetics and may be expected to take part in the teaching of elementary statistics in general mathematical courses for biologists. Preference will be given to applicants who have had first-hand experience of biological experimentation. The appointment will be subject to the Statutes and Ordinances of the University, and will be for three years in the first instance, with the possibility, for a University Lecturer, of re-appointment to the retiring age, and, for a University Demonstrator, of re-appointment for two years. The maximum tenure of a University Demonstratorship is five years.

Candidates should send eight copies (only one for overseas applicants) of their application giving details, academic qualifications and experience, list of publications, and the names of three referees to Mr. R. E. Wyatt, Secretary of the Appointments Committee of the Faculty of Biology "A," Department of Zoology, Downing Street, Cambridge, CB2 3EJ, so as to reach him not later than April 1, 1970.

(913)

For further Classified advertisements see page ix

FREE UNIVERSITY AT AMSTERDAM

In the frame of the development of the section Chemical Physiology there will be in 1970 a vacancy for a

senior lecturer

This lecturer will participate in the instruction of biochemistry for medical students. At the same time a leading position in research will be assigned to him in cooperation with the professor and the second lecturer. The research is mainly directed towards the determination of the structure, localisation, formation and degradation of sialoglycoproteins and gangliosides in the brain-tissue.

Since the Free University emanates from a protestant though not church-bound association, it is kindly requested to add some information on personal orientation in this respect.

Applicants and those who want to mention other names are invited to direct themselves to prof. dr. H. K. Oosterhuis, Laboratory of Chemical Physiology, Van der Boechorststraat 7, Amsterdam, Holland, tel. (020) 482714.

(911)

UNIVERSITY OF NEWCASTLE UPON TYNE

DEPARTMENT OF ZOOLOGY

Applications are invited for two LECTURESHIPS and one DEMONSTRATORSHIP in the Department of Zoology. The following research interests are most pertinent to the needs of the Department but others will be considered: Animal behaviour, Animal genetics, Freshwater ecology, Animal locomotion, Entomology, Developmental biology, Comparative physiology.

Salary will be at an appropriate point on the following scales, according to age, qualifications and experience:

Lecturer: £1,240 to £2,850.
Demonstrator: £1,240 to £1,470 (extendable to £1,815).

Membership of F.S.S.U. required.

Further particulars may be obtained from the Registrar, The University, Newcastle upon Tyne NE1 7RU, with whom applications (three copies), together with the names and addresses of three referees, should be lodged not later than March 28, 1970. Please quote reference Na. (946)

IRELAND

Graduates in Engineering, Chemistry or Physics

are invited to apply for vacancies as Examiner in the Patents Office. Starting salary up to £2,045 (man) depending on qualifications and experience. The authorised establishment includes higher posts carrying salaries up to £2,770. Non-contributory pension. Widows and Orphans Pension.

Essential:

(a) A degree in Electrical, Mechanical, Chemical or Agricultural Engineering, **or**

(b) an honours degree in Experimental Physics, **or**

(c) an honours degree in Chemistry with two years' experience.

Age limits: 23–45 years. Further particulars, application forms etc. from The Secretary, Civil Service Commission, 45 Upper O'Connell St., Dublin 1, Ireland.

Latest date for receiving completed application forms—April 5. 1970.

(1017)

Microbiologist

(Veterinary Virology Research)

AUSTRALIA

The Commonwealth Serum Laboratories located at Melbourne, Victoria, require a Microbiologist to participate in the wide range of research projects aimed at a better understanding of the major infectious diseases of animals in Australia.

Duties: The successful applicant will be responsible to the Head of Veterinary Research for the conduct of veterinary virus projects and the direction of professional and technical staff involved.

Qualifications: Microbiologist with post-graduate training and/or wide experience in virology.

Conditions: Excellent conditions include commencing salary in the range, Male $A6,870 to $A7,570 p.a. Female $A6,430 to $A7,130 p.a. dependent upon qualifications and experience with eligibility for Superannuation, subject to meeting certain requirements.

Applications in writing to:

Personnel Manager, Commonwealth Serum Laboratories,
45 Poplar Road, PARKVILLE, VICTORIA, AUSTRALIA.
By April 18, 1970.

(925)

INTERNATIONAL FLAME RESEARCH FOUNDATION

HEAD OF THE RESEARCH STATION IJMUIDEN, HOLLAND

Applications are invited for the post of Head of the Research Station of the International Flame Research Foundation. The successful candidate, at least 30 years of age, with a Ph.D. Degree in Physics or Engineering will lead a small team of post-doctoral investigators, working on research into combustion and heat transfer of flames, sponsored by leading international industries and universities.

He should have some years' experience in leading research teams in a University or in a research laboratory in industry.

There is also a vacancy for a post-doctoral

RESEARCH INVESTIGATOR

having an Honours or Ph.D. Degree, to join the team working on problems of combustion and physics, in particular aerodynamics and heat transfer aspects of turbulent flames.

The work at the Foundation involves frequent contacts on an international level with research scientists working in similar fields, and the Foundation encourages publication of high quality original work.

The starting salaries will be not less than, £3,500 p.a. for the Head of the Research Station, and £2,000 p.a. for a Research Investigator.

Applicants should write in the first place to:

Professor J. M. Beer, Department of Fuel Technology & Chemical Engineering, University of Sheffield, Mappin Street, Sheffield 1.

(1008)

THE FUND FOR RESEARCH ON AGEING

In order to further ageing studies in the U.K., the Fund for Research on Ageing is offering a prize of £100 in each of the years 1970, 1971, and 1972 for work performed and published in the U.K. on EXPERIMENTAL GERONTOLOGY which is deemed by the assessors to have contributed the most to the study of

THE BIOLOGY OF AGEING

It is requested that reprints for 1970 should be submitted by April 30, 1971, to Dr. John Bray, Hon. Sec., Fund for Research on Ageing, c/o Angus Campbell and Co., Ling House, Dominion Street, E.C.2. (1007)

UNIVERSITY OF LONDON

CHAIR OF MEDICAL PHYSICS AT ROYAL POSTGRADUATE MEDICAL SCHOOL

The Senate invite applications for the Chair of Medical Physics tenable at the Royal Postgraduate Medical School. Salary (clinical) in the scale £4,470 to £5,275; (non-clinical) not less than £3,780 plus £100 London Allowance. The holder of the Chair will be Head of the combined Medical Physics Department of the Royal Postgraduate Medical School and Hammersmith Hospital and will work closely with the MRC Cyclotron and Radiopathology units. He will be responsible for research and postgraduate teaching in the fields of radioisotope applications, radiation dosimetry, medical electronics and physical techniques in diagnosis. Applications (ten copies) must be received not later than April 8, 1970, by the Academic Registrar (N), University of London, Senate House, W.C.1, from whom further particulars may be obtained. (865)

QUEEN'S UNIVERSITY

KINGSTON

DEPARTMENT OF GEOGRAPHY

The Department of Geography has the following vacancies at the present time.

1. Permanent Faculty Position. From September, 1970.

Preference will be given to candidates with specialisation in geomorphology. Teaching at both undergraduate and graduate levels with opportunities for research. Salary and status are subject to negotiation and will depend upon the experience and qualifications of the candidate.

2. Visiting Positions.

One visiting professor is required for September, 1970, and preference will be given to those candidates with a specialisation in economic geography. A second position will be available in January, 1970, and there is no limitation as to interest for the position. Both appointments will be for twelve months with salary being commensurate with qualifications and experience.

3. Instructors. From September, 1970.

Two Instructors are required to supervise the teaching by graduates in introductory laboratories and tutorials. The duties will also include the teaching of one laboratory section during the summer school session. Salary in range of $8,000 per annum.

Please send full details of qualifications and experience, together with the names of three referees, to:

Professor Brian S. Osborne,
Acting Head,
Department of Geography,
Queen's University,
Kingston, Ontario. (826)

UNIVERSITY OF THE WEST INDIES

BARBADOS

Applications are invited for (a) LECTURESHIP or (b) ASSISTANT LECTURESHIP IN CHEMISTRY Division of Natural Sciences, Cave Hill, Barbados. Candidates should have special interests in inorganic or physical chemistry. Salary scales: (a) £1,550 to £2,630 per annum; (b) £1,250 to £1,410 per annum. Child allowance. F.S.S.U. Family passages; triennial study leave. Detailed applications (six copies) naming three referees by April 7, 1970, by persons living in the Americas and Caribbean area to Registrar, University of the West Indies, Kingston, 7, Jamaica, and by all others to Inter-University Council, 90/91 Tottenham Court Road, London, WIP ODT. Further particulars obtainable similarly. (862)

THE UNIVERSITY OF HULL

DEPARTMENT OF PHYSICS

Applications are invited for appointment to a **Post-Doctoral Research Fellowship in Experimental** or **Theoretical Physics.**

Salary scale: £1,330 to £1,470 per annum.

Applications (six copies), giving details of age, qualifications and experience, together with the names of three referees, should be sent by April 17, 1970, to the Registrar of the University from whom further particulars may be obtained.

(883)

NATURE

MACMILLAN (JOURNALS) LTD

No. 5237 SATURDAY MARCH 14 1970 Vol. 225

CONTENTS

Are Graduate Students Worth Keeping ? 985

Old World—Computer Policy—Satellites—Rabies—Universities—Independent University—Mathematics—Information—Pollution—Parliament in Britain—Miscellaneous Intelligence 986

New World—Keeping Plowshare Alive—Stretch Out for NASA—Space Strategy—Planets—Grand Tour—Medical Research—Education—Eclipse—College Administration—Young Scientists 992

News and Views—Towards Hybrid Plant Cells—Starts, Stops and Anti-stops—Galaxies—Nuclear Magnetic Resonance—Chemical Synthesis—Mössbauer Effect—Extra-optical Astronomy—Carbon Fibres—Palaeomagnetism—Plasmas—Solid State—Pollution 999

Value of Curiosity-oriented Research by M. Gibbons, J. R. Greer, F. R. Jevons, J. Langrish and D. S. Watkins 1005

Imperfections of Elasticity and Continental Drift by Harold Jeffreys . 1007

Positive Control of Transcription by a Bacteriophage Sigma Factor by Andrew A. Travers 1009

Involvement of a Phage T4 σ Factor and an Anti-terminator Protein in the Transcription of Early T4 Genes *in vivo* by D. A. Schmidt, A. J. Mazaitis, T. Kasai and E. K. F. Bautz 1012

Fusion of Isolated Plant Protoplasts by J. B. Power, S. E. Cummins and E. C. Cocking 1016

Ethylene, the Natural Regulator of Leaf Abscission by Michael B. Jackson and Daphne J. Osborne 1019

Effects of Actinomycin D and Cycloheximide on RNA and Protein Synthesis in Cleavage Stage Mouse Embryos by Richard J. Tasca and Nina Hillman 1022

Non-Darwinian Evolution: A Critique by Rollin C. Richmond 1025

Carbon Compounds in Apollo 11 Lunar Samples by Bartholomew Nagy, Ward M. Scott, Vincent Modzeleski, Lois Anne Nagy, Charles M. Drew, William S. McEwan, Joseph E. Thomas, Paul B. Hamilton and Harold C. Urey . . . 1028

Letters to the Editor (see overleaf) 1033

Book Reviews by R. J. S. Hookway, John Vaizey, Colin Norman, A. J. Turner, S. R. C. Hughes, A. O. L. Atkin, R. M. Dicker, D. H. Valentine, Phyllis I. Edwards, J. Shirley, R. P. Dales and Y. Spencer-Booth 1075

Obituaries—Earl Russell 1080

Correspondence—Can a Scientific Article be Libellous ? by John R. Lewis, John S. Gray, Leland W. Pollock and P. Geoffrey Moore; Should Slides be Seen Blind ? by F. J. C. Roe, R. L. Carter, E. Cotchin and G. M. Bonser; Understanding Media by R. A. Beatty; Capacity for Misunderstanding by J. W. Warren . 1081

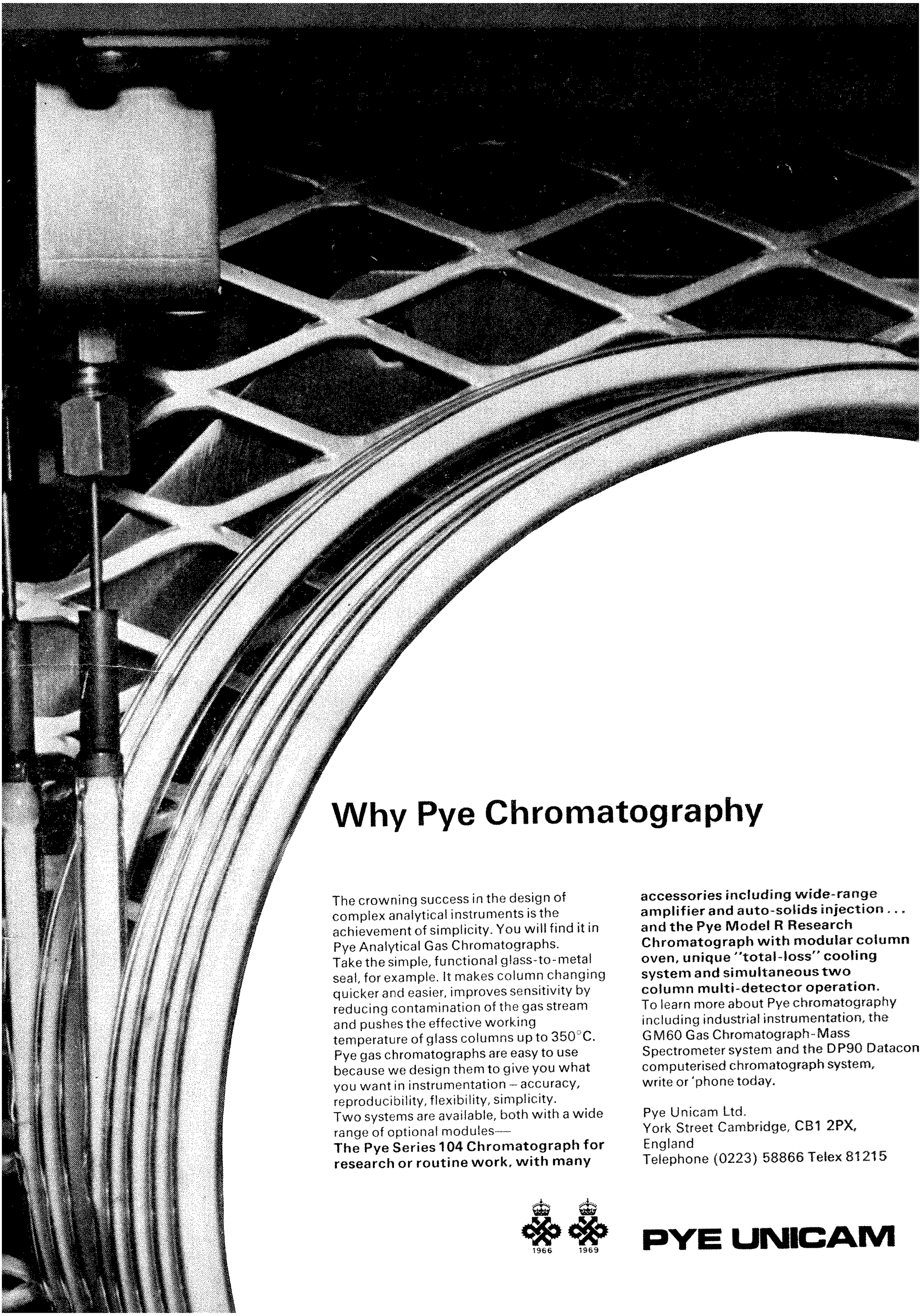
Appointments—Announcements—Sabbatical Itinerants 1082

Nature in North America: The *Nature* office in Washington DC has opened and contributors in the United States and Canada are invited to submit manuscripts for publication either to the address given below or to the present London office. The intention is that each office will be able to answer inquiries about all manuscripts and other matters. This is one of several steps by which it is hoped to provide a better service for readers and contributors.

London Office
4 Little Essex Street,
London WC2.
01-836-6633

Washington Office
711 National Press Building,
Washington DC 20004.
(202) 737-2355

(*Continued overleaf*)



Why Pye Chromatography

The crowning success in the design of complex analytical instruments is the achievement of simplicity. You will find it in Pye Analytical Gas Chromatographs.
Take the simple, functional glass-to-metal seal, for example. It makes column changing quicker and easier, improves sensitivity by reducing contamination of the gas stream and pushes the effective working temperature of glass columns up to 350°C.
Pye gas chromatographs are easy to use because we design them to give you what you want in instrumentation – accuracy, reproducibility, flexibility, simplicity.
Two systems are available, both with a wide range of optional modules—
The Pye Series 104 Chromatograph for research or routine work, with many accessories including wide-range amplifier and auto-solids injection . . . and the Pye Model R Research Chromatograph with modular column oven, unique "total-loss" cooling system and simultaneous two column multi-detector operation.
To learn more about Pye chromatography including industrial instrumentation, the GM60 Gas Chromatograph-Mass Spectrometer system and the DP90 Datacon computerised chromatograph system, write or 'phone today.

Pye Unicam Ltd.
York Street Cambridge, CB1 2PX,
England
Telephone (0223) 58866 Telex 81215

1966 1969

PYE UNICAM

LETTERS TO THE EDITOR

Page

GENERAL

Scientific inference—Statistical methods: a reply to Edwards—BIRNBAUM (New York) 1033

PHYSICAL SCIENCES

Redshift—Companion galaxies and non velocity redshifts—ARP (California Institute of Technology) . 1033

Crab Nebula—Radio spectrum at low frequencies—BRIDLE (Queen's University at Kingston, Ontario) . 1035

Cosmochronology—Detection of anomalous rhenium isotopic ratio in solar wind—MORGAN (Kentucky) . 1037

Magnetosphere—Longitudinal structure in the plasmapause—BULLOUGH and SAGREDO (Sheffield) . 1038

Carbon fibres — Strength–modulus relationship — THORNE (Rolls-Royce) 1039

Crystallography — Five-fold pseudosymmetry — BAGLEY (Bell Telephone Laboratories) . . . 1040

BIOLOGICAL SCIENCES

Unstable haemoglobins—Thermolability in various buffers—SCHNEIDERMAN, JUNGA and FAWLEY (Stanford) 1041

Haemoglobin—High resolution nuclear magnetic studies of Hb J Capetown—OGAWA, SHULMAN (Bell Telephone Laboratories), KYNOCH and LEHMANN (MRC) 1042

DNA—Tritium exchange between DNA and water—BIRD, LARK, CURNUTTE and MAXFIELD (Kansas State) 1043

Neurological disease—Measles type viruses promote spread of Papova-like virus by cell fusion—KOPROWSKI, BARBANTI-BRODANO and KATZ (Wistar Institute) 1045

Leukaemia virus—Transformed cells develop antigen before becoming malignant—GURTSEVICH, MAZURENKO, JAROVA, PROBATOVA and STEPANOVA (Institute of Experimental and Clinical Oncology, Moscow) 1047

Lysosomal enzymes—Leucine naphthylamide not appropriate as histochemical substrate for cathepsins B and B′—McDONALD, ZEITMAN and ELLIS (NASA) 1048

— Histochemical detection of cathepsin and aminopeptidase activities in rheumatoid synovia—CHAYEN, BITENSKY and POULTER (Kennedy Institute of Rheumatology) 1050

Tissue culture—Morphogenesis of cartilage from mesenchymal cells on decalcified bone matrix—URIST and NOGAMI (California) 1051

Epidermis—Uniquely oriented lipid—GOLDSMITH and BADEN (Harvard) 1052

Muscle—Myosin filaments in vertebrate smooth muscle—LOWY, POULSEN and VIBERT (Aarhus) 1053

Thrombus — Adhesion of platelets observed by scanning and transmission electron microscopy — SHEPPARD and FRENCH (Oxford) 1054

Bile salts—Inhibition of intestinal absorption of 3-O-methyl glucose—ROY, DUBOIS and PHILIPPON (Colorado) 1055

Parathyroid hormone — *In vitro* regulation by Ca^{2+} and Mg^{2+}—SHERWOOD (Harvard), HERRMAN and BASSETT (Columbia) 1056

Catecholamines—Complexes with acetylcholine relevant to mental illness—GALZIGNA (Inserm and California) 1058

Hormones and behaviour — Changes in catecholamine metabolism after psychosocial stimulation—AXELROD, MUELLER (US National Institutes of Health), HENRY and STEPHENS (Southern California) 1059

Serotonin—Involvement of giant serotonin containing neurones in synaptic links in snail brain—COTTRELL (St Andrews) 1060

Aflatoxin B1—Sheep are sensitive—ARMBRECHT, SHALKOP, ROLLINS, POHLAND and STOLOFF (US Food and Drug Administration) 1062

Vertebrate palaeontology—Bones found in Lower Trias of Australia—BARTHOLOMAI (Queensland Museum) and HOWIE (Sydney) 1063

Spermatophores—Description for *Glossina austeni*—POLLOCK (London) 1063

Mutant behaviour—Courtship in *Drosophila melanogaster* mutants—CROSSLEY (Monash) and ZUILL (Oxford) 1064

Plant hormones — Brassins, a new family from rape pollen—MITCHELL, MANDAVA, WORLEY, PLIMMER (US Department of Agriculture) and SMITH (Guelph) 1065

Pollen—Localization of antigens associated with grain wall by immunofluorescence — KNOX, HESLOP-HARRISON and REED (Wisconsin) 1066

Freshwater biology—*Hydramoeba hydroxena* found on *Hydra graysoni*—MAXWELL (London) . . 1068

Umbellulidae — Atlantic distribution extended — JAHN (US Naval Oceanographic Office) 1068

Plankton—Diatom bloom in the Black Sea reduced supply of available carbon—DEUSER (Woods Hole Oceanographic Institution) 1069

Soil fungi—Volatile factor which inhibits germination of spores—HORA and BAKER (Colorado State) . 1071

Antibacterial activity—Bactericidal effect of L-amino-acid oxidase—SKARNES (Worcester Foundation for Experimental Biology) 1072

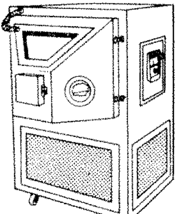
Bacteria—Alterations to cell walls in *Staphylococcus aureus* resistant to methicillin—SABATH, LEAF, GERSTEIN and FINLAND (Harvard) 1074

The titles on this page are prepared by the staff of *Nature* on the basis of the titles submitted by authors which appear in full on the pages indicated

the approved standard of preparing frozen tissue sections

LEHRER and WEDEEN

MICROTOME CRYOSTATS



The WEDEEN Cryostat has the only wide range of controlled operating temperatures from 0°C to –80°C.

The LEHRER Cryostat has a main chamber with a controlled temperature to —40°C.

Write for full details

Manufacturers of:
Lehrer and Wedeen Microtome
Cryostats and Atmo-Vac® Freeze Dryers.

Refrigeration For Science, Inc.
74 Alabama Avenue • Island Park, New York 11558

Represented by: AURIEMA INTERNATIONAL GROUP

'MICHROME'

STAINS & REAGENTS FOR BIOLOGY

Acridine orange, Aquamount, Fluormount, Lipid crimson, Matamount, May-Grünwald, Leishman, Giemsa Toluidine blue, Cresyl fast violet, Papanicolaou stains, Thioflavine S, Brilliant cresyl blue, Nile blue, Pyronin Y, Trifalgic acid, Rhodanile blue, Haplo, etc.

CATALOGUE AVAILABLE ON REQUEST

DYES FOR USE IN ALL INDUSTRIES

RESEARCH & PRODUCTION CHEMISTS: if you are seeking dyes with specific chemical and physical properties suitable for particular manufacturing and/or testing processes you are invited to contact us for supplies.

We hold stocks of an enormous variety of dyes, ready for immediate despatch, including:

Cyanine, Dicyanine, Fast Bordeaux O, Aurantia, Crystal violet, BPC-Scarlet R, BPC, Diazonium salts, Neutral violet, Lacmoid, Nile blue, Janus green, Brilliant green BPC, Rosolic acid, Methyl green, p-Ethoxy-chrysoidin, Methyl red, Nitrazine yellow, Pinacryptol green, etc.

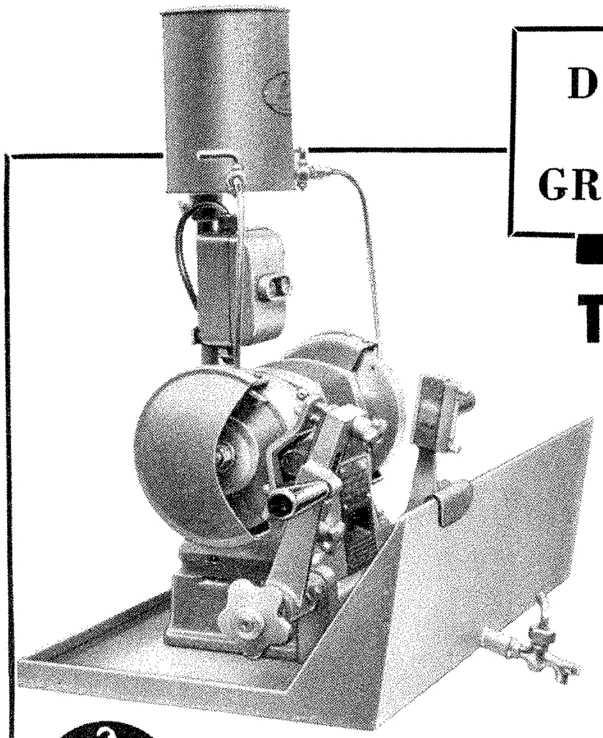
INDUSTRIAL RESEARCH ESTABLISHMENTS

are invited to make use of our professional services as consultants on the selection and use of dyes as research and production tools.

EDWARD GURR LTD

Michrome Laboratories,
42 Upper Richmond Road West, London, S.W.14
Cables: Micromlabs London SW14. Telephone: 01-876 82289

QUALITY — RELIABILITY — SERVICE
PROMPT DELIVERY



DIAMOND WHEEL CUTTING AND GRINDING MACHINE

THE MARK II UNICUTTA

The thin section machine quickly and efficiently produces thin sections for microscope study. Fitted with 6-in. dia. diamond cutting and grinding wheels.

Accessories for the machine:

- CAST IRON POLISHING PLATE
- NYLON SIMPLEX POLISHING PLATE COVERS
- EMERY DISC CLAMPING RINGS TO FIT EMERY DISCS TO POLISHING PLATE
- EMERY DISCS AND LOOSE ABRASIVES (Carborundum and Aloxite)

For full information, write or telephone:

CUTROCK ENGINEERING CO. LTD., 35 BALLARDS LANE, LONDON N.3
Telephone: FINCHLEY 5978/9

NATURE Volume 225 MARCH 14

Are Graduate Students Worth Keeping?

THE way in which it now seems to have been decided in the United States that the federal government should spend less than in the past on the direct support of graduate students in American universities will be a slap in the face for those who have been saying that such an expenditure of public money should be recognized as a necessary part of the federal government's function. The most deeply hurt should be the National Science Board, which just a year ago produced an eloquent report (see *Nature*, **221**, 800; 1969) which argued that graduate education had become so central a part of public expenditure in science that the time had even come to consider spending money not simply on individual proposals for research but also at institutions or departments where important work in education was going on. One of the mysteries of public life is that this document seems to have sunk without trace—even its authors have been only fitful advocates in the past twelve months. What has happened now, on the face of things at least, is that the Administration has repudiated its responsibility for graduate education. Why else should the National Science Foundation have found this item struck from its budget (see *Nature*, **225**, 785; 1970)? Dr W. D. McElroy says that he (and Dr L. DuBridge as well) is prepared reluctantly to give the policy a trial for a year to see what happens. So far, the National Institutes of Health seem to have resisted more successfully the pressure to dispense with traineeships for graduate students. The fact remains that in 1971, and possibly in 1970 as well, the number of graduates at American universities will be much reduced. What is to be done?

In the long run, the easiest solution is no solution at all. It is widely expected that individual investigators will try to keep research students about them by including them as research assistants on grant applications. This, as it happens, is already a necessary device in places where the statutory stipend for a graduate trainee is not sufficient to cover the cost of living. The difficulty, of course, is that if the pensioners of the National Science Foundation seek to restore the *status quo* by adding all their possible research students to their grant applications, they will add something like $9 million a year—the amount of money now saved—to the cost of the foundation's research programme or, another way of putting it, the number of grant applications that can be satisfied will be reduced by 5 per cent. So what of the possibility that universities will be able to pay for some of the research students out of local resources, either endowments or state funds? The difficulty here is that the extra strain comes at a time when universities and their sponsors are already under severe pressure. There is something to be said for the view that there was virtue in the arrangement in the thirties when large numbers of bright people were supported through their postgraduate courses by half-time assistantships, but is there any reason now to believe that hard pressed state universities will be able to support the very much larger numbers now seeking entrance to graduate schools without at least some prior warning? And is it likely that after three years of anxiety about endowments, the private universities will be able to find money for this purpose? The chances are slim; moreover, there is as yet not nearly enough experience of loans for postgraduate students to suggest that the market will allow people to come forward in the numbers to which universities have become accustomed. In short, unless there is a sudden change of heart on the part of Congress, there is likely to be at least one lean year in the recruitment to the graduate schools and then, three to five years later, a diminished output of PhDs.

Universities and academics should restrain the first natural temptation to protest that this is an unpleasantness which is intolerable. To be sure, there is every reason to believe that running down the traineeship programme is misguided and every reason to hope that it can be reversed. But at the same time, however, this is also an occasion on which universities should ask themselves whether the pattern of graduate education in the United States to which everybody is accustomed is really the best suited for the modern world. The way in which employers (or potential employers) have taken to complaining of the over-specialization of the products of the graduate schools (see *Nature*, **225**, 897; 1970) is one sign that change may be called for. Most probably, in most branches of science there is a need to fit the pattern of graduate education more accurately to the kinds of work on which graduates are eventually engaged. At present, the academic interests of supervisors and the traditions of an over-rigid curriculum are too often dominant. It may be easier for the academic world to have the cuts in the traineeships restored if they can demonstrate innovations in the pattern of graduate education which seem more appropriate to the needs not merely of the modern world but of graduate students themselves. One of the ironies of the situation which has now developed is that the National Science Foundation seems, in a gingerly fashion, it is true, to be on the point of seeking to train people whom it calls "technologists"—how easy will this be now that money is so hard to get? The second irony is that the apparently defunct report on graduate education by the National Science Board would, if it had been taken seriously, have made it much easier than at present for academics to design graduate education to suit the graduates and not to make a particular grant application seem attractive.

Reform is thus the best guarantee of a redress of present grievances, but it is unreasonable to expect that it can happen quickly. The best that universities will have to offer is some sign of a willingness to change. But there is no quarrelling with the argument that the government cannot abandon its responsibility for graduate education without putting at hazard the whole basis on which the United States has achieved its pre-eminence in science in the years since 1945. The point is that graduate students are not merely the cheap research assistants they are sometimes thought to be but, rather, the people on whom society will depend for innovation ten years from now. The fact that people are now more worried about the environment than, say, about the need to keep industrial prosperity growing is beside the point. If there were, ten years from now, a decline in the capacity of industry to transform itself—and it could happen as quickly as that—most probably it would be too expensive to make the serious improvements of the environment now sought. It is true, of course, that a reform of graduate education would work the other way, and would enhance the innovative potential of graduating students, but that is only another reason for pressing on with reform. In the meantime, the universities have only themselves on which to rely in making a case for their own survival. If they can rise successfully to the challenge, and can make a stronger and more coherent case than they have done so far for the importance of training graduates, the present crisis may be a blessing in disguise. Otherwise, it will be a disaster.

100 Years Ago

Turning now to the concluding chapters of the book, we meet with some of the most startling and suggestive ideas to be found in any modern work. The law of deviation from an average enables us to determine the general intellectual status of any nation, if we are able to estimate the ability of its most eminent men, and know approximately the amount of the population. We have these data in the case of ancient Attica ; and Mr. Galton arrives at the conclusion, that the Athenians of the age of Pericles were, on the lowest possible estimate, nearly two whole grades of ability higher than we are. With all our boasted civilisation, and the vast social and scientific problems with which we have to grapple ; with all our world-wide interests, our noble literature, and accumulated wealth ; the intellectual status of the most civilised modern nation is actually lower than it was more than two thousand years ago !

From a review by Alfred Wallace of Francis Galton's Hereditary Genius, an Inquiry into its Laws and Consequences. From Nature, **1**, 502, March 17, 1870.

OLD WORLD

COMPUTER POLICY

Mintech Under Fire Again

THE row over the government's policy of computer procurement—which began when IBM (UK) appeared before the subcommittee of the Select Committee on Science and Technology which is investigating the computer industry—hotted up last week when it was the turn of Honeywell to meet the subcommittee. The question is how large a share of the public computer market the subsidiaries of American companies such as IBM and Honeywell, which have extensive factories in Britain, can reasonably expect to corner. IBM (UK) set the ball rolling when its managing director, Mr E. R. Nixon, told the subcommittee of his belief that non-British companies have to tender 25 per cent lower than International Computers Limited (ICL) to get government orders (see *Nature*, **225**, 889; 1970). The IBM team stressed how unfair this is by going on to list the benefits bestowed by IBM (UK) on Britain; £33 million of the company's revenue of £125 million in 1968 came from exports, and although imports of £38 million led to an unfavourable contribution to the balance of payments, the situation seems to have been reversed last year.

Last week's witnesses from Honeywell Ltd, led by chairman and managing director Mr L. R. Price, started from a firmer base and went farther than IBM in its criticism—in the twelve months up to September last year, £16·3 million of Honeywell's sales of £37 million went to export, compared with an import bill of £11·8 million. But it is not in fact government policy that Honeywell is complaining of so much as that the stated policy is apparently not being followed. The Honeywell team brought to the notice of the subcommittee several statements by government spokesmen affirming that subsidiaries of non-British firms manufacturing in Britain stand on an equal footing with British companies such as ICL when it comes to orders for the public sector, which is fine as far as Honeywell is concerned, but Mr Price and his colleagues alleged that the figures tell a different story. Out of 75 systems in central government, 69 were made by ICL, four by IBM and two by Univac.

Taken to task by the subcommittee, the Honeywell representatives agreed that in individual cases their machines might have been unsuccessful for reasons of compatibility, price or performance. But Mr Price and his colleagues said that this is not the whole story because the figures for the public sector have to be compared with Honeywell's more respectable share of the private market, where the Ministry of Technology has no influence. Honeywell's share of sales to the entire public sector which includes local authorities and universities is a little more than one-third of its share of the private market. In its written evidence to the subcommittee, the company states that "It is apparent that foreign-owned companies, whether or not they manufacture in Britain, are largely excluded from the increasingly important public sector of the market".

The government practice of sometimes inviting tenders from ICL only, raised by Mr Nixon of IBM, also came under fire, and the Honeywell team thought that the practice was probably on the increase. But they agreed with Mr Eric Lubbock of the subcommittee that single-tendering is more honest than requesting tenders when there is no intention of ordering, which the Honeywell team suspected might have been happening. Mr Price thought that some cases of single-tendering were completely correct when the choice was a foregone conclusion for reasons of compatibility of computers, but he nevertheless believed that Honeywell's share of the market indicates that they are being discriminated against.

Honeywell's estimate of the British market for both business and scientific machines rises from £150 million in 1968 to £170 million in 1969, £210 million in 1970 and £260 million in 1971. With much of the growth expected to be in the public sector, Honeywell believes the government ought to practice what it preaches about the position of foreign-owned firms which manufacture in Britain, especially those with a positive trade balance. For one thing, it would help if government departments made known their reasons for choosing a particular computer. The subcommittee will also be trying to discover precisely what are the guidelines for computer purchase laid down by the Ministry of Technology which seem to produce such a discrepancy between theory and practice. These are questions to be reserved for the Minister of Technology who is expected to appear later in this enquiry which is already noted for the searching questions coming from the subcommittee.

SATELLITES

Aquiver to Join the Space Club

Britain took a step nearer becoming the fifth member of the "space club" last week with the successful launching of the Black Arrow 3-stage satellite launch vehicle from Woomera on its second flight test. On the first launching, made last summer, the rocket had to be destroyed because of a failure in an electrical circuit in the control system. The Mintech team in charge was sufficiently confident that the trouble had been located and put right to move on to the second of the three scheduled flight tests without holding up the timetable for a repetition. They were justified by satisfactory performance in all the three stages last week.

At the next firing later this year there will also be a trial of the satellite injection system on the third stage so that, if successful, a nominal satellite will go into orbit. This will then put Britain square with the Japanese whose small satellite launcher orbited a nominal payload for the first time last month (*Nature*, **225**, 678; 1970), although the satellite ceased to transmit a few hours after launch because of a failure in the electrical supply system. Meanwhile, France is expected this week to launch her fifth satellite with a French built rocket. This will be the first satellite to be launched from the new French rocket range, on the equator at Kourou, French Guiana.

Black Arrow's planned payload capacity is of the order of 100 kg when launched north into a polar orbit, or 150 kg if launched easterly from an equatorial range. This is slightly in excess of the payloads of the comparable French and Japanese launchers. The purpose of the programme, finally given the go-ahead by the government three years ago, is to provide a modest technological capability in satellite launching and construction and associated activities. Three satellites have so far been commissioned, of which the first, essentially a "housekeeping" vehicle, should be orbited in 1971. Black Arrow satellites are to provide an orbiting test bed for trying out and proving various experimental ideas and components. Among the likely candidates are communications satellite components and instrumentation for operational weather satellites. So far the Black Arrow budget has been running at about £3 million a year which is expected to rise to £5 million from now on.

The launch vehicle itself has been developed from the proved components and technology of the highly successful British Black Knight high altitude sounding rocket that carried out a re-entry test programme for the Ministry of Aviation in the early 60s. It achieved the then unbeaten record of 21 successful test launchings out of 21. Black Knight was developed by the Saunders Roe aircraft company, based in the Isle of Wight, which was later taken over by the division of Westlands that has become the British Hovercraft Corporation. This group is the prime contractor for Black Knight's successor, Black Arrow, with the curious result that the British satellite is to be launched into space through the efforts of an industry associated with hovering and at heights of only a few feet at that.

RABIES

End to Compromise

A ban on the import of dogs, cats and all other felines and canines into Great Britain was announced last week by Mr Cledwyn Hughes, Minister of Agriculture. The ban will be waived only for animals from Ireland, the Channel Islands and the Isle of Man. Mr Hughes is also setting up a committee of enquiry on rabies which will review the whole quarantine scene, but in the meantime imported animals that are already in the country will have to complete twelve months in kennels before they can go free.

The British quarantine regulations have now been overhauled twice in three months. Last December the length of quarantine went up from six to eight months after a rabid dog, not long out of kennels, ran wild in Camberley (see *Nature*, **224**, 634 and 1049), and at the same time a number of more exotic species were forbidden entry to Britain. The new restrictions were prompted by a mongrel which died from rabies at Newmarket on February 27, nine months after arriving from Pakistan.

Mr Hughes seems to have taken the only course open to him in order to preserve Britain's enviable record of freedom from rabies since 1922; a second large increase in the quarantine period without cutting imports would not have been practical. The alarming spread of rabies in wildlife on the European continent is one justification for being strict. Mr Hughes's case also rests on the uncertainty about the incubation period of rabies, but leaves room for relaxing stringency later by asking the committee of enquiry "to review the policy and precautions against rabies in Great Britain and to make recommendations". Chaired by Mr Ronald Waterhouse, QC, the committee is expected to report within a year but may speak sooner if it finds an urgent need for more action.

The government will be paying for the extra four months' quarantine, but response to the changes has been ambivalent. The Royal Society for the Prevention of Cruelty to Animals said that "the minister had, in making his proposals, consulted the best veterinary opinion in the country, and . . . we can only accept the regulations", although "since quarantine represents a hardship for animals, we find it disturbing that the period should increase from eight to twelve months". The secretary of the Canine Defence League, Mr A. H. Roosmalecocq, thought the ban might be justified as a temporary measure, although he considered twelve months' quarantine "unnecessarily drastic" and suspected the minister's advisers were "covering up while they do their sums again". Taken at face value the prohibition covers laboratory animals just as much as pets, although when the order is actually made it may allow some exceptions for research; at the time of his announcement Mr Hughes was not able to say what would happen. The Medical Research Council, at least, is not worried by any potential loss, for it either breeds its own cats and dogs or buys them from accredited British breeders, and says it "certainly wouldn't import them under any circumstances".

One obstacle to complete safety remains, however, and that is smuggling. The maximum fine for smuggling an animal into Britain is £200—increased from £50 in 1967—and may well prove too small to deter a really desperate dog-lover. In practice, fines have been nowhere near the maximum, and some magistrates have been known to impose derisory penalties of £5 or so. Here, it seems, the committee of enquiry might find one of its priorities.

UNIVERSITIES

Teaching of Physics

FEW physicists can feel indifferent to the way physics is taught and a lively audience arrived last week at the physics exhibition in Alexandra Palace, London, to hear Professor E. J. Burge, of the Chelsea College of Science and Technology, proclaim there are at present far too many examinations. He thought that some more continuous form of assessment should be introduced, including oral tests. But he was against attempts to dismember physics as a subject. "I believe that physics is an academic discipline that holds together as a whole, and efforts to turn it into a series of service courses will be vigorously opposed."

Professor E. H. S. Burhop of University College, London, took a rather different view. He foresaw the emergence of a new subject comprised of topics from both physics and chemistry. University College has recently introduced a joint chemistry and physics course, which is no soft option, he said. But the pattern of education is changing rapidly. If the proposed Qualifying and Further examinations (intended to replace O and A level) come into being, physics will only be taught at school as part of the physical sciences, Professor Burhop said. With the government eager to keep down the cost per head of education, there could be a lot of wisdom in introducing four year courses for bright students and teaching the less academically inclined for only two years.

On postgraduate teaching, Professor Burhop thought there was value in the present emphasis laid on teaching postgraduates to dissect a specific problem, to scan and assess literature and perform specific functions like data analysis, but conceded that a lack of general technique was proving a handicap to those going into industry. A physicist might now finish his PhD as an expert in, say, bubble chambers or counters and with considerable incentive to continue working in the same field. This would have to change, he said. But industry for its part was still too ready to treat physicists as technicians.

Professor Burhop also criticized the Institute of Physics and the Physical Society which, he said, was far too timid in creating a dialogue between industrial and academic physicists. He also thought the Science Research Council was wrong to increase the proportion of postgraduate grants given for advanced studentships rather than for MSc and PhD courses. For one thing, staff was short and these courses often turned out to be very uninspiring. But the SRC's CAPS awards (Co-operative Awards in Pure Science) which give a student one supervisor in industry and one at university were proving a success in providing more "industry oriented" physicists.

UNIVERSITIES

Revamping the Vets

THE way in which veterinary science is taught in Britain needs a thorough overhaul—this, at least, was the conclusion of a two-day conference held last week by the Royal College of Veterinary Surgeons. The college, which officially supervises university veterinary courses, has asked the government to set up an interdepartmental inquiry into the education and future role of the veterinary profession, a request which is being urgently considered.

The chief disadvantage of the present system, it seems, is that the universities are failing to produce the flexible, well-educated type of veterinary scientist who is needed. The system, which has remained largely unchanged for the past thirty years, is becoming increasingly inappropriate in view of the widening range of veterinary activities. On the clinical side, general practitioners are being expected to cope with the problems of large herds, and particularly the problems of disease prevention in intensive animal production systems. There is a growing demand for clinical species specialists but as yet very few opportunities for specialist training. Commercial activities such as the development and testing of new medicines, food additives and agricultural sprays are becoming increasingly important. More graduates are needed in research, particularly in new fields such as veterinary epidemiology, but here also there are no facilities for postgraduate teaching. This point did not escape the attention of the Swann committee, which in its report on the use of antibiotics in veterinary medicine recommended that university departments of veterinary epidemiology should be set up.

Most of the speakers at the conference agreed that the universities or research establishments should offer many more postgraduate courses. Dr J. T. Stamp from the Moredun Research Institute in Edinburgh suggested that, following the normal five-year undergraduate course, all students intending to go into practice or other clinical fields should have to take an extra year of postgraduate professional training. For research scientists, Dr K. N. Burns from the Agricultural Research Council suggested that a period spent in practice would probably be helpful, but that the transfer to further research training should be made easy. There seemed to be general agreement that the first two or three years of the undergraduate course should provide a broad scientific training, and possibly be taken in conjunction with other medical and dental students, while the next two or three years should provide the necessary vocational training. Sandwich courses were suggested, by Dr J. T. Done from the Ministry of Agriculture, as a means for giving the students a feel for veterinary practice.

INDEPENDENT UNIVERSITY

£15 Million to Launch

by our Education Correspondent

To launch a self-financing university that is free from state control is a difficult business. That, if nothing else, is the first conclusion to have been drawn by the planning board of the proposed Independent University. Not only are supporters of the university laid open to the criticism that it will be merely an extension of the public school with industrial control in the place of state control, but they are now faced with the fact that the estimated cost of launching the university has trebled in just over a year. A policy document put out by the planning board suggests that £15 million will be needed to get the university off the ground. In January 1969, when the idea was first mooted, it was thought that £5 million would do the job.

The supporters of the university are not despondent, however. They believe that £15 million "should not be beyond the pocket of a wealthy nation concerned to preserve the best in its university tradition", and they hope that this money will come from endowments, loans, guarantees and gifts in kind. Once over the first few years it is intended that the university will be self-financing. Students will have to pay economic fees ranging from about £650–£700 a year for arts students to £1,050–£1,100 for science students. These figures do not include maintenance, but the planning board can see no reason why independent university students should not qualify for grants from public funds. It also argues that a system of loans would help such students to finance themselves, and would prevent the university from having to take only children from rich families, and therefore from becoming merely an extension to public school. The cost to the student over three years would be nearly £5,000.

One criticism often levelled at the proposed Independent University is that it will find difficulty in attracting staff of sufficiently high quality. But the planning board's answer to this seems to be that the university will not be bound by UGC scales of pay, and will place great emphasis on teaching ability when selecting staff. The planning board also believes that the university should be located on the outskirts of London, and the secretary of the board, Mr MacCallum Scott, indicated that some sites are already being considered. Although a site in the provinces cannot be ruled out, the planning board suggests that proximity to London's facilities will offer great advantages.

Before the university can award degrees, it must first receive a royal charter, and there seems little chance of this for several years. It seems, therefore, that if the university does get off the ground, it will be at least four or five years before it starts to operate, and even longer before it reaches its proposed size of 3,000 students.

MATHEMATICS

Differentials in Low Gear

Too little research on differential equations is being done in Britain, according to the interim report of the panel set up by the mathematics committee of the Science Research Council to review both pure and applied work on differential equations. There is not much research activity in Britain on most aspects of ordinary differential equations, for example, and the panel finds the amount of study of non-linear partial differential equations to be "most unsatisfactory". To rectify the situation amounts virtually to the introduction of new subjects to Britain, and the panel lists three ways in which this might be achieved—by year-long symposia, by a long term programme of visitors from abroad, and by the exchange of personnel with experienced overseas groups.

Established in June 1967 under the chairmanship of Professor I. N. Sneddon of the University of Glasgow, the panel is expected to continue regular meetings for a further two or three years. The interim report includes the familiar homily about poor communications between universities and industry, which the panel feels is hampering the development of such topics as control theory, for instance. In general, what is required on the industrial side is a better formulation of industrial problems, and a readiness of university mathematicians to be more involved in the application of differential equations. How this ideal is to be achieved the panel recognizes to be a problem with no easy solution, but it suggests the trial of methods such as the joint study group between universities and industry which is being tried by the Oxford Mathematical Institute, and the publication of a comprehensive survey of techniques.

To give the subject of differential equations a boost the SRC has already begun to set aside twelve research studentships per year for graduates working in this field, and the report recommends that this experiment be continued for five years. The panel says that over the next five years or so there should be one large symposium each year on some aspect of differential equations (one has already been held at the University of Warwick) and they favour more universities increasing their interest in this field.

INFORMATION

Literature Taped

from a Correspondent

THOSE biologists who count as wasted the time spent tracking references to their particular research interest through the paper jungle of literature search indices will welcome news of an experiment, currently in progress in Britain, which may presage an end to their labours. The experiment, which is financed by the Office of Scientific and Technical Information (OSTI), aims to ascertain just how valuable a computerized literature searching service could be to biologists.

The two year project has been established at Oxford University, in close collaboration with the Experimental Information Unit already based there, and a subsidiary experiment is being developed along identical lines at Nottingham. Three liaison scientists, two in Oxford and one in Nottingham, have been appointed and the first stages of the experiment are already under way. The project centres around BA-Previews, magnetic computer tapes on which are recorded all the items of literature surveyed in the journal *Biological Abstracts* which appears twice a month. BioSciences Information Service (BIOSIS), the American non-profit-making organization which publishes *Biological Abstracts*, sees these taped previews as the latest advance in the battle to rationalize and classify the ever mounting torrent of biological literature. The tapes have therefore been made available to OSTI for one year free of charge in return for a feedback of information from the British project. The tapes can be searched quickly and efficiently by a computer programmed to provide a print-out of references corresponding to a particular "user profile". This is a series of keywords which the computer can recognize in several different permutations and combinations so designed that items of specific interest only are selected from the ten thousand or so on each preview tape. Retrieval systems of this nature have been available to chemists, physicists and medical workers for some time—MEDLARS, the Medical Literature Analysis and Retrieval System is one example—but such a facility will be a considerable novelty to biologists.

The immediate aim of the liaison officers at Oxford and Nottingham is to recruit a limited number of biologists both from the universities and from kindred establishments. From information provided by these selected users it is hoped to find out how effectively the literature is scanned by the abstracting service, how best to formulate subject profiles for biologists, and, most important, to compare the performance of the computer-based service with more conventional methods of information retrieval.

BIOSIS hopes eventually to extend the range of its BA-Preview tapes, at present covering only current material, to include the contents of all *Biological Abstracts* back to 1927; the prospect of having the entire literature survey for a review prepared in minutes by computer is surely enough for most biologists to hope that the OSTI experiment proves successful.

POLLUTION

Why Lead gets into Petrol

THE furore over the presence of lead in petrol and the announcement of lead-free petrols by major oil companies have arisen not so much because of the health risk, which is uncertain, but because of the harm wrought by lead compounds in exhaust gases on the antipollution devices which manufacturers are being urged to fit to cars in order to trap the more noxious products. Although lead, in the form which it is added to petrols—tetraethyl lead, $Pb(C_2H_5)_4$—is undoubtedly poisonous, and nobody is ever happy about the presence of lead compounds in the atmosphere, there seems to be no evidence so far that the amount of lead released from engines is hazardous. There is, however, some concern that the background level of lead from exhausts might conceivably be building up, which is why some people are calling for a reduction in the amount of tetraethyl lead in petrol so as to keep in step with the increase in the car population.

The presence of lead in petrol is felt to be of concern because of an indirect effect on the pollution problem. In theory the exhaust products of a petrol engine should be CO_2 and H_2O, but in practice, of course, pollutants such as CO, oxides of nitrogen, and unburnt and partially burnt hydrocarbons are released. One way to trap some of these undesirable gases is to fit catalytic gas treaters which look rather like an extra silencer into the exhaust system, but unfortunately the lead compounds in the exhaust gases, presumably for the most part oxides, poison the catalyst and make its lifetime unacceptably short. If the life of the catalyst is too brief, the gas treaters are not likely to be replaced often enough to keep the pollution level down.

The value of adding tetraethyl lead to petrol was first realized by Thomas Midgley, Jun., in the United States in 1922, and now quantities up to 4·8 ml. per UK gallon are added, usually as a mixture of tetraethyl and tetramethyl lead, when the products of the refinery are blended to produce the required petrol. Its role is as an anti-knock compound, to prevent the unpleasant effects of explosive burning ahead of the flame front in the combustion chamber. What ought to happen is that the flame front propagates smoothly across the combustion chamber from ignition at the spark plug, but sometimes the gas mixture in front of the flame is compressed enough for spontaneous ignition to take place. Besides the unpleasant noise, the shock wave so produced is damaging to the engine. It is still not clear exactly how the addition of tetraethyl lead inhibits the uncontrolled burning which is the cause of knocking.

The addition of tetraethyl lead can improve a petrol by as much as six octane numbers, which is hard to compensate for by other means. The alternative to additives for knock reduction is to lower the pressure in the combustion chamber, in other words to reduce the compression ratio. This allows petrols with octane numbers as low as 91 to be used, which probably can be produced fairly easily without lead. This seems to be the direction which is being taken in Detroit with compression ratios being lowered to around 7:1. Unfortunately the problem is less tractable in Europe, where motor car engines tend to be smaller than in North America, so that reductions in the normal compression ratios (usually around 9 or 10:1) and commensurate reductions in the octane number of petrols would be more seriously felt. The production of petrols with high octane numbers without adding tetraethyl lead is more difficult; other additives with anti-knock properties are known, but none seem to be as good as lead, and the alternatives (such as certain aromatic compounds) could be just as much a nuisance from the pollution point of view. Increasing the octane number by the addition of synthetic hydrocarbons is ruled out because of the expense of breaking down the crude oil and rebuilding in the desired form. It seems that in the long run motorists will have to get used to lower compression ratios.

Parliament in Britain

Gas Centrifuge Agreement

The agreement to develop the gas centrifuge process for enriching uranium, signed last week by Great Britain, Holland and West Germany, may later be extended to include Italy and Belgium. Mr David Luard, Joint Under Secretary, Foreign and Commonwealth Affairs, said that discussions are taking place with these countries, and both have formally expressed interest in joining the agreement. Mr Luard also gave an assurance that stringent safeguards and security arrangements have been written into the agreement. (Written answers, March 2.)

Polytechnics

Eighteen major building projects for polytechnics, at a total cost of £6·2 million, have been given the go-ahead for 1970–71. Between 1967 and 1970, further education building programmes have included projects worth £17·7 million at colleges now forming part of designated or proposed polytechnics. These figures were given by Mr Gerald Fowler, Minister of State for Education and Science, and later the Secretary of State, Mr Edward Short, said that the proposed polytechnic at Huddersfield will probably be designated on September 1, 1970. Fourteen polytechnics have so far been designated. (Written answers, March 5.)

Fluoridation

The government may be forced to bring in legislation to compel local authorities to introduce fluoride into water supplies. This warning was given by Mr Richard Crossman, Secretary of State for the Social Services, after he had said that however much time, money and trouble are taken, eyes that are determinedly closed cannot be opened. His department, he said, has spent £300 this year to promote the case for fluoride in water supplies, but Mr Frank Judd asked for higher priority to be given to supplying authentic and appropriate information to local authorities on fluoridation. Mr Judd said that a number of authorities are taking half baked decisions on the basis of totally inadequate information. (Oral answers, March 9.)

Pneumoconiosis

The greater weight of respirable dust particles in South Wales coalmines may be the reason why the incidence of pneumoconiosis in the South Wales coalfield is almost twice as high as in other British coalmines. Accordingly, from the end of this month, new dust standards, based on the weight of dust in the atmosphere, will be introduced in all mines operated by the National Coal Board. This announcement was made by Lord Delacourt-Smith. (Oral answers, Lords, March 5.)

Strategic Nuclear Deterrent

Mr Denis Healey, Secretary of State for Defence, said that the government has no plans for modernizing Great Britain's nuclear deterrent, although the effectiveness of the Polaris system is kept constantly under review. Sir Ian Orr-Ewing asked whether reports that the government has refused to modernize Polaris missiles are true, but Mr Healey replied that steps had already been taken to improve the penetration capability of Polaris following the news of a Soviet ABM system. (Oral answers, March 4.)

Miscellaneous Intelligence

"The real purpose of the book I am writing is to say the human race is not so bad, after all. It can produce cancer workers. I have tremendous admiration for them; I think they are doing absolutely marvellous work; and I am proud to be a member of the same race"—Bernard Glemser in *Man Against Cancer* (Bodley Head, London, 1970). "Were it not for the tremendous amounts of money available, wrung from a susceptible public by an obviously emotional appeal, I suspect that very few scientists would be working directly on cancer. It seems to me that the mechanism of cancer is most unlikely to be discovered until pure biologists have unravelled a great deal more about the fundamental processes which go on inside a normal living cell. Most competent biologists probably realize this and avoid working directly on the problem of cancer. As a result, many research workers in the cancer field, I fear, are equipped with more dedication and money than scientific ability"—David Horrobin in *Science Is God* (Medical and Technical Publishing, Aylesbury, 1970).

According to a list prepared by Professor A. M. Liquori, head of the Laboratory of Physical Chemistry and Electrochemistry at Rome University, his department published no less than 63 papers during 1967–69. Professor Liquori is author or co-author of 42 of the 63 papers.

The view that the Irish are unduly partial to alcohol has been irrefrageably refuted. Figures compiled by the Economic and Social Research Institute in Dublin and reported in the *Journal of the Irish Medical Association* (**63**, 39; 1970) reveal the sober truth that Ireland is among the least bibulous of nations, consuming each year only 102 pints of beer a head compared with the Englishman's 164 pints and the German's 224. This being the case, how can the myth of Irish drunkenness have arisen? Possibly from the untypical propensities of the Irish immigrants to the United States, the Dublin Institute cannily suggests. Perhaps—but under what kind of influence could James Joyce have been when he wrote "Ireland sober is Ireland stiff".

Is the civilized world really awake to the true perils of cannabis? In South Africa they are not deceived. According to Mr Richard Neville, the editor of OZ (in *Play Power*, Cape, London, 1970), a South African textbook of criminology warns that "in extreme cases marijuana can so destroy a man's character that he mixes freely with persons of another race".

The following genealogical conundrum is posed in a current issue of *Zootecnica e Veterinaria* (**11–12**, 281; 1969): "Chauries have originated from Yaks (female is known as Nak) and Khirkoo and Jolung bulls on lower hill Zebu cattle. The female of Yak which is commonly known as Nak produces chauries when crossed with a bull locally known as Khirkoo and Jolung. Chauries are further subdivided into two types depending on different combinations of parents. Chauries obtained by crossing Nak (mother) with Jolung or Khirkoo bulls (father) is called as DIMJU and is supposed to be of a better quality than the one called URUNG which is obtained by crossing lower hill cows (Zebu cattle) with Yaks." Dimju jolung a chaury urungn't you the nak?

NEW WORLD

Keeping Plowshare Alive

THE stiff upper lip which the Atomic Energy Commission has kept for the news that Project Plowshare will be cut almost in half in the coming financial year seemed to tremble a little last week when the director of the Division of Peaceful Nuclear Explosions, Mr John S. Kelly, gave an account of his plans to the Joint Committee on Atomic Energy. For one thing, Mr Kelly seems to be hoping that news of the Russian development of peaceful nuclear explosions now being made available to a technical panel at the International Atomic Energy Agency in Vienna will help to win back support for his programme. Dr Michael M. May, director of the Lawrence Radiation Laboratory at Livermore, California, went so far, at the authorization hearings on the AEC's budget for 1971, as to suggest how an extra $10 million could usefully be spent in 1971.

Over the years, Project Plowshare has been under increasing financial pressure. In the financial year 1967–68, the budget was a heady $17·8 million—this was the year in which the AEC exploded a row of nuclear charges so as to simulate a section of what might yet be a sea level canal across the Isthmus of Panama. (The commission studying that proposal is due to report at the end of 1970.) A year ago, the AEC asked for $29 million for the current fiscal year and received just half that amount—$14·5 million. In a statement that has just been made public in a report by the Joint Committee on its Plowshare investigations last year, the AEC says that it had been hoping for $44 million in the new budget—more than five times as much as it has been given—and that it foresaw a programme of roughly the same size and cost for at least the next four years.

The chief effect of the new financial restrictions in the coming year is that there will be no experiments with nuclear explosions intended for excavation but only laboratory analysis of work already carried out. By the same test, the AEC will have to postpone its plans for making elements with atomic number greater than 104 by the use of nuclear explosions, although some theoretical studies in the design of nuclear explosives are intended. The only explosion will be that of a device intended for underground engineering—this will take up $1·3 million. The object is to develop a nuclear device more suitable than those now available for stimulating the flow of natural gas from underground reservoirs, principally by reducing the amount of tritium produced in the explosion. Mr Kelly said last week that it should by 1973 be possible to use "improved explosives" producing amounts of tritium about one per cent of those found in the gas from Project Gasbuggy, the underground explosion in December 1967 in New Mexico. Another objective is to produce a nuclear device small enough to be lowered down a conventional drill pipe six or eight inches across.

The most obvious weakness in the case for Plowshare is that no overwhelming economic case has yet been put forward. The results so far available from the Gasbuggy programme are encouraging enough—Mr Kelly said last week that the project had been a success "by almost any criteria", and that the underground explosion had increased the amount of gas which it would be possible to extract in twenty years from the reservoir to 900 million cubic feet, five times the capacity of similar wells operated conventionally. With gas selling at the well head at 13·3 cents per thousand cubic feet, this implies a total value of more than $100,000. Even though the cost of distribution is an increasing fraction of the total cost of selling natural gas, it is unlikely that such a comparatively small increase in the productivity of a well will bring hydrocarbon companies clamouring for Plowshare projects. The fact is that it is not yet clear how safely natural gas laden with tritium could be fed into the public distribution system—Gasbuggy gas is at least 25 times as heavily laden with radioactivity as the distributors would like to see it, with the result that the material now being bled from the reservoir is burnt on the spot. Perhaps more will be known of these problems when the AEC uses $100,000 of its new budget to drill a hole towards the underground cavern left by Project Rulison, 8,000 feet below western Colorado. Another puzzle to be solved is that the pressure in the Rulison cavern seems to have increased rapidly in the last three months of 1969 until it had reached more than one ton per square inch by the end of the year, presumably because of the release of gas from elsewhere in the reservoir, but Mr Kelly said it is now

AEC BUDGET
(in thousands of dollars)

	1969 actual	1970 est.	1971 est.
Raw materials	100,904	50,800	18,016
Special nuclear materials	328,664	321,360	348,518
Weapons	816,279	808,150	841,760
Reactor development	443,399	424,000	432,034
Physical research	273,944	278,180	274,430
Biology and medicine	88,779	89,450	88,300
Training, education and information	16,250	15,068	12,780
Isotopes development	6,718	5,890	6,000
Civilian applications of nuclear explosives	12,959	14,500	8,000
Communities	6,334	9,981	7,844
Regulation	9,316	11,725	12,672
Programme direction and administration	96,217	108,701	111,128
Security investigations	7,178	8,530	8,370
Cost of work for others	21,063	42,881	24,246
Adjustment to prior year costs	−560	—	—
Total programme costs	2,227,444	2,189,216	2,194,098

stabilized. Until these questions are resolved, Mr Kelly's claim last week that nuclear stimulation of underground gas wells could make available some 317,000 million cubic feet of gas, more than the present proved reserves in the United States, will no doubt remain a paper promise—and the chairman of the joint committee, Mr Chet Holifield, wanted in any case to know why 50,000 million cubic feet of gas should be shipped to Japan each year if the shortage of natural gas was as serious as Mr Kelly said.

In the new straitened circumstances, most of what Plowshare has to spend will go on research, partly in the design of improved explosives, partly in the better understanding of the mechanical effects of nuclear explosions and partly in trying to anticipate the production of radioactivity in novel circumstances (such as in copper bearing strata) and the production of geothermal heat.

This said, it seems clear that the reduction of effort will have serious consequences, particularly at Livermore. Mr Kelly said last week that a delay of a year or more in the cratering experiments would mean that the project would lose engineers and scientists. Dr May said that there would be a good many scientists among the eighty out of two hundred people who would be leaving Livermore, a laboratory already under pressure because of a seven per cent reduction in the weapons programme. This argument no doubt gave point to Dr May's programme of supplementary work, including a second underground explosion, the drilling of extra holes for geophysical exploration around the Rulison reservoir, experimental studies of an existing underground gas reservoir to determine leaks, studies of other existing cavities and of the proposals which are, according to Dr May, streaming in to the AEC.

The extent to which tales of what the Russians are doing will melt the heart of Congress remains to be seen. According to Mr Theos J. Thompson, an AEC commissioner, the Soviet Union has already carried out several peaceful nuclear explosions as part of a development programme by the State Committee on Atomic Energy. The meeting in February, the second between the Soviet Union and the United States, showed that the Russians have already carried out five excavation experiments, two of them with rows of explosives intended to form a trench, and a 25 kiloton underground explosion intended to produce an underground gas reservoir, while there are also plans to stimulate natural gas and oil production. Although this development seems to indicate a change of policy in the Soviet Union since the early sixties when there was vigorous criticism of the American plan for Plowshare, there is nothing in what Mr Thompson said last week to suggest that the Russians have significantly improved on experiments already carried out in the United States, or that they have been able to avoid some of the difficulties in the way of Plowshare. Indeed, talk of diverting rivers to balance water evaporation from the Caspian Sea probably has the same status in the Soviet Union as the American plan for a sea level canal across Central America.

One of the ironies of the AEC's new programme is that comparisons between the United States and the Soviet Union are chiefly responsible for the most striking, if modest, increase in expenditure—the proposals for more spending on thermonuclear research machines. Last week, Mr Paul McDaniel, the director of the division of research at the Atomic Energy Commission, explained that the AEC has embarked on a programme of machine building intended to replicate and to extend the work done in the Soviet Union with the Tokamak devices. Already, work is under way at the Oak Ridge National Laboratory and at the Princeton Plasma Physics Laboratory on the construction of two machines. Dr Robert Hirsch, the manager of the programme, told the Joint Committee that the Princeton project, involving the construction of a stellarator device, will cost $200,000 and "will duplicate the T3 plasma". The Oak Ridge machine will be similar to another Tokamak design except that the magnetic field will be produced by a one-turn coil so that asymmetry will be excluded. This machine should operate with a magnetic pressure twice that of its Russian analogue, so that there will be some hint as to how the scaling laws go and the magnetic coil will be easily replaceable so that it will be possible to check the influence of different parameters.

SPENDING ON PHYSICAL RESEARCH
(in thousands of dollars)

	1969 actual	1970 est.	1971 est.
High energy physics	118,630	120,530	119,450
Medium energy physics	11,266	12,790	13,140
Low energy physics	29,620	29,480	28,860
Mathematics and computer research	5,686	5,800	5,410
Chemistry research	54,482	53,970	51,980
Metallurgy and materials research	27,719	27,930	26,980
Controlled thermonuclear research	26,541	27,680	29,610
Total	273,944	278,180	274,430

Two other proposals which the AEC will follow include a machine at MIT (ALCATOR) which will allow magnetic fields of 130,000 gauss (four times that of the highest fields used in the Tokamak experiments) and an experimental device called Doublet II, intended for the Gulf General Atomic Corporation at San Diego. It also seems likely that the AEC will contribute to a proposal by the University of Texas, financed largely with $350,000 of university money.

Mr Hirsch went further than the long-standing promise of Dr Amasa S. Bishop, who has been in charge of the thermonuclear programme for the past four years but who is now succeeded by Professor Roy Gould, of the California Institute of Technology, that the feasibility of commercial nuclear power would be demonstrated by 1978, in saying that it would be apparent "sooner than 1978" that commercial thermonuclear power is feasible. "We now have several new tools in our hands. People of the CTR community think we are now at a point at which we can move forward at a steady pace." Even so, the AEC is not counting on the fashionable interest in laser research to help it towards this goal—although Mr McDaniel said that work was continuing on the use of laser light to irradiate specks of material, the ionized vapour of which would be contained in a conventional magnetic field configuration, it had already abandoned the notion that a thermonuclear machine might be constructed by a sequence of micro explosions triggered off by a laser.

Stretch Out for NASA

By now, it is clear that the total impact of the stringent budget under which the National Aeronautics and Space Administration labours will be a further postponement of some of the projects that had been advertised in the past few years. In evidence submitted to the Committee on Science and Astronautics of the House of Representatives two weeks ago, Dr John E. Naugle explained that the agency will in the coming year be taking steps to complete its plans for putting satellites in orbits about the Earth to practical use. At the same time, he was able to say that one of the objectives for the seventies "will be to understand the problems of the ecology of the Earth"—one of the ways of drawing attention to good intentions in the present climate.

In the year ahead, expenditure on scientific work and the applications of satellites will actually increase (if the budget is agreed) by about 50 per cent to $550 million. Physics and astronomy will be dealt with as in the current year, and will take roughly 20 per cent of the total. A somewhat larger sum will go on lunar and planetary exploration, although the total for this work will be rather less than in the current year. The bioscience programme and the bioscience satellites will be tapered off, but space applications will get more attention—and about $150 million—than in the past.

One of the projects for the year ahead is the construction of two advanced technology satellites (ATS F and G), to be launched respectively in 1973 and 1975. Each of these will weigh nearly a ton and one important innovation will be a steerable antenna beam which can be directed to within a tenth of a degree of any chosen point on the Earth. The satellites will also be equipped with lasers for communicating not merely with stations on the ground but with other satellites. One objective of the experiments will be to test the technology of air traffic control by satellite as well as to obtain information about the propagation of millimetre waves. The first of these satellites will be used for the cooperative programme in the broadcasting of television programmes that has been agreed with the Indian Government. For reasons of economy, Dr Naugle said, it has been necessary to delay the first of the launchings from 1972 to 1973 and the second from 1974 to 1975.

In the coming months, however, there will be a launching of the satellite called the Cooperative Applications Satellite in which an attempt will be made to track instrumented balloons from the satellite. This is a project in which American and French scientists will collaborate, with the French providing the spacecraft and the balloons but not the rocket. This experiment should provide some of the information on which decisions about the usefulness of satellites in aircraft control can be made.

On the use of satellites for geodetic purposes, Dr Naugle said that it is planned to launch a third satellite into an orbit inclined at 20° to the equator early in 1972. The objective here is to complement the results obtained with earlier satellites at higher inclinations, but it is also intended that there should be a radio altimeter for measuring directly the mean sea level of the ocean.

The use of Earth satellites for observation of the surface of the Earth is plainly dear to NASA's heart, although it seems not yet entirely clear how these possibilities will be exploited. One important part of the programme ahead is to make observations of the Earth from aircraft, although the photographs taken by the Apollo 9 astronauts seem to have given people plenty to chew over. In the year ahead, NASA is planning to continue with the design of two satellites intended to put into application some of the uses already suggested—the first of the two satellites should be launched in the first half of 1972. In meteorology, according to Dr Naugle, the most hopeful development has been the proof that instruments carried in satellites can usefully provide a record of temperature at all heights in the atmosphere, and this is the way in which NASA will put its chief emphasis.

In NASA's plans for the exploration of the solar system, the biggest development is a negative one—the decision to postpone from 1973 to 1975 the landing of a package of instruments on Mars. There will, however, be two Mariner spacecraft aimed at orbits about Mars in May 1971 (due to reach the planet in November). The first of these will be equipped to provide wide angle photographs of the surface of the planet, with a resolution of between 2 and 3 kilometres, for a period of about three months. The second satellite will be designed so as to make repeated observations of a few selected sites on the surface in a search for periodic phenomena (see page 995).

Dr Naugle explained to the House committee that the Viking project, "the largest single project" in the exploration of the solar system, is now at the point at which 165 proposed experiments have been whittled down to 25, selected last December. In the immediate future, work on the rocket will consist only of the development of those systems, concerned chiefly with landing and navigation, where progress is necessarily slow. It has, however, been decided that the rocket to Mars will be equipped with instruments to help select a good landing site, with instruments to determine the vertical profile of the martian atmosphere, to photograph the site, to analyse samples of surface material and also to seek out living forms as determined by photosynthesis and respiration. So far, there has been no postponement of the plan to launch a rocket towards Venus and Mercury in 1973. The chief object here is to learn something of the atmosphere of Mercury. The collaborative programme with West Germany to launch a rocket (Helios) to within the orbit of Mercury is also continuing. According to Dr Naugle, however, the most exciting of the projects now on hand is the launching of two Pioneer rockets towards Jupiter (page 996). The plan is that a prototype will be built and tested within the next year and that the completed rockets should be launched in March 1972 and April 1973 (each rocket will take just under two years to reach Jupiter).

Among the projects for the exploration of the region near the Earth, NASA has plainly great hopes for a number of orbiting observatories, both for the Sun and more distant objects in the universe. A great deal of what it plans to spend on this work will, however, go on the telescope package which is being designed for the Apollo applications programme.

SPACE STRATEGY

Almost a Blank Cheque

THE long awaited statement of government policy on space, made public last weekend by President Nixon, is not so much a declaration of purpose as a framework within which the National Aeronautics and Space Administration will be able to carry out most, although not quite all, of the schemes on which hearts have been set. To some, no doubt, it will be disappointing that the journey to Mars is described merely as a "longer range goal we should keep in mind as we proceed with our exploration of the planets". On the other hand, the President has made a firm commitment to the schemes for sending an unmanned rocket on a grand tour of the outer planets, using the gravitational field of one to help pave the way to the next—a trick which will be feasible in the late seventies but not thereafter until some time in the twenty-first century. One of the potentially most interesting features of the strategy now outlined is an undertaking to "encourage greater international cooperation in space".

The President's starting point last weekend was that "having completed that long stride into the future which has been our objective for the last decade, we must now define new goals which make sense for the seventies". He was anxious that the space programme should not stagnate but, at the same time, said "that we should not try to do everything at once".

The declaration of policy is said to be based on a consideration of the report of the space task group under Vice-President Agnew (see *Nature* **223**, 1297; 1969), and in practice it consists of an endorsement of most of the options there spelled out except the mission to Mars. The guiding principle, President Nixon says, was that the space programme should satisfy the hankering after exploration, that it should help to provide scientific understanding and, in particular, "should be attentive" to what scientists might suggest; third, that the space programme should exploit practical applications.

In practice, the President said that exploration of the Moon should continue even after the Apollo programme is completed, that there should be "bold exploration of the planets and the universe" with the grand tour as a starting point, an attempt to reduce the cost of space operations, which implies approval for the scheme to develop a space shuttle, an attempt to extend peoples' capacity to live and work in space, which implies approval for the experimental space station on which NASA is working, the increased applications of space technology in fields such as air traffic control and navigation as well as in meteorology and telecommunications, and the deliberate involvement of other countries than the United States.

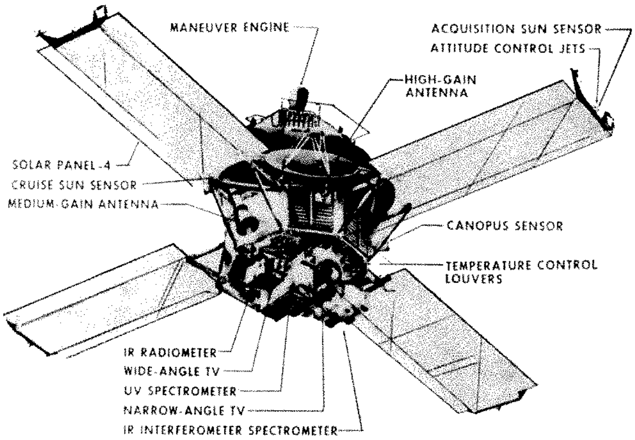
This declaration of policy will almost certainly imply that the budget of NASA will not in future shrink below $3,000 million a year. Indeed, as the space shuttle project moves from design to construction in 1972 and 1973 it may well be necessary to spend more on implementing the package which the President has put together.

To this extent, the new policy should provide NASA with a greater sense of security in the months ahead as well as with one or two exciting schemes on which to work.

PLANETS

Mars Progress Report

ONE reason why the plan to send two unmanned spacecraft into Mars orbit next year has not suffered in the wave of budget cuts is that it is based on the well-tried Mariner design whose most recent success was the close approach to Mars last summer. On the other hand, the more ambitious Viking project to soft-land two capsules on the surface of Mars has been postponed for two years from 1973 to 1975. The delay is expected to increase the $750 million cost of the Viking project by $150 million. Half of the increased expenditure will cover a rise in wages, and half the extra costs incurred by the less favourable position of Mars in 1975. Experiments for the Viking capsules and for the orbiting spacecraft from which they will be ejected were chosen last year (see *Nature*, **225**, 124; 1970), and none of the British proposals was selected.



The 1971 Mariner spacecraft.

The National Aeronautics and Space Administration has now published more details of next year's missions which will pave the way for Viking by identifying particularly interesting areas on Mars. Two spacecraft will be launched to reach Mars on November 14 (Mariner 8) and on November 24 (Mariner 9). The spacecraft are basically the same as the Mariners which achieved the close approach to Mars last summer, with the addition of a small engine to power the velocity change of 3,400 miles per hour for insertion into Mars orbit. Each spacecraft will weigh about 2,200 lb, which includes 970 lb of fuel. The 125 lb of scientific instrumentation on board will be made up of a 50 mm wide-angle television camera, and a 500 mm telephoto camera for detailed work, an infrared radiometer to measure surface temperatures, an ultraviolet spectrometer to identify the composition of the atmosphere, and an infrared interferometer spectrometer to look at constituents of the atmosphere and surface. Then there will be the usual close observations of the telemetry signals to improve estimates of various astronomical constants and for further information about the atmosphere of Mars.

The chief aim of Mariner 8 is to provide the maps of the surface which planetary astronomers badly need. It is already known that the density of cratering on Mars varies widely from place to place, for example, and good maps are essential for work on crater statistics. The plan is for the wide-angle television camera to cover the band between 40° north and 60° south with a resolution of two-thirds of a mile. The primary object of Mariner 9 is to search for changes in the appearance of surface features with time, in particular the wave of darkening which is visible from the Earth. Thus the Mariner 9 orbit will be such that the cameras can photograph the same area of the surface every fourth day.

GRAND TOUR

Planetary Billiards Approved

The preliminary exploration of the planets beyond Mars for which President Nixon has now given his approval fulfils at least two of the major recommendations made by the Space Science Board of the National Academy of Sciences in a report last year on the programme of exploration of the outer solar system (see *Nature*, **223**, 661; 1969). Among other things, the board urged that advantage be taken of a favourable configuration of the planets to dispatch probes to Pluto by way of Jupiter and Saturn in 1977, and to Neptune via Jupiter and Uranus in 1979. These proposals have now been agreed to. Coupled with flights to Jupiter alone, planned for 1972, the most urgent of the board's recommendations for the study of the outer planets in the 1970s will be carried out.

The reason for the haste is, of course, the once-in-a-lifetime opportunity of a particular alignment of the planets. The alignment, which will not recur for 180 years, allows the close approach to each planet to swing the probe on to the next with roughly the amount of power needed to reach Jupiter. Planning for the project, which has been going on at the Advanced Studies Office of the Jet Propulsion Laboratory, is based on a 1,200 lb spacecraft which could be launched by a Titan-Centaur rocket. Power for the spacecraft will probably come from a radioisotope thermoelectric generator, and clearly the spacecraft's appearance is going to be dominated by a large parabolic aerial for communications with Earth. With radio signals taking four hours to reach the Earth from Neptune, a new breed of spacecraft is going to be required with plenty of redundancy built into its circuits.

The justification for going to the outer planets is, of course, that without better knowledge, in particular of their constitution, it is difficult to theorize about the early history of the solar system, especially about the composition of the material from which the solar system condensed. It is not yet known what instruments the probes will carry, but the payload will probably include cameras, ultraviolet and infrared detectors for studying planetary atmospheres, and radiation detectors. It will also be important to take full advantage of the spacecraft to probe the interplanetary medium, and one problem may be to ensure that it remains magnetically clean after passing through the Jovian field. There is also the question of designing instruments which will not be ruined by the adverse conditions expected near Jupiter—a high flux of energetic particles, and possibly dust clouds. Another problem is whether the spacecraft should be complicated by the addition of entry probes to study planetary atmospheres.

It is clear that these are not going to be missions with quick results. It will take seven or eight years to reach Pluto, for example, but this is better than the 41 years which a direct flight would involve. Neptune will take 8·9 years, compared with 18 years by direct flight, and Jupiter (which will be the most important planet for the missions) will be reached after just under two years.

MEDICAL RESEARCH

Good Fairy for the NIH

The National Institutes of Health have fared best among the scientifically oriented agencies of the United States Government out of the new budget (see *Nature*, **225**, 582; 1970), and now seem like also to profit from the way in which Congress has forced the Administration to accept a somewhat larger budget for the Department of Health, Education and Welfare than is welcome. Although it is not finally certain that the President will refrain from vetoing the legislation giving spending authority for the current year's budget, the chances are that the National Institutes of Health will finish up more than $10 million better off than they had hoped. This should allow a little relief from the present pressures on the budget even if the extra money is likely to be chopped off the budget for the year which begins in July, when the National Institutes of Health as a whole are hoping to grow by $66 million to $1,526 million for the year.

Among the operations of the National Institutes of Health, the National Cancer Institute and the National Heart and Lung Institute will grow most steadily, at least if the budget gets through Congress unscathed, the former by $29 million to $201 million and the second by $19 million to $171 million. A less spectacular beneficiary of this now unfamiliar open-handedness is the National Institute of Dental Research, which has an extra $6 million in the new budget. The parts of the NIH programme which have grown like this are related in one way or another to the Administration's hope that it will be possible to put paid to a number of social problems by careful spending on health research. It remains a remarkable feature of the NIH budget, however, that it has turned out to be possible to sustain, although not increase, the amount of money which the NIH will be able to commit to educational activities. Other agencies have found themselves forced to abandon or severely curtail educational programmes—the National Science Foundation's traineeship programme and the NASA University Sustaining Programme are among the more spectacular casualties (see page 985). It appears that the NIH have been able

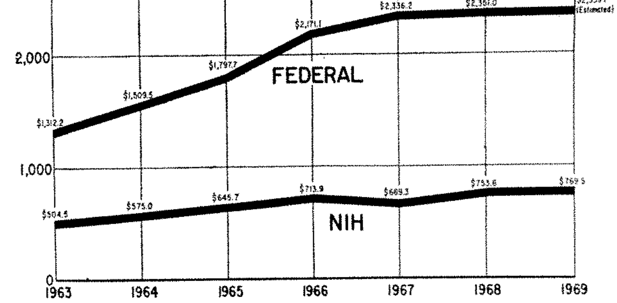


Fig. 1. Federal and NIH support for academic science, including both research support and finance for students and training programmes.

to keep their own educational programmes intact by vigorously complaining that curtailment would reduce the numbers of trained doctors at a time when shortages already serious are likely to become even more apparent.

Although the budget of the NIH as a whole has continued to grow over the years, the direct benefit to research and to universities has tended to stagnate for several years. Thus the total amount to be spent on research grants if the new ship comes home will be $546 million, nearly £10 million less than two years ago. Much of the increase to be expected will be spent on what is called collaborative research and development, chiefly by letting contracts. (An extra $50 million is earmarked for this purpose, while the cost of research carried out at the national institutes themselves will increase by merely $10 million.) With the sustained growth of the NIH since the early sixties, contracts have played an increasingly important part in the pattern of research financing, not merely no doubt because there are limits to the work which can be done by a necessarily limited group of people, but also because there is more scope for this method of working in circumstances when research programmes are aimed at specific goals.

EDUCATION

New Plans for Students

URGED on by the ubiquitous Mr Daniel P. Moynihan, who has been in hot water in the past few days for suggesting in a private memorandum that the colour problem would profit from a period of "benign neglect", President Nixon announced last week a programme intended to improve the quality of education in American schools. One important development is the setting up of a National Institute of Education intended to carry out and to sponsor educational research to the tune of as much as $250 million a year. There is also a Commission on School Finance to study the financial problems of the school system, public and otherwise. In the hope of replacing some of the welfare projects of the last Administration, Project Headstart in particular, by more effective programmes, President Nixon has also suggested a programme of financial assistance, costing $200 million in the year ahead, to help states and local communities teach children to read and there is to be another programme of what are called "child development projects".

The National Institute of Education is plainly destined to become the focus for most kinds of federally sponsored educational research in the United States. Much of its work will be concerned with curriculum development and with the evaluation of new kinds of curricula. Much of the work will be undertaken by universities and other organizations under contract, but it is intended that there should be a National Advisory Council to guide the development of policy. Among the particular subjects mentioned in the message to Congress on March 3 were the search for more effective means of "compensatory education", the search for new methods of putting flesh on the bones of Dr James E. Allen's slogan "Right to Read", partly by helping school libraries to buy books and by helping school systems provide special teaching programmes, and the investigation of the use of television in education.

One of the oddest features of this proposal is that it now seems somewhat old fashioned. Ten years ago, curriculum development was all the rage and programmes such as that of the Physical Sciences Study Committee were widely acclaimed. More recently, in the mid-sixties, there seemed to be great enthusiasm for the application of television and management techniques to the learning situation. In the past few years, however, much of the enthusiasm for these programmes seems to have been turned in other directions, as if the first wave of really self-conscious curriculum development had been diffused into the practical problems of how best to organize the application of the new techniques, still far from complete—a tendency no doubt accelerated by the shortage of funds in the educational budget. The efforts of the National Science Foundation in this direction have been shrinking for the past few years. Although the Administration is now plainly concerned with a different part of the educational system from that in which curriculum development began in the nineteen fifties, the success of the National Institute of Education will plainly be intimately dependent on the chance that able people are ready to participate.

The proposals for helping with the analysis of the financial plight of school systems, through a commission already appointed, are still somewhat an unknown quantity. At present, states are responsible for 38 per cent of the cost of operating school systems, and 54 per cent of the cost is met from local funds. The federal sources provide only 8 per cent. One of the problems to be tackled is to find some way of channelling more federal money into the school systems, but there have already been complaints that President Nixon's promise to make a study does not do away with the urgent need for financial assistance to prevent some school systems from collapsing altogether. The fact that the United States spends more on education than the rest of the world put together, according to President Nixon's statement, may not be a sufficient guarantee of health. On the other hand, it seems to be agreed that there will be great profit if great uncertainty in some of the schemes that are being talked about for helping to improve the educational environment of those not yet at school.

ECLIPSE

Observations Galore

THE solar eclipse last Saturday was a great success and, if these things were regulated by the wishes of astronomers and even of the general public, there would evidently be more of them. In Mexico, the 300 observational astronomers who had gathered for the occasion were provided with almost perfect conditions for observing the eclipse. As the track crossed Florida and the coast of Georgia, clouds obscured the view, but as it reached the cleverly sited station of the National Aeronautics and Space Administration at Langley air force base and Wallops Island in Virginia, conditions could not have been improved on. At Langley air force base, photography of the flash spectrum of the chromosphere is said to have been successful. At Wallops Island, the staff managed somehow to launch 31 sounding rockets before, during and after the total eclipse. The only failure was the loss of a naval research laboratory payload intended to return flash spectra of the chromosphere in the ultraviolet—the Aerobee rocket functioned perfectly but the payload disappeared. One of the Nike-Cajun rockets intended for ozone measurement also failed to function, while the second of two airglow experiments was cancelled when the first failed.

In these circumstances, the star turn at Wallops Island was the international experiment involving teams from Imperial College, London, the Culham Laboratory of the UK Atomic Energy Authority, Harvard University and York University, Toronto. Dr R. J. Speer, of Imperial College, said after recovery of the film package that he and his colleagues had been surprised and delighted by the quality of the films recovered. He explained that the experiment was designed to provide ultraviolet spectra of the Sun at three-second intervals in two ranges from 85 to 200 nanometres and from 210 to 310 nanometres. In practice, Dr Speer said, the rocket had functioned perfectly and the apogee had been placed accurately within the ten kilometre cube at the instant of totality. The package of film recovered was found to include fifty complete spectra, only slightly fogged, representing the ultraviolet output of the Sun at intervals of three seconds of time between each of which the Moon had moved 1·3 seconds of arc. Since the chromosphere is about 10 seconds of arc thick, the sequences of films will make it possible to reconstruct the ultraviolet emission of the chromosphere in great detail.

Dr Speer was full of praise for the way in which the rocket operation had been mounted and the equipment itself had performed. He said that this was the first occasion on which the guidance system has been flown—the principle is to lock on to the nearly eclipsed Sun and then to follow with inertial guidance during totality. By good chance, it seems, the rocket happened to be stationary at the point of the second contact, which means that the interpretation of these photographs will be simplified. Dr Speer also praised the way in which an expeditious recovery of the package made it possible to start work on development of the spectra in just over five hours after the eclipse—an important consideration with ultraviolet sensitive film in which the sensitive grains lie on the surface and are easily damaged.

COLLEGE ADMINISTRATION

Scientists as Presidents

Two distinguished scientists will in future be presidents of two colleges which have been more than a little disturbed by the troubles of the past two years. The most unexpected appointment is that of Dr Robert E. Marshak, the theoretical physicist from the University of Rochester, who has been appointed president of City College, New York. Dr Marshak, who is 53, began his research career at Cornell, where he worked with Bethe on thermonuclear reactions in stars, among other things, but has been at the University of Rochester since 1938 and has worked on the penetrating components of cosmic rays. In the past few years, he has been an outspoken critic of university administrations for their unwillingness to admit students into full participation in university government and in this spirit he resigned from the faculty senate of the University of Rochester as recently as February 13. He said after his appointment on February 27 that he proposed delegating a good deal of the administration of the City College, which has some 20,000 students, to a provost yet to be appointed and that he would try to sponsor ways in which the university could help with the social improvement of depressed parts of the city.

Dartmouth College, New Hampshire, is a somewhat more sedate institution, even though there was some trouble there some years ago. The new president is Dr John G. Kemeny, professor of mathematics and philosophy at Dartmouth for the past fifteen years. Dr Kemeny, a Hungarian by birth, has been much involved in the development of time sharing computers.

YOUNG SCIENTISTS

More Scholarships for Precocity

Like an intellectual version of the Miss America competition, the twenty-ninth search for talent among budding scientists in United States high schools ended successfully last week with the presentation of awards ranging from $4,000 to $10,000 to ten young people between fifteen and seventeen, two of them girls. A large roomful of people, described by Dr Glen Seaborg, chairman of the Atomic Energy Commission, as "everybody who is anybody in science", shared with the thirty unsuccessful finalists in the room the sight of the winners being called to the limelight in reverse order, $4,000 people first and the $10,000 winner, Kirk A. Shinsky of Allentown, Pennsylvania, last of all. The occasion was a great success for New York, the state which provided seven out of the ten winners. The Bronk High School for Science produced no fewer than three of these.

The annual Science Talent Search is organized by Science Services but financed by the Westinghouse Corporation. Mrs Dorothy Shriver, an assistant director of Science Services responsible for the organization of the programme, said there were this year almost 3,000 initial applications from high school students. Applicants provide a written account of a scientific project which is then supplemented by information from teachers and by a "scientific aptitude test". Mrs Shriver explained this week that this test will in future be replaced by the scores obtained in the national scholastic aptitude tests, chiefly because experience has shown that there is very little difference between the two yardsticks of performance even though the test which has in past years been designed for the talent search has consisted almost entirely of scientific questions.

The 3,000 applicants were this year winnowed down to 300, the dossiers of whom were rescrutinized so as to produce a short-list of 40 young people who then spent a week in Washington seeing some sights and some dignitaries. The ten winners were chosen by the seven judges after personal interview. Mrs Shriver says that the judges are more concerned with promise than achievement, but she acknowledges that it is necessarily hard to distinguish between scientific aptitude and general intelligence. Four years ago, a twenty-five year follow-up study among the people who had been the first winners suggested that ninety per cent had gone on to take higher degrees and that seventy per cent had remained in science.

NEWS AND VIEWS

Towards Hybrid Plant Cells

BOTANISTS are lucky that they can do with an easy conscience things that bring down the wrath of public opinion on their zoological colleagues. Professor Cocking and his co-workers at Nottingham, who this week report the first successful steps towards cell fusion in plants (page 1016), can expect unalloyed admiration and gratitude for their labours. They may have laid the foundations for a vast new programme of genetic engineering, but even the sternest guardian of morals must welcome the eventual development of new and better varieties of crop plants, and is unlikely to object to any monster plants that may be produced in the meantime.

The achievement of the team in Nottingham has been to release the contents of plant cells from their hard retaining walls, and to induce the resulting cytoplasmic masses, known as protoplasts, to fuse together in pairs. Although this has been done before, fusion has not been reproducible as it is now, and protoplasts were obtained by mechanical manipulation, far less sophisticated than Cocking's precisely defined mixture of enzymes. The most significant factor for plant breeders is that fusion has now been achieved between protoplasts from different species.

Of course, this does not mean that a hybrid plant cell has been produced in the laboratory, for there is yet no sign of fusion of the nuclei contained in the fused protoplasts. And even when that has been achieved it will still be necessary to generate a new cell wall around the fused protoplasts. At present there is no indication as to when these vital steps will be taken, but it is certain that no efforts will be spared to hasten the day.

When the day comes, the interest of geneticists will focus principally on fusion between plants such as Cocking *et al.* have been using—oats, maize and barley—which cannot be hybridized in field or greenhouse by conventional methods. Once hybrid cells are ready there should be little difficulty in growing them into mature plants, for there is a vast store of knowledge, gathered all over the world, about the conditions required to induce single plant cells to differentiate and develop in the same way as a normal embryo within a seed.

Just as disease resistance can be introduced into wheat from other species, so there are hopes that, starting with the techniques developed by Cocking and others, it will be possible to endow maize, barley and oats, for example, with useful characters from species with which they cannot be mated successfully.

The returns from such work would be slow to come, as they always are with plant breeding, but they would certainly be significant from the point of view of world agriculture. Further progress in plant cell fusion will undoubtedly be awaited eagerly. There may be much to be learned from the zoologists who have already fused mammalian cells successfully although they are in no position to grow their hybrid cells into mature animals, for which most people are thankful. On the other hand, it might be said to be the social responsibility of Professor Cocking and his colleagues to continue with their work as quickly as possible.

Starts, Stops and Anti-stops

THE outsider must find it a great consolation that the concepts of molecular biology are a great deal simpler than the language, or rather shorthand, the professionals use to communicate them. Current ideas about the positive control of the process of genetic transcription, copying genetic information from DNA to RNA, for example, are much simpler than a glance at the reports by Travers (page 1009) and Bautz's group (page 1012) might suggest. It is little more than a year since these two groups, working at Harvard and Rutgers Universities, reported in *Nature* (**221**, 43; 1969) the discovery of the so-called sigma factor, a protein in *E. coli* which transiently associates with the enzyme RNA polymerase and confers on it the ability to discriminate between genes. Sigma factor in effect tells RNA polymerase which genes it can copy and so exerts positive control over genetic expression. Since the discovery of the *E. coli* sigma factor, several analogous factors made by bacteriophages have been discovered, and we now have a fairly detailed picture of the involvement of sigma factors in the replication of T4 coliphage.

Immediately after an *E. coli* cell has been infected with T4 DNA the bacterial RNA polymerase programmed by the *E. coli* sigma factor starts to copy phage RNA from a set of so-called pre-early phage genes. Travers has shown that one of these genes specifies a new phage specific sigma factor. Once made, this displaces, by a completely obscure mechanism, the *E. coli* sigma. As a result the RNA polymerase is subverted to copying phage instead of *E. coli* genes and the replication of the phage continues at the expense of the host bacterium. At some later stage in the replication cycle, either a second phage specific sigma factor is made, or the first phage sigma and the host core RNA polymerase are modified to permit expression of more so-called late phage genes. Details of how this change is achieved are, however, obscure.

Sigma factors dictate the specificity of the start of RNA synthesis, but what signal tells the enzyme where to stop synthesis ? If sigma factors act as capital letters, what are the full stops ? Last year Roberts reported from Harvard (*Nature*, **224**, 1168; 1969) the discovery of another factor, rho, which somehow tells an RNA polymerase molecule it has reached the end of a particular unit of genetic information and should stop making RNA. In theory, sigma and rho factors working together could provide a complete positive control mechanism to regulate sequential gene expression. Bautz and his colleagues (see page 1012), however, suggest that there may be more to the process than that. Like Travers, but less directly, they find that T4 sigma factor allows the polymerase to read genes which cannot be read if the enzyme is programmed by *E. coli* sigma. But they also find that some T4 genes are read very early in infection even though they are expressed as proteins only late in infection when all the *E. coli* sigma factor has disappeared. They suggest that this very early reading of late genes occurs because of a failure in the normal termination process. They envisage that at the outset of infection *E. coli* sigma and polymerase initiate the synthesis of the first phage RNAs to be made. These include, of course, the RNA specifying the T4 sigma factor. But instead of stopping RNA synthesis at the correct points the enzyme sometimes reads through the earliest genes and goes on to copy into RNA some of the genes expressed much later in infection when the T4 sigma is active. Such read-through must be the result of a failure in the *E. coli* termination mechanism; rho cannot be functioning properly. Bautz *et al.* suggest that one of the first phage proteins to be made together with the phage sigma is an anti-terminator which somehow stops the terminator, rho, from acting. To prove their point they must now isolate the putative anti-terminator.

GALAXIES

Ejected Clouds in Milky Way

from our Cosmology Correspondent

THERE is now increasing evidence that the centre of our own galaxy has at some time been the site of violent events comparable with those seen in Seyfert galaxies. The latest addition to this evidence comes from a survey of the high velocity component of the 21 cm profiles seen in the region $10° < l < 350°$, $-5° < b < +5°$ which has been carried out by P. C. Van der Kruit using the 25 m telescope at Dwingeloo (*Astron. Astrophys.*, **4**, 462; 1970). Van der Kruit has observed many of the well known features which lie in the galactic plane, but features which are not associated with the structure of the plane are all found to have velocities greater than would be expected from the rotation of the galaxy.

Almost all the material is expanding away from the nucleus, except for that in the nuclear disk, which is rotating very rapidly. At negative longitudes these features are seen above the galactic plane, and at positive longitudes below it, suggesting that clouds have been ejected from the nucleus in two roughly opposite directions, at an angle to the plane which may be the same as that of the secondary ridge of continuum radiation found by Kerr and Sinclair (*Nature*, **212**, 166; 1966). Van der Kruit favours the idea that this matter has been ejected rather than that it is falling into the nucleus, and suggests that the energy in the required explosions would be as large as 10^{57} ergs, comparable with that seen in radio galaxies, and that they occurred 10^7 years ago. Many of the features of the ejected clouds can be explained satisfactorily in terms of the clouds seen emitted from Seyfert galaxies, provided that it is accepted that in our own galaxy the clouds have had time to expand to diameters about an order of magnitude larger than the clouds in Seyfert galaxies, with corresponding reduction in their outward velocities and temperatures.

Following the observations in the far infrared which support the hypothesis that the nucleus of our galaxy is similar to those of Seyfert galaxies (E. E. Becklin and G. Neugebauer (*Astrophys. J. Lett.*, **157**, L31; 1969) this evidence at radio wavelengths does much to encourage the idea that all galaxies pass through such an active stage in their evolution.

NUCLEAR MAGNETIC RESONANCE

In the Crystal Ball

from our Molecular Biology Correspondent

ANYONE wishing to lay a modest bet on scientific trends for the seventies might do well to give a thought to ^{13}C nuclear magnetic resonance of biological molecules. The first few cautious exploratory offerings in the literature may amount as yet to a cloud on the horizon no bigger than a man's hand, but the prospects are clear enough. In the first place, the chemical shifts are very much larger than for protons, and the possibilities for assignment and resolution of resonances are correspondingly greater. Second, the use of ^{13}C should make it possible to extend the method to macromolecules of much greater size than is feasible for proton resonance, and finally it has been demonstrated that there are good prospects of working on native materials, taking advantage of the natural abundance of the ^{13}C isotope.

This feature, which may in many cases eliminate the limitations and tedium of preparation of ^{13}C-labelled materials, is brought out in two articles that explore the ^{13}C-resonance spectra of natural nucleotides. Dorman and Roberts (*Proc. US Nat. Acad. Sci.*, **65**, 19; 1970) have examined a series of nucleotides in aqueous solution. Simply by means of comparisons of different sugars and pyrimidines, all the sugar and pyrimidine carbon resonances can be assigned; purine frequencies are assigned, at least in part, with the aid of procedures (heteronuclear decoupling) which make it possible to establish whether protons are attached to the nuclei from which particular resonances arise. The ^{13}C spectra of the common nucleosides have been analysed in an accompanying article by Jones *et al.* (*ibid.*, 27), which supports and completes the assignments, and emphasizes the remarkable degree of discrimination between carbon nuclei in only slightly different environments.

Another exploratory study, this time on amino-acids and some peptides, comes from Horsley, Sternlicht and Cohen (*J. Amer. Chem. Soc.*, **92**, 680; 1970). The materials were prepared from cultures of algae grown from a ^{13}C-enriched source. The chemical shifts are again many times greater than those for protons. It is well established that the chemical shift of a ^{13}C frequency can be derived in terms of the nature of the carbon skeleton, with quantitatively defined values for the α-carbon, β-carbon and so on as far as the ϵ-position, when a proton on the α-carbon is replaced by a methyl group. By this means, and by comparison of the different amino-acids, Horsley *et al.* have made an essentially complete assignment of the entire spectrum. It has already been shown that there are small but measurable sequence-dependent differences in frequency in peptides, and Horsley *et al.* envisage the likelihood that peptide carbonyl resonances of proteins will reflect the local chain conformation. One now awaits ^{13}C resonance studies on enzymes.

A different use of an isotope in NMR has the aim of clearing the thicket of the proton magnetic resonance spectrum of proteins, so as to focus on selected residues only. If an enzyme is prepared from cells supplied with selected deuterated amino-acids, the number of proton resonances in the spectrum can be reduced at will. This method has been applied to staphylococcal nuclease by Putter *et al.* (*Proc. US Nat. Acad. Sci.*, **64**, 1396; 1969). Five derivatives, containing protons only in two, three or four species of amino-acid, were prepared. With the tryptophan residues deuterated, the aromatic region of the spectrum emerges starkly simple from the mush of overlapping bands present in the native enzyme. The tyrosine and histidine frequencies can be identified easily. The aliphatic region is also much simpler, and, for example, four peaks apparently corresponding to the methyl groups of the four methionine residues are resolved. The twenty-three lysines, with their many protons, remain difficult to disentangle, however, and it would require chemical synthesis or hybridization of non-covalently associating fragments from deuterated and undeuterated molecules to make any further impression. To the fertile mind many applications of selective deuteration will suggest themselves.

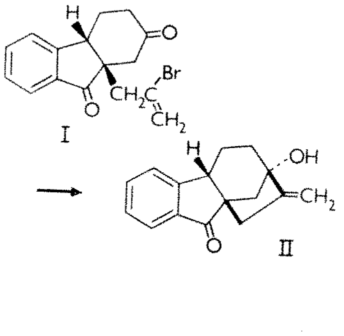
CHEMICAL SYNTHESIS

New Cyclization Reactions

from our Organic Chemistry Correspondent

THE formation of a new carbon–carbon bond by reaction between a Grignard reagent and a carbonyl compound is well known. But although this reaction can be utilized to bring about efficient coupling between two molecules, no effective intramolecular counterpart has been developed.

A practical solution to this problem has now been found which utilizes the organo-copper species di-*n*-butylcopperlithium (Corey *et al.*, *J. Amer. Chem. Soc.*, **92**, 395, 396; 1970). This reagent attacks a vinyl bromide or iodide more readily than it does an isolated ketone function. The possibility of effecting cyclization when both functions are present in the same molecule is exemplified by the conversion, in high yield, of the bromodiketone (I) into the keto-alcohol (II), which has the carbon skeleton of the plant hormone gibberellic acid. Cyclization proceeds selectively to give the desired new five-membered ring; protection of the second carbonyl group is unnecessary.



Similar cyclizations can be carried out with saturated haloketones, but yields are disappointing (about 20–30 per cent). An alternative procedure, also reported by Corey *et al.*, however, overcomes this difficulty. The reagent of choice is now the dianion derived from nickel tetraphenylporphine and two equivalents of lithium naphthalene. With this, in tetrahydrofuran, 7-iodo-2-heptanone is cyclized to 1-methylcyclohexanol in 88 per cent yield. A detailed mechanistic picture of these valuable new transformations, however, is not yet clear.

MÖSSBAUER EFFECT

Liquid Crystals Join the Club

THE latest surprise to emerge from the select group of materials known as liquid crystals is an observation of the Mössbauer effect in a smectic liquid crystal. D. L. Ulrich, J. M. Wilson and W. A. Resch (*Phys. Rev. Lett.*, **24**, 355; 1970) have introduced atoms of ^{57}Fe into a smectic liquid crystal with a low solid to smectic transition temperature and have obtained the familiar shapes of Mössbauer spectra produced through the recoil-free emission of gamma rays from the unstable ion.

This experiment opens up a valuable new way of studying liquid crystals. The peculiarity of these materials is, of course, that the long rod-like molecules have some of the translational and rotational degrees of freedom of a liquid and yet retain a kind of crystalline order in the manner of a solid. What is interesting is that for the Mössbauer effect to occur there must be strong binding between atoms to provide the recoilless emission of low energy gamma rays, and it seems that the layered structure of the smectic liquid crystal is rigid enough for this to happen. In normal liquids the atomic binding is, of course, too weak, but the Mössbauer effect has been demonstrated in glycerine, the high viscosity of which is thought to be due to hydrogen bonding. There is no evidence, however, of hydrogen bonding in liquid crystal systems.

Ulrich *et al.* observed the changes in Mössbauer spectra as a sample was taken through two phase transitions, solid to smectic liquid crystal and smectic to nematic liquid crystal. An abrupt reduction in peak heights was seen at the first transition and at the second the Mössbauer effect vanished completely as the molecules gained an additional degree of translational freedom. The ^{57}Fe atoms had been introduced to the system through a small quantity of solute. The effect of the liquid crystal in aligning the solute molecules was evident from the changes in symmetry of the spectrum when the sample was shaken and then taken through a heating and cooling cycle.

The Mössbauer effect has been steadily finding new applications since its discovery in 1958. During the past six or seven years a mass of information has been accumulated on hyperfine interactions, and more recently several industrial applications have been gaining a hold. The presence of different phases can be recognized by the superimposition of spectra; for example, in supposedly aluminium doped yttrium iron garnet (YIG) the presence of a precipitate of pure YIG can be recognized from the Mössbauer spectrum. The determination of the ferrous to ferric ratio in sintered iron ore mixtures is being used to estimate the optimum conditions for operating blast furnaces, and at the Atomic Energy Research Establishment at Harwell Mössbauer spectroscopy has proved a valuable tool in the production of a series of aluminium garnets.

EXTRA-OPTICAL ASTRONOMY

X-ray and Microwave Progress

from our Cosmology Correspondent

REASONABLY accurate measures of the positions of X-ray sources are in most cases essential before any identification of the object with an optical or radio source can be attempted. It is therefore particularly welcome that W. Mayer, H. V. Bradt and S. Rappaport have determined fairly good positions for twelve X-ray sources, all lying in the intensity range 1·5–8 keV, and all observed on the same rocket flight on July 26, 1968.

By making a rapid sequence of still stellar photographs, the orientation of the rocket was recorded at any time in the flight, and the accuracy of the alignment is confirmed by the detection of the well known source Sco X-1 with a position error of less than 2′·5 (*Astrophys. J. Lett.*, **159**, L115; 1970). For each of these twelve objects, the positions given by Mayer *et al.* are better than those previously known, in some cases considerably so. But none of the sources seem to exhibit the irregular variation seen in some of the more closely studied sources, such as Sco X-1, because the observed intensities on this flight are roughly comparable with those seen several years before.

In the same issue of *Astrophys. J. Lett.* (**159**, L123; 1970) comes news of improved information about the other important class of new objects recently detected as a result of the expansion of astronomy either side of the optical range. P. R. Schwartz and A. M. Barrett have surveyed 134 infrared sources in a search for microwave H_2O emission, and have found this in three new sources, NML Cygni, V Herculis and W Hydrae. Two of these three stars are long period variables, as is becoming almost standard in such cases, but there are considerable differences between their other properties, both at microwave and infrared wavelengths. VY Canis Majoris and R Aquilae, previously known H_2O sources, were also studied. Like V Herculis (a Mira variable) and W Hydrae (a semi-regular variable) R Aquilae varies in intensity, although in detail its spectrum is unlike those of each of the other variables. Perhaps the most important similarity is that in each case the radial velocities of the radio H_2O lines lie between the radial velocities of the H_2O emission and absorption lines at visual wavelengths, although closer to that of the visual absorption line. Although this offers a hint as to the details of the maser action producing these lines, no entirely satisfactory model of these sources is likely to emerge until more intensive studies of the stars have been carried out.

CARBON FIBRES

Fine Structures in Ribbons

from our Materials Science Correspondent

THE results of an intensive programme of research into the detailed structure of high strength carbon fibres, in progress for the past three years in the laboratories of Union Carbide Research Associates in Brussels, have been summarized recently by Fourdeux, Hérinckx, Perret and Ruland (*CR Acad. Sci.*, **269**, 1597; 1969). Most of their work was done with 'Thornel' fibres, made from cellulose by Union Carbide, but studies carried out elsewhere on fibres made by the British process from polyacrylonitrile (PAN) have shown that both types of fibre have very similar structures. Techniques used included X-ray and electron diffraction, small-angle X-ray scattering, electron microscopy and potassium intercalation.

The conclusion is that high strength carbon fibres consist of two dimensional ribbons of trigonally bonded carbon sheets, highly oriented but somewhat twisted, with long, narrow, lens-shaped cavities making up between 5 and 30 per cent of the total volume, and entirely isolated from each other. The carbon ribbons form very shallow parallel stacks between 30 Å and 100 Å thick. This, however, does not imply that even within these slender stacks the individual layers are put together in a graphitic stacking sequence: the structure is parallel but in arbitrary azimuth, properly described as turbostatic. The fact that the fibres are able to form a complete sequence of potassium intercalation compounds in spite of the highly irregular structure is taken by Fourdeux *et al.* as evidence that there are essentially no cross-links between adjacent layers and specifically that no sp^3 carbon atoms are present. Presumably, because parallel stacks do form, they must be held together by weak van der Waals bonds.

The conclusion that cross-links are absent is parallel to the view taken by Halpin and Jenkins (*Proc. Roy. Soc.*, A, **313**, 421; 1969) with respect to vitreous carbon, which is made in the same way as fibres by pyrolysis of polymers. Halpin and Jenkins reached their conclusion on the basis of the ready formation of potassium intercalation compounds. Fibres and vitreous carbon thus differ only in the presence or absence of preferred orientation of the *c* axis, and (possibly) in the presence of cavities in fibres. Nothing seems to be known about the presence or absence of cavities in vitreous carbon. Fourdeux and her colleagues point out that carbon fibres are wholly non-graphitic (as is vitreous carbon) and that the references in the literature to "graphitizing temperatures" should be taken as metaphorical.

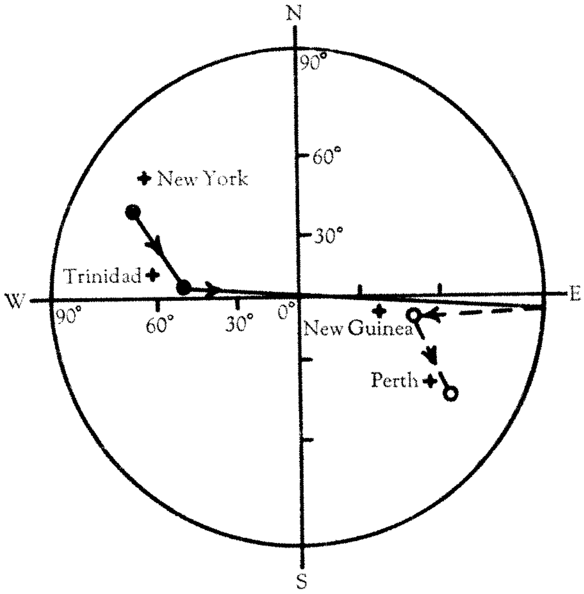
Another approach to the problem, using the time-of-flight neutron diffraction technique, has been taken by Sinclair, Wedgwood, Harris and Egelstaff (UKAEA Report, AERE-R 6052, HMSO, 1969). Using Harwell (PAN) fibres, they, too, found a stack thickness of 100 Å down to less than 50 Å, depending on the mode of manufacture, with alignment as good as 3°–4° (again in agreement with the best Union Carbide results). Attempts to detect cavities by small-angle neutron scattering have so far been vitiated by parasitic scattering from other sources. Sinclair *et al.* regard the technique as sufficiently promising to warrant further experiments.

PALAEOMAGNETISM

Another Transition Zone

from our Geomagnetism Correspondent

One of the classic pieces of evidence for reversals of the Earth's magnetic field is the existence of transition zones—sets of rocks apparently magnetized when the field was actually reversing from one polarity to the other. Unfortunately, the time the field spends in the process of reversing is but a few per cent of the time spent in the fully normal or reversed states, which is why only a handful of well defined transition zones have been recorded.



The migration of the ancient geomagnetic pole from normal to reversed states, as determined from Indian sediments.

A newly discovered transition zone is thus good news for palaeomagnetists in general and proponents of field reversal in particular. Such a zone has now been reported by Pullaiah and Verma (*Phys. Earth Planet. Interiors*, **2**, 158; 1970) in the Tirupati sandstone formation (Lower to Middle Cretaceous) from Godavary Valley, India. To be sure, there are only two samples within the transition zone proper, which means that the new zone is not the best known example. On the other hand, the stability of the magnetizations of the two samples has been studied in great detail, which cannot be said of some of those transition zones discovered when palaeomagnetism was in its infancy. Thermal and a.f. demagnetization thus leave no doubts that the observed magnetizations are primary; and petrographic examination revealed no evidence of mineralogical differences between normal and reversed samples, and thus no hint of self-reversal.

PLASMAS

Instabilities in the Solar Wind

from our Magnetosphere Correspondent

An outstanding problem in dealing with the solar wind concerns the temperatures of the electrons and protons, which are observed to differ considerably (electrons are hotter by a factor of about 2·5), but not as much as they would according to the crude theoretical model. The plasma is cooled by adiabatic expansion as it flows away from the Sun, and thermal conduction which is important is much more effective for electrons than protons. Collisions are barely important and so the standard transport coefficients are of dubious validity, a conclusion which also follows the fact that the thermal velocity of the electrons exceeds the wind speed, while the proton thermal velocity does not. Collisions are insufficiently frequent to make the temperature isotropic, and the anisotropy is aligned with the magnetic field.

It seems that heat must be transferred from the electrons to the protons by means of some unstable waves, since the collisional transfer is negligible, and this is confirmed by the observed ratio of temperatures. Such a transfer reduces the temperature gradient of the protons and increases that of the electrons. Forslund (*J. Geophys. Res.*, **75**, 17; 1970) has shown that heat conduction in the electrons is itself a source of instability for several plasma wave modes. Associated with the heat flow is a skewing of the proton and electron distribution function (integrated over $v_\perp$ to give a reduced distribution over $v_\parallel$). The outgoing electrons have a bigger velocity spread than those ingoing and consequently the peak of the distribution occurs at a lower outward velocity than the mean velocity. If there is no electric current, the mean velocity of the protons is the same as that of the electrons, and then the peaks of the proton and electron distributions are separated and waves may be amplified in essentially the same way as when a current flows in a plasma of uniform temperature.

Forslund estimates growth rates for the various modes at various distances from the Sun. Ion acoustic waves have high growth rates and the magnetic field is weak enough to be neglected here, but the waves are damped if the temperature ratio approaches unity. They probably start at ten solar radii and dominate until the temperature ratio drops to the critical value. The resulting heating of the protons occurs in the tail of the proton distribution.

Where the temperature ratio is not large other modes involving the magnetic field may be important, and their stability depends also on β, the ratio of plasma pressure to magnetic pressure, which increases with distance from the Sun. The first to go unstable may be the less familiar electrostatic ion cyclotron mode, the growth rate of which is also proportional to $\partial f/\partial v_\parallel$ at the parallel phase velocity. These waves may heat the protons a little farther out by gyroresonance. Far out, at about one astronomical unit where β is high, the fast magneto-acoustic mode may be amplified at frequencies near the proton gyrofrequency. The stability condition is complicated by the importance of the magnetic force. More general electromagnetic waves, the stability of which depends on cyclotron harmonic resonances, should also be considered.

A quantitative quasilinear treatment of the energy transfer remains to be carried out and it may well be necessary to use a more detailed model of the distribution, but the way seems clear for the construction of an adequate theoretical model. It is already clear that macroscopically the rate of heat transfer from electrons to protons increases with the magnitude of the electron temperature gradient and this could have interesting dynamic effects.

SOLID STATE

Electroluminescent Devices

from a Correspondent

In twenty years the transistor has almost ousted the simple electronic vacuum tube and a similar usurpation may be facing the cathode ray tube. Although such a development would take at least twenty years, devices for producing the visual effect are becoming commercially interesting, in particular the electroluminescent (EL) lamp which is making an impact as a simple indicating device. A smooth transition is in progress from a first stage in which a reasonably efficient, convenient, reliable and cheap solid state visible light source is being produced, to a second stage where modest display requirements will be fulfilled by combining an array of EL lamps with fully integrated memory and addressing circuitry. It should then be possible to decide whether to continue towards a solid state display which will match and even surpass the cathode ray tube. With such grand plans, it is not surprising that the symposium on electroluminescent solid state devices arranged by the Institute of Physics and the Physical Society and the Institute of Electrical Engineers attracted 250 delegates to London on March 4.

Discussing gallium arsenide phosphide alloys, Dr C. E. E. Stewart (Standard Telephone Laboratories, Harlow) showed that not only II–VI materials have native defects, while Dr G. Lidgard (Plessey, Towcester) showed how the scanning electron microscope can be used for optimizing the combination of good minority carrier injection with efficient luminescence in gallium phosphide.

The wisdom of the almost total commitment of world effort to the light emitting diode rather than the electroluminescent panel is questionable, as Mr A. Vecht (Woolwich Polytechnic) showed when he summarized recent advances by his group working on zinc sulphide d.c. EL panels. Greater light output at 100 V d.c., a green alternative to the usual orange-yellow and no loss in brightness at constant input power during 500 hours of testing, emphasized the seriousness of the challenge. But it is becoming increasingly important to understand these devices sufficiently to decide whether the present poor half-life at constant voltage is curable, or whether it represents a fundamental limitation. Dr R. L. Rouse (GEC, Wembley) showed that such an understanding has not yet been reached when discussing the voltage dependence of ZnS d.c. EL panels.

The gallium arsenide infrared lamp coated with an up-converting phosphor is an interesting recent development. Dr W. A. Shand (Signals Research and Development Establishment, Christchurch) talked about the optimization of these phosphors. But the highlight of the day was a review of possible new tetrahedrally bonded materials for electroluminescence by Dr C. Hilsum (Royal Radar Establishment, Malvern). He thought that gallium nitride showed the greatest promise.

Practical problems of optimization and addressing were considered by Dr C. Gooch (Services Electronics Research Laboratory, Baldock), who felt that the immediate future depended on the commercial success with gallium phosphide and gallium arsenide phosphide lamps. The problem of integration of EL devices with silicon circuitry has been approached rather unusually by Dr J. Morant (University of Durham). He has found a phosphor (willemite) which is epitaxially compatible with silicon and is now hoping to make it efficiently electroluminescent.

POLLUTION

Teach-in at Edinburgh

from a Correspondent

On March 6, Edinburgh University was the venue for a teach-in at which scientists, economists, moral philosophers, politicians and representatives of industry discussed pollution with students and the public. This probably indicates a significant change in attitudes towards pollution since ten years ago, say, when reports of the effects of certain types of pollution were just reaching the public. The mood of the meeting was therefore one of "what are we going to do about it, when and how ?" It was good to see polluters and polluted facing up to one another on the same panel, so that the audience, consisting of some eight hundred people, was able to hear both sides of the problem.

Professor Alan Williams (University of York) pointed out that economics concerns things people value, and that a general achievement of a sense of values is difficult. For example, the cost of saving a few lives which might otherwise be lost due to a polluted water supply could be diverted to building a hospital to save many lives. It is dangerous, too, to take decisions on existing costs; in the end, what value will posterity place on action taken now ? Certainly, Canon Hugh Montefiore (University Church, Cambridge) felt that it was our moral duty to leave the Earth as we found it.

Some members of parliament were a little naive in their approach and alarmingly unaware of the inefficiency of some of the existing pollution legislation. Mr David Steele (MP for Roxburgh, Selkirk and Peebles), however, pointed out that it had taken general disasters to bring in certain legislation on pollution, and we must not wait for another disaster before doing anything further. Dr John Dunwoody (Parliamentary Under-Secretary, Ministry of Health and Social Security) agreed with this point, and felt that it was no use waiting for agreements on international control before action was taken. We must impose national controls, he said. The present position concerning the restriction of DDT indicates that this idea is shared by other nations.

One thing is certain, as Mr Roland Glover (Scottish Marine Biological Association), representing the Natural Environment Research Council, stressed. "It is no longer sensible to try to study ecology without considering man and society as significant components of the natural system. It will be necessary to improve and develop the environmental sciences as a whole so that our understanding of nature will be sufficient for us to diagnose and predict all the problems, of which pollution is but one, that are created by the changing pattern of society. The detection and measurement of the effects of pollution in the field will always be difficult, and often impossible, without a thorough understanding of the baselines of natural variation."

23 suggestions for gifts!

GIFTS FOR ACTIVE MINDS—

both indoors and outdoors at prices from 10/6 to £35. As only three are subject to a few shillings purchase tax they offer good value for money.

Write for a copy of this interesting brochure to-day.

W. A. QUINN LTD.

12 FULWOOD PLACE, HIGH HOLBORN, LONDON, W.C.1.
Telephone: 01-405 5268

"I can say...
'laboratory reagents for fluorescent antibody techniques, complement fixation tests, identification of bacteria and viruses, haematology, pregnancy diagnosis, buffering, radio-immunoassay, quality control sera, microtitrations and veterinary diagnosis'
in 12·5 seconds!
Beat that if you can!"

"Wellcome"

Quick, too, is the delivery of all these laboratory diagnostics from Wellcome Reagents Limited. They are subjected to rigorous quality control, their performance is consistent and they are backed by Wellcome Research Laboratories' own original immunological work.

Wellcome Reagents Limited
Wellcome Research Laboratories Beckenham Kent BR3 3BS

wright ACRYLIC chromatography columns

versatile inexpensive laboratory columns for chromatographic and gel filtration procedures employing aqueous solvents

Simple to use—unique patented design in acrylic and nylon with sintered plastics bed support.
Minimal dead space with low-volume connections. Diameters selected to produce cross-sectional area of 2, 4, 8, & 16 sq. cms.
Fixed or adjustable columns with or without jackets.
Standard lengths of 30, 60 and 90 cms.
Columns easily lengthened by joining.
Prompt delivery of standard columns from stock.
Preparative columns with bed volumes up to 50 litres are available to special order

Prices from **£5**

Phone or write for detailed catalogue to Dept. CC1/N

wright SCIENTIFIC LIMITED 3 Lower Road, Kenley, Surrey, CR2 5NH *Tel:* 01-668 4611

LOCARTE FLUORIMETER

Excitation wavelengths commencing at 240mμ

Now incorporating these novel features:

- **Output socket for chart recorder.**
- **Flow-through cuvettes.**
- **Automated fluorimetry with special converter unit for direct connection to most automatic analysing equipment.**
- **Stability ensured with fully regulated special Mercury Arc and Zinc lamp. Zinc lamp specially developed for plasma cortisol estimations.**

In laboratories throughout the world the Locarte Fluorimeter is saving time while providing unparalleled results. Important work demands precision. An extensive range of filters is available.

With an instrument so sensitive that monochromatic filter can be used on both primary and secondary sides many laboratories have extended their sphere of work. With an instrument so fast in operation your costs are reduced.

RAPID measurement of fluorescence (solution or filter paper).

SENSITIVITY: Minimum cuvette 'blank' allows full use of 13 stage photomultiplier and allows estimations well below 0·001 μg./ml. Quinine Sulphate; 0·001 μg./ml. Serotonin (measuring the fluorescence in the Ultra Violet at 335 mμ) and 0·1 μg. of Cortisol on filter paper.

REPRODUCIBILITY SPEED AND ACCURACY in all your fluorimetric estimations.

Locarte Precision equipment includes: Fraction Collectors, H.V. Electrophoresis, U.V. Handlamps, Recording Densitometers, Desalters, Column Scanners, Pipette Washers, Amino Acid Analysers.

For further information please quote NA/F

THE LOCARTE COMPANY

24 Emperors Gate, London SW7. FREmantle 1712

Value of Curiosity-oriented Research

by

M. GIBBONS
J. R. GREER
F. R. JEVONS
J. LANGRISH
D. S. WATKINS

Department of Liberal Studies in Science,
University of Manchester

Is the most profit to be had from research inspired by curiosity or by foresight of practical applications? A recent suggestion that the answer could be found by evaluating curiosity-oriented projects has proved hard to follow up.

A WAY to approach the problem of quantifying the economic benefits from curiosity-oriented research has recently been suggested by Byatt and Cohen[1]. It consists essentially of identifying key discoveries which have had profitable applications and then estimating the economic effects of notional marginal delays in the timing of these discoveries; that is, of attempting to assess how much less wealth, suitably discounted to a common year, would have arisen if, because of a smaller scale of effort in particular areas of research, certain discoveries had been made later than they actually were.

We report in this article some studies which formed part of a project* to assess the feasibility of this approach by trying to apply it to some recent examples of technological innovation. The examples were chosen after due consultation with the Working Group on the Economic Benefits of Scientific Research which has been established by the Council for Scientific Policy. The principal areas selected were the Chorleywood bread process, the float glass process and cryogenics. Some other areas of innovation were also considered more briefly.

The term "curiosity-oriented" used by Byatt and Cohen is clearer than "pure" or "basic", indicating research justified by curiosity about topics for which no application is apparent. According to Byatt and Cohen (benefit (*f*) of their article, paragraph 2), ideas discovered during curiosity-oriented research, which "tend to arise unpredictably", are sometimes applied later in industry. This is contrasted with "mission-oriented" research, defined by Byatt and Cohen (benefit (*c*)) as "research in fields whose application is evident".

Thus work cannot properly be described as curiosity-oriented if it can be shown that some application was envisaged; though naturally this does not imply that scientists necessarily lose interest just because there is a practical goal in view. The term "basic" is commonly used in a wider sense to include fundamental investigations of topics with a quite obvious application.

These distinctions are not just semantic subtleties. They are relevant to the possible policy problem of the extent to which basic research should be funded regardless of whether social or economic benefits seem relatively likely. The alternative is to try to identify topics which have obvious social and economic value and selectively support work in them, even though this work may be quite basic.

Chorleywood Bread Process

The principal feature of this process[2] is the replacement of a lengthy period of dough "development" involving fermentation by a combination of a controlled amount of intense mechanical work and the use of certain chemicals known as "improvers".

The effect of mechanical work was described by mission-oriented researchers[3] in the United States in 1926, thirty-five years before the Chorleywood process was announced by the British Baking Industries Research Association (BBIRA). No commercial application resulted until J. C. Baker in the United States was able to combine the effects of mechanical work with the use of improvers. Baker carried out careful systematic studies of a mission-oriented nature and no large input of curiosity-oriented research was involved[4].

Baker's "Do-Maker" process was not suitable for the needs of the British bakers or housewives, but the Chorleywood process is now used by more than two-thirds of the British baking industry. The Chorleywood process uses ascorbic acid (vitamin C) as a fast acting improver in addition to conventional improvers which were discovered in the 1920s by accident and by empirical research. The commercial availability of ascorbic acid depends on research in organic chemistry carried out in the 1930s but, had ascorbic acid not been available, other fast acting improvers could have been used[5].

The following quotations from Byatt and Cohen[1] indicate the main points of their argument.

2.14. "The economic value of the research derives from the total economic surplus—i.e., the total economic benefit derived from the final product or process, less the costs of applied research, development, investment and manufacturing. This surplus is a wider concept than the profit of an individual firm. It includes the profits of firms, and indeed the money, costs and receipts of manufacturing firms may, in a number of cases, be good measures of the economic benefit to society as a whole."

2.26. "The slowing down of the exploitation of new products and processes would usually result in an economic loss and an acceleration of the exploitation will usually result in economic gain. The extent of the change in the net economic benefit to society, when appropriately discounted, can be compared with that change in expenditure on research which results in this change. Only if the increase in net economic benefit from all the industries associated with the scientific discovery, when discounted, is equal to or exceeds the increased expenditures on research, will the extra expenditure have been worthwhile".

---

* The studies reported here were part of a larger project carried out in collaboration with the R and D Research Unit of the Manchester Business School where, under the direction of A. W. Pearson, work was carried out on the estimation of the economic benefits from the Chorleywood and float glass processes. J. R. G. worked at the unit. Work on the economic benefits from cryogenics was carried out by J. S. Metcalfe, of the Department of Economics, University of Manchester.

The technical knowledge forming the background to the research which produced the Chorleywood process was available in the 1930s, but could not have been used in Britain before the 1950s when chemical additives in flour and bread were allowed after studies by BBIRA.

While the allocation of greater resources to BBIRA might conceivably have brought about the development of the Chorleywood process slightly earlier, there is nothing to suggest that greater curiosity-oriented research effort would have had any accelerating effect.

Float Glass and Cryogenics

The float glass[6] process seems to be a typical "technological discovery" in that it cannot be described in terms of the application of a specific discovery made by academic or other curiosity-oriented scientists. The concept of using molten tin for the manufacture of flat glass was patented in America in 1902[7]; Pilkington had to spend seven years and £4 million on development work, however, before announcing the new process in 1959.

After the process had been developed empirically to the stage of producing satisfactory glass, scientists became involved to increase the understanding of the process and to solve technological problems; for example, the cause of a surface bloom was traced to a layer of tin dissolved in the glass. Such work made use of certain fundamental concepts and analytical techniques which may have derived from curiosity-oriented research; but it does not seem possible to apply the concept of marginal delay to these connexions between science and technology, which occurred after the initial development of the process.

We chose cryogenics to complement bread and glass manufacture, because it seemed *a priori* more likely to show dependence on curiosity-oriented research. We concentrated on the cryogenic gas-separation industry because it is too early to assess the economic impact of work at temperatures below about 20 K.

The history of low temperature research yielded one case that seemed at first to lend itself to Byatt–Cohen analysis. The Joule–Thomson effect[8], discovered in 1852, was in 1895 made the basis of a gas liquefaction process by Linde[9] in Germany and independently by Hampson in England[10–12]. It is at least plausible to argue, however, that a delay in the discovery might have accelerated rather than delayed its application. The Joule–Thomson effect was assimilated into science as a small scale effect and it required considerable insight on the part of Hampson and Linde to see it, as it were, "out of context" as part of a powerful industrial process. Had it been discovered after the failure of the Pictet[13] and Cailletet[14] approaches to liquefy oxygen on any but the very small scale, its potentialities as the basis of an industrial process might have been realized somewhat earlier.

Much of the explanation for the timing of the development of the industrial process seems to lie in market rather than technical factors: the realization of a large potential market for oxygen, to meet which only a relatively costly chemical process was available (ref. 15 and personal communications from personnel of the British Oxygen Co, formerly Brins Oxygen Co).

Other Innovations

Of several other major innovations considered more briefly in the course of this project, it seems that only nuclear power and silicones can legitimately be considered to be based on specific discoveries of curiosity-oriented research. In those two cases, the important influence of war-time development work makes it difficult to apply the concept of notional marginal delay; the timing of the innovations may have been determined more by the Second World War than by the timing of the curiosity-oriented discoveries. The massive effort put into the development of an atomic bomb is well known. Silicones could be said to depend on specific curiosity-oriented discoveries made by Kipping in 1904 and 1908. Silicones, however, were first produced commercially in 1943 by Dow–Corning to satisfy military needs and post-war production was based not on one of Kipping's reactions but on a synthesis developed by Rochow working for General Electric[16].

In several other cases, however, curiosity-oriented research may have been involved in the sense that knowledge and techniques were passed on through the educational process and made available for the solution of industrial problems via the supply of scientifically trained manpower. An example of such a problem is given in connexion with float glass.

In a further search for innovations which could be directly linked to curiosity-oriented research, issues of the *New Scientist* for the period January to June 1968 were surveyed, concentrating particularly on the Science in Industry feature. Of a sample of seventy-four British innovations thus obtained, fifteen (20 per cent) were considered possibly susceptible to Byatt–Cohen analysis, but in fourteen of these the chief reason for categorizing them thus was lack of precise knowledge.

Byatt–Cohen Hard to Find

From the studies we have summarized, we conclude that Byatt–Cohen type innovations are quite difficult to find, let alone investigate. Interactions between science and technology are usually too complex for the method of notional marginal delay. Only rarely is it possible to pinpoint specific curiosity-oriented discoveries from which wealth-producing applications are derived. Even when this can be done, other factors affecting the timing of innovations, such as market factors or war-time pressures, obscure the effects of possible delays in discoveries.

It should be emphasized that we do not conclude that curiosity-oriented research is useless in economic terms. On the contrary, in view of the importance of the problem, we feel that further work should be directed to exploring various other avenues through which curiosity-oriented research may lead to economic benefits.

We thank Drs Byatt and Cohen for making available pre-publication copies of their article, and them and other members of the secretariat of the Council for Scientific Policy for helpful discussions. J. R. G., D. S. W. and J. L. were supported by the DES.

[1] Byatt, I. C. R., and Cohen, A. V., *An Attempt to Quantify the Economic Benefits of Scientific Research*, Science Policy Studies No. 4 (HMSO, London, 1969).

[2] Elton, G. A. H., *Proc. Roy. Austral. Chem. Inst.*, **32**, 25 (1965).

[3] Swanson, C. O., and Working, E. B., *Cereal Chem.*, **3**, 65 (1926).

[4] Baker, J. C., *Bakers Weekly*, **161**, No. 11, 60 (1954).

[5] Jorgensen, H., *US Patent* 2,149,682 (1939).

[6] Pilkington, L. A. B., *The Glass Industry*, **44**, No. 2, 80 (1963).

[7] Heal, W. E., *US Patent* 710,357 (1902).

[8] Joule, J. P., and Thomson, W., *Phil. Mag.*, series 4, **4**, 481 (1852).

[9] Linde, R., *German Patent* 88,824 (1895).

[10] Hampson, W., *J. Soc. Chem. Ind.*, 411 (1898).

[11] Hampson, W., *Industrial Gases* (*Quarterly*), September 1921, and others.

[12] Hampson, W., *UK Patent* 10,165 (1895).

[13] Pictet, R., *CR Acad. Sci.*, **85**, 1214, 1220 (1877).

[14] Cailletet, L., *Comp. Rend.*, **85**, 815 (1877).

[15] Monopolies and Restrictive Practices Commission, *Report on Supply of Certain Industrial and Medical Gases* (HMSO, London, 1965).

[16] Rochow, E. G., *Chemistry of the Silicones*, second ed. (Wiley, New York, 1950).

Imperfections of Elasticity and Continental Drift

by
HAROLD JEFFREYS
St John's College,
Cambridge

Imperfection of elasticity in the Earth follows a law that forbids convection and continental drift.

CONTINENTAL drift is the subject of so much discussion that I think some remarks about the history of the hypothesis are worth making. Wegener attempted an explanation involving a small force acting on a floating continent and tending to push it towards the equator. As a meteorologist he thought that the Earth's rotation would convert this into a westerly drift. It did not occur to him that the viscosity of the Earth was so high that its effect would overwhelm that of rotation, and the effect, if any, would be to spread the continents symmetrically about the equator. He also assumed that the viscosity extended right up to the ocean floor; but if this were so the ocean floor would be flat. In fact, the inequalities are of the same order as those of the land surface.

Wegener claimed that vertical movement was impossible, supposing a form of isostasy, according to which excess height is compensated exactly by extra thickness of light matter, leaving the continents floating like icebergs. Even in his time it was known that gravity departed from the values calculated on this hypothesis by about ten times the errors of observation; much larger departures have been found since. Furthermore, geologists were familiar with deep sediments. They supposed that the sea floor could be depressed as the sediments accumulated, so as to leave little disturbance of gravity. Two geologists, Morley Davies and E. M. Anderson, however, showed that the depression produced in this way would not be at all adequate. At most about 2 km of sediments could be deposited in water originally 1 km deep. To get 6 km of sediments we should need 3 km of water, possibly a great deal more, on the continental shelf. Such thicknesses are known.

Little is heard now about Wegener's mechanism. But Holmes, followed by Meinesz, used convection currents. These had the advantage that not much was known about their theory. But in the past few years a great deal of relevant information has appeared. The fundamental matter is the behaviour of solids under continued shear stress. If this is great enough they break at near 90° or 45° to the greatest principal stress, sometimes with and sometimes without deformation nearby. This happens in rocks when lengths are changed by something like 1 part in 1,000 and is familiar enough to geologists. But there is evidence of some sort of imperfection of elasticity at extensions of the order of 10^{-6} or less. Early workers, on various materials, found continuous creep at low stresses, behaving like $\log t$ or $t^{1/3}$. These met a difficulty, for the rate of increase of strain would be infinite at zero time. But C. Lomnitz noted two things. One was that at extensions of the order of 10^{-4}–10^{-5} the stress–strain relation was linear; if so, extrapolation to smaller stresses would probably be justified. The other was that if a constant shear stress P is applied at $t=0$, and the shear is

$$\varepsilon = \frac{P}{\mu}\left[1+q\log\left(1+at\right)\right]$$

where μ is the rigidity and q and a are constants, the creep when at is large approximates to $q \log (at)$ times the initial yield. But at small at, $d\varepsilon/dt$ has a finite limit. This is one of the obvious things that take a very long time for somebody to notice. For time 1 s, estimates of at from 10^3 to 10^5 exist, but in any case 1 can be neglected in comparison with at for any motions of geophysical interest.

The power law can be adapted similarly

$$\varepsilon = \frac{P}{\mu}\left[1+\frac{q}{\alpha}\left\{\left(1+at\right)^{\alpha}-1\right\}\right]$$

If $\alpha=1$ the creep becomes $(P/\mu)qat$, so that it varies linearly with time as for ordinary viscosity (elasticoviscosity). For a forced vibration of period T the lag is as T^{α}, tending to a constant as $\alpha \rightarrow 0$. The amplitude of a wave travelling a wavelength contains an exponent as $(\text{period})^{\alpha}$; of one travelling a given distance as $(\text{period})^{-1+\alpha}$, with a factor depending on distance alone. In addition there is a small increase in the time of travel except for $\alpha=1$.

Damping of Waves

There has been a great deal of recent work on the damping of surface waves and free vibrations. Nearly all of it assumes the logarithmic form, giving the damping in a period independent of period. In addition the free nutation of the Earth's axis (otherwise the 14-monthly variation of latitude) shows damping with a time of relaxation of the order of 30 years. Stuart Crampin and I have shown that this, with $\alpha=0$, would ruin seismology[1]. If a sharp transverse wave is generated, its beginning at distance 80° would be spread out over about 70 s, and it would be unreadable. On the approximately correct assumption that it reaches half its ultimate amplitude in 2 s, α has to be about 0·2. The data used on the logarithmic law cover periods from about 10 to 2,000 s, and $(200)^{0 \cdot 2}$ is about 3, so that failure to detect $\alpha>0$ from seismological data alone is not hard to understand. The range of period covered, when the free nutation is taken into account, is by a factor of 10^7.

Convection

The equations for the initiation of convection in a fluid are well known. Viscosity has a controlling effect. When the viscosity term is replaced by any law with $\alpha<1$ it is found that the equations cannot be made to balance. The essential point is that to maintain steady upward and downward currents there must be steady horizontal flow between them, which must be maintained by differences of pressure. But if the differences of pressure are constant the rates of flow according to any law with $\alpha<1$ will die down, and there is a contradiction. Convection demands the elasticoviscous law, which is contradicted by all the available evidence. Similarly, to maintain continental drift, in any way, at a constant rate would require a steadily increasing force.

It has been suggested that the law might break down and approximate to elasticoviscosity when still longer intervals of time are considered. The best way to test a law is to apply it as far as possible beyond the original data and see what happens. One test is on the gravity anomalies disclosed by the work on artificial satellites.

With any law of imperfection of elasticity at small stresses these should be subsiding, and the rate of subsidence would be much more rapid for the widest-spread anomalies. If an anomaly of 100 km in horizontal extent has had time to be reduced to half its original amount, an anomaly of 3,000 km would be reduced to 10^{-9} of its original amount. But the greatest discoveries resulting from work with artificial satellites are the uncompensated second and third harmonics in the gravitational field. This difficulty disappears with the modified Lomnitz law. In 10^8 years a harmonic of degree 2 would be about halved, and one of wavelength 10^8 km perhaps reduced by 10 per cent. The law does not explain the approximate compensation of mountain systems, but the stresses are of the order needed for fracture anyhow.

Using the Moon for Further Checks

The principal evidence for any imperfection of elasticity at very small stresses is from the fact that the Moon keeps a constant face to the Earth. With the same values of q and α as for the Earth, Crampin and I find that this could easily be achieved in 10^7 years. In addition the Moon is dynamically triaxial and the chief effect of this is the inclination of its axis to the normal to the ecliptic. With the same values the difference of the moments of inertia would have decreased by about 10 per cent in 3×10^9 years. But if we assume elasticoviscosity to fit the damping of the free nutation of the Earth the difference would have been halved in the past 200 years, the interval of reasonably accurate observation.

Because of triaxiality the Moon theoretically has three free oscillations with periods of about 1, 40 and 800 months in the directions of its axes. These have not been detected. With the same constants they would have times of relaxation of the order of 10^6 years, so there is no difficulty in understanding why they have not been found.

The criterion that the law should be tested by application far beyond the original data is therefore satisfied. It is worth pointing out, however, that the data concern a free oscillation of the whole of the Earth's shell and a wave that has gone nearly to its base, so that the results must be interpreted as average properties for the shell. There is a good deal of evidence from surface waves that qa^a in the top 100 km or so is of the order of ten times this value. The data are enough to show that α is nowhere near 1. This would imply that so far as this type of imperfection of elasticity is concerned the elasticity becomes more nearly perfect with depth, contrary to what has usually been expected.

---

[1] Jeffreys, H., and Crampin, S., *Mon. Not. Roy. Astron. Soc.* (in the press).

Some Needs of Geophysics

by Harold Jeffreys

MUCH further work is needed before we can understand several important facts, some of which have been known for fifty years or more.

First, we need a general survey of gravity, such that every point of the surface is within 5° of a place where gravity has been measured. Artificial satellites have given good determinations of the gravitational potential at least to all harmonics of degree 4, and far better than gravity surveys alone can give. But gravity can still give valuable information for the contributions from the higher harmonics to the distribution of potential over limited regions, notably for the correction of estimates of the mean radius of the Earth for deflexion of the vertical. The function that arises in Stokes's theorem keeps the same sign over long intervals of distance, so that unsurveyed regions are liable to make considerable contributions over regions where gravity is well observed. But if Stokes's function is modified by omission of harmonics up to degree 4 it keeps the same sign over much shorter distances and the contributions from the higher harmonics would be much better determined.

Second, on any theory of mountain formation it is hard to understand why so many of the major mountain chains are in western China or near its borders. A factor of 2 in the apparent crumpling on different great circles would be easy to understand, but the ratio is more like 5 or possibly 10. It might be due to specially great thickness of the lighter upper layers there; this could be tested by a gravity survey.

Third, since the present standard times of travel of seismic waves were produced, the number of observations of P (longitudinal waves) has greatly increased, chiefly through the use of new stations with instruments of high magnification and short period (1 s or so). This has been mainly for the rapid and accurate determination of epicentres. But the accuracy for S (transverse waves) has diminished. The reason is that the prevailing periods in S are of 4 s to 7 s and the new instruments are insensitive to them. In many respects S is more interesting than P, and the new stations should be equipped with instruments capable of recording these periods. This applies specially to the phase SKS, which provides the most direct evidence on velocities at small depths in the core.

Provisionally I think that the cost of this work would be between 1 and 10 per cent of that of sending up one artificial satellite and 0·01 per cent of that of a landing on the Moon.

Fourth, with any law of imperfection of elasticity, if vertical movements are due to differences of surface load, they should be fairly directly related to gravity anomalies. So far as I know no comparison has ever been made except for Fenno-Scandia, and there is no evidence that the conclusions drawn from it are general; there is a good deal of evidence against it. Geodesists have a habit of speaking of any vertical movement as isostatic, which begs the question of spontaneous internal change. We need a detailed comparison.

Fifth, we know a great deal about elasticity when the stresses are well below the strength, and it is not difficult to adapt the results to any linear law of imperfect elasticity. But near the elastic limit the stress–strain relations are far from linear, even before fracture. The great distortions found in geology are understood only to the extent that something like them can be obtained in the laboratory and can be seen in the convolutions of boulder clay, where we have more direct knowledge of the forces acting. A quantitative theory of plastic instability is needed. A simple case is that of a plastic rod under tension ('Plasticine' will do). If it becomes slightly thinner in one place, the tensile force per unit area is increased there, and flow becomes faster, until the specimen is thin enough there to break.

Positive Control of Transcription by a Bacteriophage Sigma Factor

by
ANDREW A. TRAVERS
The Biological Laboratories,
Harvard University

A sigma specificity factor induced by infection with bacteriophage T4 directs the *in vitro* synthesis of RNA species corresponding to those switched on *in vivo* about 2 min after infection.

DURING the course of bacteriophage T4 development, different classes of viral RNA are synthesized in a defined temporal sequence[1-5]. The earliest studies[1-3] distinguished two principal classes of T4-specific RNA: early and late, transcribed predominantly before and during DNA replication, respectively. More recent work[4,5] has shown, however, that early RNA can itself be separated into at least two distinct classes. The first of these, class I or pre-early, appears immediately after phage infection while the second, class II or early, is switched on about 2 min after infection at 30° C. The synthesis of all classes of phage RNA, except pre-early RNA, is dependent on phage specific protein synthesis[4,5], indicating that the switching on of RNA species characteristic of later stages of the infection cycle requires at least one component of the transcription machinery to be phage-coded.

Recently a molecular mechanism for such positive control of transcription was suggested by the elucidation of the role of the RNA polymerase σ factor[6,7]. This factor directs the accurate initiation of selective transcription by the core RNA polymerase, the enzymatic machinery for synthesizing RNA (refs. 8, 9). For example, with T4 DNA as template, σ factor directs the initiation of pre-early RNA species *in vitro*[8,10,11]. Because the host core polymerase is conserved, at least in part, throughout the infection cycle (ref. 12 and personal communication from C. G. Goff), it seemed probable that phage coded factors analogous to σ might direct the synthesis of the later species of RNA. Such a factor was recently identified in T4 infected cells[13]. This factor, termed σ^{T4}, stimulates transcription only on T-even phage DNA and even depresses the non-specific synthesis by the core polymerase on other DNA templates. An additional alteration of the host transcription machinery after infection with phage T4 is the modification of the α subunit of the core polymerase[14-16]. We show here that σ^{T4} directs the synthesis of early RNA and further that the specificity of initiation is determined primarily by the type of factor and is not affected by the modification of the core polymerase.

Isolation of σ^{T4}

Because the original procedure described for the isolation of a T4-coded sigma factor is unsuitable for processing large quantities of cells, we have developed an alternative method. The cells are grown, infected and harvested as previously described and are then lysed by grinding briefly with alumina instead of by a lysozyme–freeze/thaw treatment. After removal of alumina and cell debris by centrifugation, the crude cell extract is fractionated into a supernatant fraction and a ribosomal pellet. This pellet is then washed with a buffer of high ionic strength to elute absorbed protein. With both methods of lysis the σ^{T4} activity is normally found preferentially in the ribosomal wash, although in about 30 per cent of all preparations the factor was uniformly distributed between the ribosomal pellet and the supernatant fraction. The σ^{T4} factor can be separated from the core polymerase by fractionation of the ribosomal wash by precipitation with ammonium sulphate (Table 1). Most of the RNA polymerase activity is recovered in the fraction precipitating between 25 and 40 per cent saturation with ammonium sulphate. This polymerase preparation transcribes calf thymus DNA well and exhibits a low but significant activity on T4 DNA. It thus probably contains mainly core polymerase with a small amount of σ^{T4}. Addition of the fraction precipitating between 40 and 55 per cent saturation to the polymerase fraction greatly enhances the activity on T4 DNA, however, while RNA synthesis on calf thymus DNA is depressed. Because the 40–55 per cent cut has negligible RNA polymerase activity it must contain σ^{T4}, and was used as a source of this factor in these experiments.

Table 1. TEMPLATE SPECIFICITY OF TRANSCRIPTION DURING PURIFICATION OF σ^{T4}

Fraction DNA template:	^{14}C-AMP incorporated (pmoles/0·1 ml.) T4 (37 μg/ml.)	Calf thymus (37 μg/ml.)	Ratio of activity, T4 DNA/calf thymus DNA
Ribosomal wash (42 μg)	132	36	3·7
Ammonium sulphate precipitates:			
25–40 per cent (16 μg)	64	352	0·18
40–55 per cent (13 μg)	23	15	—
25–40 per cent cut (16 μg) + 40–55 per cent cut (13 μg)	248	69	3·6

To separate core polymerase and σ^{T4} by fractional precipitation with ammonium sulphate, the ribosomal wash from T4 infected cells was prepared as previously described[13] except that the cells were lysed by grinding them for 2 min with twice the cell wet weight of alumina. The paste of alumina and lysed cells was resuspended in 1 volume of standard buffer (0·01 M Tris-HCl, *p*H 7·9, at 25° C, 0·005 M $MgCl_2$, 0·005 M 2-mercaptoethanol, 0·0001 M EDTA, 0·05 M KCl) per wet weight of cells. 10 ml. of ribosomal wash was brought to 25 per cent saturation with ammonium sulphate by the addition of a saturated solution, *p*H 8, and allowed to stand 15 min at 4° C. The solution was centrifuged and the supernatant was then brought to 40 per cent saturation with ammonium sulphate, allowed to equilibrate for 15 min, and finally centrifuged. The pellet was redissolved in 1·5 ml. standard buffer while the second supernatant was brought to 55 per cent saturation with ammonium sulphate. Once again the precipitate was collected by centrifugation and redissolved in 1·5 ml. standard buffer. The redissolved pellets were assayed for RNA polymerase activity on calf thymus and T4 DNA as previously described[13]. The background incorporation in the absence of added DNA has been subtracted from all data; in no case did this background exceed 9 per cent of the maximum observed incorporation. The amounts of protein added to the assay mixture are indicated in parentheses.

Asymmetric Transcription by Reconstituted System

Previously we have shown that the crude mixture of T4 factor and RNA polymerase in the ribosomal wash transcribes mature T4 DNA in a highly asymmetric manner, only the l-strand of the T4 DNA being copied[13]. Bautz *et al.*[8] have demonstrated, however, that the core polymerase, whether modified or not, transcribes T4 DNA as though RNA chains were initiated almost at random along the entire DNA molecule. By measuring the asymmetry of RNA synthesis in such reconstituted systems, we have asked whether the addition of σ^{T4} to core polymerase restores the specificity of transcription of the T4 genome. The results (Table 2) show that when σ^{T4} directs RNA synthesis either by host core polymerase or by T4-modified core polymerase more than 90 per cent of the RNA product hybridizes to the l-strand of T4 DNA. Similarly, RNA synthesized by either host or modified core polymerase under the direction of the host σ factor also hybridizes almost exclusively to the l-strand.

σ^{T4} directs the Synthesis of Early RNA

During normal phage development, RNA complementary to the l-strand of T4 DNA is synthesized at all times

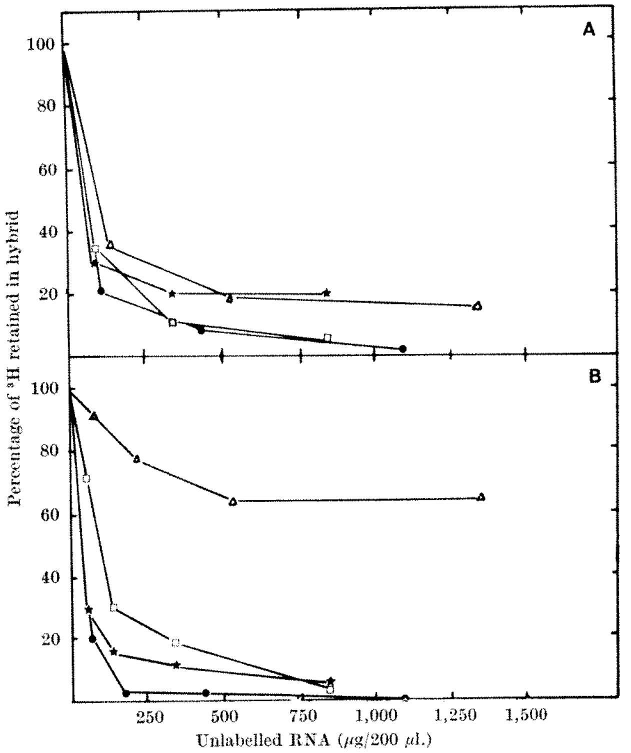


Fig. 1. RNA–DNA hybridization competition experiments comparing σ^{T4} directed T4 RNA and *E. coli* σ directed RNA with *in vivo* T4 RNA. The *in vivo* RNA was extracted from T4 infected cells at 3 min, 8 min and 16 min after infection by the method of Bolle *et al.*[25]. T4 CM RNA was extracted 3 min after infection from cells to which chloramphenicol (100 µg) had been added 1 min before infection. T4 ^{3}H-RNA was prepared as described in the legend to Table 2. Hybridization of *in vitro* T4 ^{3}H-RNA to 10 µg/ml. T4 l-strand in the presence of increasing amounts of RNA was performed as described in the legend to Table 2. *A*, Competition of RNA synthesized *in vitro* by complex of *E. coli* σ and *E. coli* core polymerase. Input, 2,680 c.p.m.; hybridization efficiency, 24 per cent. *B*, Competition of RNA synthesized *in vitro* by σ^{T4} and T4 core polymerase. Input, 2,180 c.p.m.; hybridization efficiency, 31 per cent. Competitor RNA species: CM RNA (△); 3 min RNA (□); 8 min RNA (●); 16 min RNA (★).

after infection whereas transcription from the r-strand does not become significant until 10 min after infection[17]. To determine whether σ^{T4} directed the synthesis *in vitro* of RNA species containing nucleotide sequences homologous to those in *in vivo* T4 RNA species, we competed labelled σ^{T4} directed RNA and also host σ directed RNA with unlabelled *in vivo* T4 RNA isolated at 3 min, 8 min and 16 min after infection. In addition, competition was carried out with T4 RNA isolated from cells treated with chloramphenicol (CM) before infection. CM RNA is identical to pre-early RNA, and presumably only the host transcription machinery is required for its synthesis *in vivo*. The results (Fig. 1) show that whereas CM RNA is a relatively poor competitor of σ^{T4} directed RNA, only competing for about 40 per cent of the radioactive label, the RNAs at 3 min, 8 min and 16 min are good competitors, virtually 100 per cent competition being observed. Because the efficiency of hybridization in conditions of DNA excess is high (about 80–90 per cent) this complete competition demonstrates that all the nucleotide sequences in σ^{T4} directed RNA are homologous to *in vivo* messages. The corollary of this conclusion is that little or no antimessenger is synthesized in the presence of σ^{T4}. By marked contrast, when core polymerase transcribes T4 DNA in the absence of any σ factor, about 30 per cent of the resulting RNA is self complementary[8]. Unlike σ^{T4} directed RNA, host σ directed RNA was well competed by CM RNA. Similarly 3 min and 8 min RNA were good competitors but 16 min RNA had slightly diminished competing capacity. Maximum competition was achieved by 3 min RNA where more than 97 per cent of the labelled RNA could be diluted out.

Table 2. ASYMMETRY OF *in vitro* TRANSCRIPTION BY RECONSTITUTED CORE POLYMERASE–FACTOR COMPLEXES

Core polymerase	σ factor	Input c.p.m.	Hybridization r-strand (c.p.m.)	Hybridization l-strand (c.p.m.)	Hybridization (ratio r : l)
E. coli	*E. coli*	1,340	45	1,019	4·2 : 95·8
E. coli	T4	1,810	5	1,532	0·3 : 99·7
T4	*E. coli*	1,260	16	1,109	1·4 : 98·6
T4	T4	1,090	18	775	2·3 : 97·7

T4 SH-RNA was prepared as previously described[13], the labelled precursor being ^{3}H-ATP (specific activity 24 Ci/mmole). The reaction mixtures contained per ml., as appropriate, *Escherichia coli* core polymerase, 25 µg; *E. coli* σ, 50 µg; T4 core polymerase, 36 µg; σ^{T4} (as the 40–55 per cent ammonium sulphate fraction), 156 µg. The core polymerases were prepared by the method of Burgess[24]. Incubation of the reaction mixtures was for 4 min at 37° C. Aliquots of the ^{3}H-RNA were annealed in 0·03 M sodium citrate–0·3 M NaCl for 4 h at 66° C with 5 µg/ml. of either the l or r-strand of T4 DNA in a reaction volume of 200 µl. The DNA–RNA hybrids were measured as previously described[13]. Data have been corrected for radioactivity retained by the nitrocellulose filter in the absence of added DNA. These backgrounds were in the range of 10–20 c.p.m.

The difference in the ability of host σ directed RNA and σ^{T4} directed RNA to be competed by CM RNA confirms the previous observation[13] that the two σ factors direct the synthesis of qualitatively different sets of RNA. The good competition of host directed RNA by CM RNA strongly supports the demonstration by Bautz *et al.*[8] that σ directs the synthesis of pre-early RNA. Because most of the σ^{T4} directed RNA species are competed by 3 min RNA but not by CM RNA, we conclude that σ^{T4} must direct the synthesis of early RNA which is switched on between 1·75 min and 2·5 min after infection[4,5].

Modification of Core Polymerase does not affect Specificity

We have shown that the host core + host σ and T4-modified core + σ^{T4} complexes synthesize different classes of T4 RNA. The recognition unit for the correct initiation of transcription is probably the complex of core polymerase and factor, so we asked whether it was necessary to change both components in order to change the specificity of RNA synthesis. Accordingly we tested RNA synthesized *in vitro* by host core + σ^{T4} or T4 modified core + host σ reconstituted complexes by competing these RNA samples with *in vivo* T4 RNA. The results (Fig. 2) demonstrate

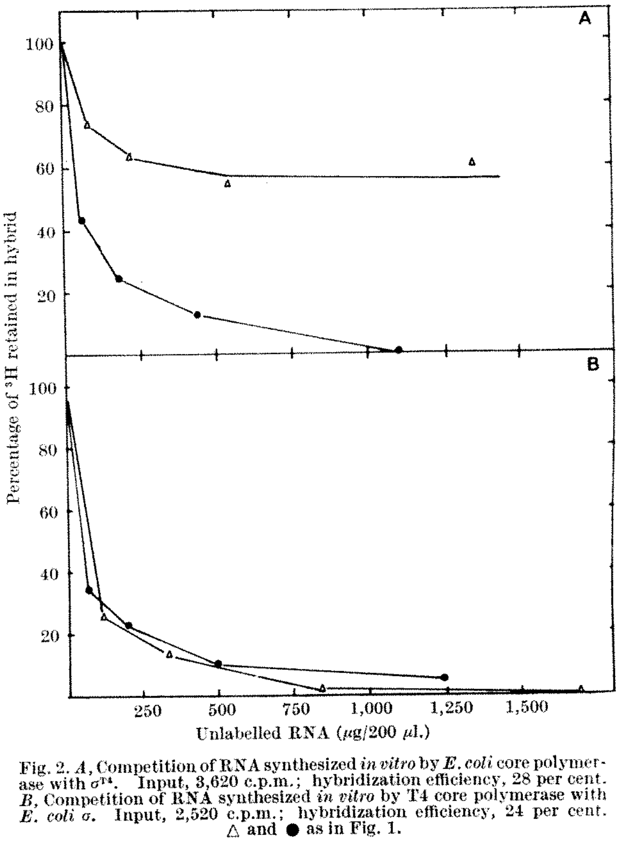


Fig. 2. *A*, Competition of RNA synthesized *in vitro* by *E. coli* core polymerase with σ^{T4}. Input, 3,620 c.p.m.; hybridization efficiency, 28 per cent. *B*, Competition of RNA synthesized *in vitro* by T4 core polymerase with *E. coli* σ. Input, 2,520 c.p.m.; hybridization efficiency, 24 per cent. △ and ● as in Fig. 1.

that σ^{T4} directs host core polymerase to synthesize early RNA and host σ directs T4 modified core polymerase to synthesize pre-early RNA. The amount of competition by the *in vivo* species is almost identical in this case to that observed during competition of RNA synthesized by homologous core–factor complexes. Thus it is clear that in this system the specificity of transcription is determined primarily by the type of factor and not by the modification of the core.

The Role of σ^{T4}

Two models have been proposed to explain why different classes of phage messenger RNA appear sequentially early in the infection cycle[4,5,10]. This effect could arise either from sequential non-synchronous initiation of different transcription units or from the propagation of transcription along synchronously initiated transcription units of appreciable length. The experiments reported here clearly strongly support the first hypothesis. σ^{T4} must act at the level of initiation, for in addition to stimulating RNA synthesis on T4 DNA it depresses random initiation by core polymerase on such templates as T7 and calf thymus DNA (ref. 13). Milanesi *et al.*[10] have shown, however, that the sequential appearance of pre-early and early RNA *in vivo* can be exactly paralleled *in vitro* using only the host complex of factor and core together with T4 DNA. This observation can be explained by the lack of natural RNA chain termination in this highly purified *in vitro* system, because in the presence of the RNA synthesis termination factor, ρ (ref. 18), host σ directs the synthesis of pre-early RNA but without it both pre-early and early RNA sequences are transcribed (Table 3). That in the absence of ρ, host σ initiated core polymerase does read through into early sequences is confirmed by the observation that host σ directed RNA synthesized for 10 min at 37° C is a good competitor of σ^{T4} directed RNA whereas host σ directed RNA synthesized for 5 min competes similarly to CM RNA (Fig. 3). All these results strongly suggest that pre-early and early RNA sequences occur on adjacent regions of the T4 chromosome and, further, that their arrangement must be such that the promoters for early RNA species are close to the termination signals for pre-early RNA sequences. Kasai and Bautz[19] have shown that promoters for pre-early species occur at widely separated points on the T4 genome and also that early genes are similarly scattered. Both host σ and σ^{T4} must therefore each direct the synthesis of several different T4 RNA species.

Table 3. EFFECT OF ϱ ON HOST σ DIRECTED RNA SYNTHESIS *in vitro*

In vitro RNA	Competition by *in vivo* RNA CM RNA	8 min RNA
E. coli σ and core polymerase + ϱ	89	94
E. coli σ and core polymerase alone	51	93

T4 ^{14}C-RNA was synthesized *in vitro* in the presence and absence of purified ϱ factor for 20 min at 37° C. The reaction mixtures contained per ml. as appropriate: *E. coli* complex of core polymerase and σ, 30 μg; ϱ factor, 20 μg; T4 DNA, 15 μg. Other conditions were as specified in the legend to Table 2 except that [KCl] = 0·1 M. The reactions were terminated by the addition of phenol and the ^{14}C-RNA was annealed to 10 μg/ml. T4 l-strand in the presence of increasing amounts of unlabelled *in vivo* T4 RNA. Hybridization was performed as described in the legend to Fig. 2. The figures for extent of competition by CM RNA (1,350 μg/200 μl.), and 8 min RNA (1,100 μg/200 μl.) are plateau values. The experiment was performed in collaboration with Dr J. Roberts.

Do host σ and σ^{T4} direct the synthesis of independent or overlapping sets of RNA species ? Because host σ directed RNA is not merely a subset of σ^{T4} directed RNA (ref. 13), the 40 per cent competition of σ^{T4} directed *in vitro* T4 RNA by *in vivo* CM RNA suggests that the sets are overlapping. Support for this idea is provided by the results of Hosoda and Levinthal[20], who have shown that whereas some of the T4 proteins that are synthesized immediately after phage infection are switched off after 8 min at 25° C, others continue to be synthesized until 20 min. These latter proteins behave similarly in this respect to another class of proteins which is switched on about 3 min after infection and which is probably coded by early RNA species. It is perhaps significant that when cells are infected with a T4 phage mutant in gene 55 (such a phage is unable to synthesize late RNA (ref. 21)) the RNA synthesized between 8 min and 11 min after infection is quantitatively similar in its ability to be competed by CM RNA (ref. 5) to σ^{T4} directed *in vitro* RNA. Thus the characteristics of *in vivo* transcription in this phage can be explained simply by assuming that σ^{T4} is responsible for directing all transcription after the host σ factor ceases to function.

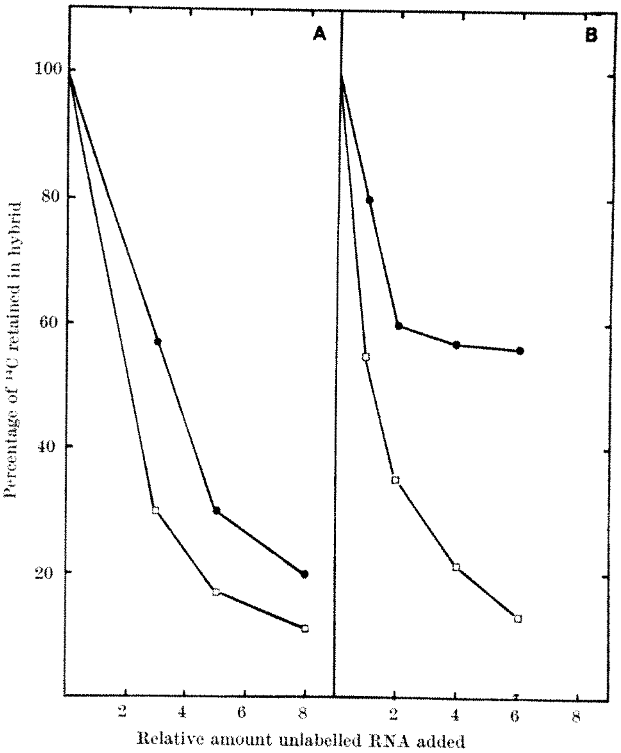


Fig. 3. Readthrough by *E. coli* σ initiated RNA polymerase into sequences transcribed by σ^{T4} initiated polymerase. ^{14}C-labelled precursor was ^{14}C-ATP (specific activity 35 Ci/mole) and unlabelled RNA samples were synthesized in parallel tubes as described in the legend to Table 2. In each case ^{14}C-RNA was annealed to 0·2 μg/ml. T4 l-strand in the presence of increasing amounts of unlabelled RNA. *A*, □, Competition of 10 min ^{14}C-labelled *E. coli* σ directed RNA by unlabelled 10 min *E. coli* σ directed RNA; input, 3,410 c.p.m.; hybridization efficiency, 33 per cent. ●, Competition of 5 min ^{14}C-labelled T4 σ directed RNA by unlabelled 10 min *E. coli* directed RNA; input, 2,880 c.p.m.; hybridization efficiency, 27 per cent. *B*, □, competition of 5 min ^{14}C-labelled *E. coli* σ directed RNA by unlabelled 5 min *E. coli* σ directed RNA; input, 3,200 c.p.m.; hybridization efficiency 31 per cent. ●, Competition of 5 min ^{14}C-labelled T4 σ directed RNA by unlabelled 5 min *E. coli* σ directed RNA; input, 2,880 c.p.m.; hybridization efficiency, 27 per cent. The times given are the length of synthesis at 37° C.

We can tentatively formulate a model for the events which take place immediately after infection by phage T4. On injection of the phage DNA into the bacterium the host core–factor complex rapidly binds to this DNA (ref. 22) and initiates the synthesis of pre-early RNA. Among the proteins coded by this RNA would be the σ^{T4} and possibly the enzyme responsible for the modification of the core polymerase. σ^{T4} then directs the synthesis of delayed early RNA together with certain immediate early RNA species. Meanwhile, the synthesis of those pre-early species not directed by the T4 factor would be switched off[19]. This switch-off would result from the functional replacement of the host factor by the T4 factor. The mechanism of such a replacement is obscure, but it is noteworthy that host σ factor activity is only detectable in T4 infected cells immediately after infection and is not found at late times[13–15]. One possibility is that the modification of the core polymerase reduces the affinity for the host factor, thereby allowing the T4 factor to bind preferentially to the core polymerase.

The only experimental finding which is not compatible with this model is the timing of the appearance of the σ^{T4} factor in infected cells. *In vivo*, early RNA is switched on about 2 min after infection at 30° C (refs. 4 and 5), while the T4 sigma factor cannot be detected until after 5 min from infection[13]. It remains possible, however, that the assay for the factor is not sufficiently sensitive to detect it earlier. Indeed, Schmidt, Mazaitis and Bautz (personal communication) have tentatively identified an early promoter in the rIIB region which is active *in vivo* about 2 min after infection and which is not read by the *E. coli* σ factor[8]. The obvious conclusion is that this early promoter site is recognized by a phage-specific factor.

The experiments reported here confirm the model of positive gene control[13,23] in which transcription can be regulated by altering the initiation specificity of RNA polymerase. This specificity is determined primarily by the σ factors and is altered during the development of phage T4 and also T7 (ref. 23) by the substitution of a phage-induced factor for the host factor.

I thank Mrs Christine Roberts for technical assistance and Professors J. D. Watson, W. Gilbert and K. Weber and Dr J. Roberts and C. Goff for discussions. This work was carried out during the tenure of a Damon Runyon Memorial fellowship and was supported in part by a grant from the US Public Health Service.

Received February 10, 1970.

[1] Kano-Sueoka, T., and Spiegelman, S., *Proc. US Nat. Acad. Sci.*, **48**, 1942 (1962).

[2] Khesin, R. B., Gorlenko, Zh. M., Shemyakin, M. F., Bass, I. A., and Prozorov, A. A., *Biokhimiya*, **28**, 1070 (1963).

[3] Hall, B. D., Nygaard, A. P., and Green, M. H., *J. Mol. Biol.*, **9**, 143 (1964).

[4] Grasso, R. J., and Buchanan, J. M., *Nature*, **224**, 882 (1969).

[5] Salser, W., Boller, A., and Epstein, R., *J. Mol. Biol.* (in the press).

[6] Travers, A. A., and Burgess, R. R., *Nature*, **222**, 537 (1969).

[7] Burgess, R. R., Travers, A. A., Dunn, J. J., and Bautz, E. K. F., *Nature*, **221**, 43 (1969).

[8] Bautz, E. K. F., Bautz, F. A., and Dunn, J. J., *Nature*, **223**, 1022 (1969).

[9] Goff, C. G., and Minkley, E. G., in *First Lepetit Colloquium on RNA Polymerase and Transcription* (North-Holland, Amsterdam, 1970).

[10] Milanesi, G., Brody, E. N., and Geiduschek, E. P., *Nature*, **221**, 1014 (1969).

[11] Crouch, R., Hall, B. D., and Hager, G., *Nature*, **223**, 476 (1969).

[12] Haselkorn, R., Vogel, M., and Brown, R. D., *Nature*, **221**, 836 (1969).

[13] Travers, A. A., *Nature*, **223**, 1107 (1969).

[14] Walter, G., Seifert, W., and Zillig, W., *Biochem. Biophys. Res. Commun.*, **30**, 240 (1968).

[15] Bautz, E. K. F., and Dunn, J. J., *Biochem. Biophys. Res. Commun.*, **34**, 230 (1969).

[16] Seifert, W., Qasba, P., Walter, G., Palm, P., Schachner, M., and Zillig, W., *Europ. J. Biochem.*, **9**, 319 (1969).

[17] Guha, A., and Szybalski, W., *Virology*, **34**, 608 (1968).

[18] Roberts, J. W., *Nature*, **224**, 1168 (1969).

[19] Kasai, T., and Bautz, E. K. F., *J. Mol. Biol.*, **41**, 401 (1969).

[20] Hosoda, J., and Levinthal, C., *Virology*, **34**, 709 (1968).

[21] Bolle, A., Epstein, R. H., Salser, W., and Geiduschek, E. P., *J. Mol. Biol.*, **33**, 339 (1968).

[22] Oleson, A. E., Pipsa, J. P., and Buchanan, J. M., *Proc. US Nat. Acad. Sci.*, **63**, 473 (1969).

[23] Summers, W. C., and Siegel, R. B., *Nature*, **223**, 1111 (1969).

[24] Burgess, R. R., *J. Biol. Chem.*, **244**, 6160 (1969).

[25] Bolle, A., Epstein, R. H., Salser, W., and Geiduschek, E. P., *J. Mol. Biol.*, **31**, 325 (1968).

Involvement of a Phage T4 σ Factor and an Anti-terminator Protein in the Transcription of Early T4 Genes *in vivo*

by

D. A. SCHMIDT
A. J. MAZAITIS*
T. KASAI
E. K. F. BAUTZ

Institute of Microbiology,
Rutgers University,
New Brunswick, New Jersey

The existence of an rIIB promoter site not recognized by the *Escherichia coli* σ factor is demonstrated. Early synthesis of lysozyme messengers seems to result from the lack of termination of pre-early transcription.

STUDIES on the regulation of transcription of the bacteriophage T4 genome have indicated that at least two distinct classes of genes are transcribed in succession before the onset of phage DNA replication[1,2]. The discovery of the RNA polymerase σ factor which is required for proper transcription of T4 DNA *in vitro*[3] suggested that the expression of the first class of genes (the pre-early genes) would be under the control of this host factor. When this was confirmed[4], it seemed very likely that the second class of genes (the early genes) would be under the control of another—a phage-specific—σ factor. The reported finding of a σ-like factor in phage-infected *E. coli* cells[5], having most of the expected properties of a hypothetical early T4 factor, has strongly supported this idea, especially because it was also observed that addition of chloramphenicol at or before the time of infection prevented transcription of the early genes[2,6,7]. Thus the discovery of transcription factors seemed to explain the regulatory mechanisms involved in prereplicative RNA synthesis in a most satisfactory way.

The fact that *E. coli* RNA polymerase *in vitro* mimics the *in vivo* situation—in that it transcribes sequentially first the pre-early and then the early genes[8]—suggests, however, that the two classes of genes are interspersed and that RNA polymerase reads past the boundaries between pre-early and early genes. The chloramphenicol data could then be interpreted as indicating the existence of transcriptional termination signals between pre-early and early genes which are recognized neither *in vitro*, because of the lack of a termination factor in highly purified RNA polymerase, nor *in vivo*, because of the phage-directed synthesis of an anti-terminator protein which inhibits the host's termination factor. Indeed, addition of a termination protein[9] to *E. coli* RNA polymerase results in the *in vitro* transcription of only pre-early T4 genes (personal communication from A. A. Travers). There are therefore two diverging lines of evidence leading to two different hypotheses explaining the transcription switch from pre-early to early genes, namely the T4 σ factor and T4 anti-terminator hypotheses.

Previous studies in this laboratory on the time-dependent transcription of genetically defined regions of the T4 genome demonstrated that of the three regions studied, *e*, rII and D_2, none seemed to be regulated identically with either of the other two[1]. Here we report further data on the transcription of these regions, and

---

* Present address: Department of Biochemistry, New York University School of Medicine.

present evidence that (1) transcription of the rIIB cistron is at least partially under the control of an early promoter site, and (2) the early transcription of the *e* gene is probably attributable to non-termination of transcription of a neighbouring pre-early gene. To explain these results, we have to conclude that both the recognition of a second set of promoter sites as well as the lack of termination are sharing in the controls which operate in prereplicative RNA synthesis.

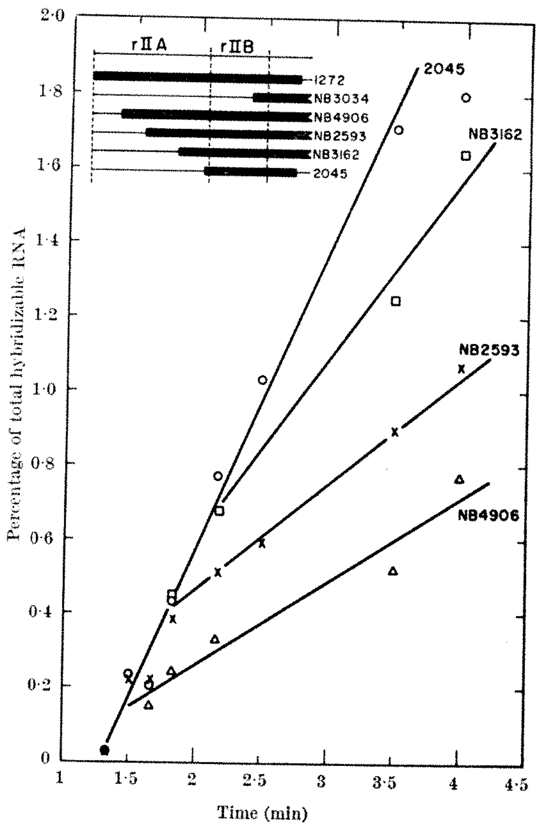


Fig. 1. Kinetics of transcription of the rIIA cistron. *E. coli* BA cells, grown with aeration in minimal salts (MS) plus glucose medium at 37° C to 5×10^8 cells/ml., were poured on to 0·2–0·5 volume of finely crushed frozen MS, centrifuged, resuspended in a small volume of cold MS, and kept at 0° C. After addition of tryptophan solution, the bacteria were each infected with 5–8 T4 wild-type phages, kept in the cold for 90 s to allow phage adsorption, and diluted 1 : 5 or 1 : 10 to 5×10^8 cells/ml. by simultaneously blowing aliquots of the infection mixture from cold pipettes into each of several aerating, pre-warmed samples of MS + glucose to give a final temperature of 37° C. The time of dilution was considered to be zero time with respect to transcription because cells pulsed during the adsorption period did not incorporate tritiated uracil into T4-specific RNA. At various times after dilution, 0·3 mCi of 5-^{3}H-uridine or 6-^{3}H-uracil (New England Nuclear, 28·3 and 8·9 Ci/mmole, respectively) per 25 ml. of culture was added to one of the suspensions. Pulses were terminated 1 min after addition of tritium; collection of cells and extraction of RNA were as described by E. K. F. B. and Hall[11]. Subsequent procedures were essentially as described by K. T. and E. K. F. B.[1]. The columns contained r1272 DNA in the upper layer and rNB3034 DNA in the lower layer. The segment-specific RNA was tested in triplicate on r1272, rNB3034, rNB4906 (△), rNB2593 (×), rNB3162 (□), and r2045 (○) DNA filters. The percentage pure segment-specific RNA obtained was calculated as described[1]. This value multiplied by, for example, the fraction of the radioactivity (c.p.m.) on 4906–1272/3034–1272 filters gives the percentage of total hybridizable RNA homologous with nucleotide sequences of the rIIA cistron present in the DNA of deletion mutant rNB4906. Radioactivity retained on DNA filters was corrected for background by subtraction of counts on the blank filter. The times indicated were the times of termination of pulses.

Sequential Transcription of the rIIA Cistron

Studies on frameshift mutations in connexion with a mutant deleting the cistron divide between the rIIA and B cistrons have shown that the rII region is transcribed and translated from left to right on the genetic map, that is, in the direction of rIIA to rIIB[10]. We have attempted to determine the exact time at which different sections of the rIIA cistron are initially transcribed at 37° C in order (1) to confirm the genetic data, and (2) to estimate the time it takes an RNA polymerase molecule to read the rIIA cistron. Knowing the physical size of the rIIA cistron from electron microscopy of artificial heteroduplexes of r$^+$ and deletion DNA (H. Bujard, A. J. M., and E. K. F. B., manuscript in preparation), such an estimate would allow a calculation of the chain growth rate of RNA *in vivo*. Fig. 1 shows the relative amounts of rIIA-specific RNA found at different times after infection. The first rIIA-specific fragments found all hybridize both to DNA of a deletion mutant possessing only the first quarter of the rIIA cistron and to DNA of a mutant possessing most of this region, indicating that this RNA all came from the left end of rIIA (see insert, Fig. 1). Half a minute later, some rIIA-specific RNA is found which does not hybridize with the DNA of deletion mutants missing the right half of the rIIA cistron. From these data we estimate that it takes about 60 ± 10 s for the entire cistron to be transcribed. Because the length of the rIIA cistron is about 2,000 base pairs (H. Bujard, A. J. M., and E. K. F. B., manuscript in preparation), the chain growth rate is 34 ± 6 nucleotides/s at 37° C. This estimate lies between the 28 nucleotides/s reported for T4-infected cells[12] and a value of 43 nucleotides/s reported for uninfected *E. coli*[13].

An Early Promoter for the rIIB Cistron

The timing of transcription of the rIIA cistron is compatible with several alternatives, the two most likely being: (1) the function of the region to the left of rIIA is pre-early, and the polymerase which traverses this pre-early region continues to transcribe sequentially the early function rII genes, with the result that transcription of the rII region would be under the control of a pre-early promoter, presumably recognized by the *E. coli* σ factor. Alternatively, (2) transcription of the rIIA cistron is under the control of an early promoter, located near the left end of the gene, which is recognized by a new T4 σ factor. In the latter case, the approximately 80 s lag between the time of infection and the beginning of rII transcription would indicate the time required for the first T4 factors to be synthesized. (It is of interest to note that, according to our estimate of the RNA chain growth rate, the maximum size of a polypeptide which could be synthesized in this time span would be between 8×10^4 and 10×10^4 daltons, provided the transcription and translation proceed almost simultaneously.)

According to the first alternative, transcription of the rIIB cistron would necessarily have to commence only after completion of the first rIIA messengers, that is, the first rIIB-specific RNA fragments should not be found until about 2·25–2·5 min after infection. According to the second alternative, however, transcription of rIIB could start as early as that of rIIA if the B cistron has its own early promoter site. It was of interest therefore to see whether the rIIB cistron became transcribed before transcription of rIIA was completed. For this purpose we purified pulse-labelled RNA samples homologous to the DNA region missing in mutant rPB240 (or rPB28) but present in rNB3034. The RNA was tested for hybridizability with DNA of mutants rPB240 (or rPB28), r2045, and rNB3034 to determine what proportion of the RNA originated from approximately the right half (or right third) of rIIA and from rIIB (see insert, Fig. 2). Fig. 2 demonstrates that transcription of the rIIB cistron starts before the RNA polymerase has travelled halfway through the rIIA cistron; this can only be interpreted as indicating the existence of a separate promoter site serving the rIIB cistron. This promoter site is likely to be located between rIIA and rIIB because transcription of the entire chromosomal segment (including the distal D_2 region[1]) seems to proceed unidirectionally from left to right[10].

Some rIIA and rIIB Messengers are Polycistronic

Because of the evidence for the existence of a promoter site between the rIIA and B cistrons, we could expect that the two genes are transcribed separately, either mono-

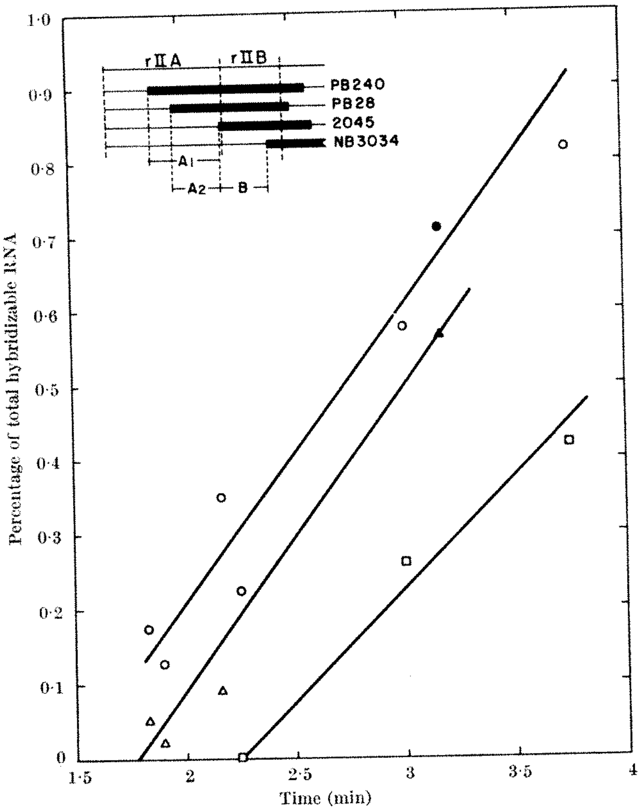


Fig. 2. Kinetics of transcription of adjacent portions of the rIIA and B cistrons. Procedures were as described in the legend to Fig. 1. Columns contained either rPB240 DNA in the upper layer and rNB3034 DNA in the lower layer, or were triple-layered, containing rPB28, r2045, and rNB3034 DNA, from top to bottom. DNA filters used were rPB240 or rPB28, depending on which DNA was used in the column, rNB3034, and r2045. The percentage of total hybridizable RNA corresponding to segment B is ○; segment A_1, △; segment A_2, □. Solid figures are the averaged results of duplicate determinations. The times indicated are times of termination of pulses.

cistronically or polycistronically with other adjacent genes. To test whether rIIA and B messengers are ever part of the same polynucleotide chain, we have done the experiment outlined in Fig. 3. RNA pulse-labelled from 2 to 5 min after infection was extracted by the hot phenol procedure[14] to obtain high molecular weight RNA. This RNA was passed through a double layer DNA column (step 1) containing DNA of the deletion r1272 in the upper and rPT8 in the lower column layer. RNA homologous with DNA of the B cistron should not find complementary sequences in either of the two layers and thus be lost from the column unless it were part of the same polynucleotide chain as rIIA RNA, which should be retained in the layer containing rPT8 DNA. The RNA eluted from rPT8 DNA with low ionic strength salt solution was then subjected to sonication (step 2) which produces 4S RNA fragments 60–100 nucleotides in length[1]. The fragments were then re-run through a second double layer DNA column (step 3) containing in the top layer a mixture of rA105 and r638 DNAs which feature all DNA sequences except those of the B cistron. RNA bound to r^+ DNA in the lower layer was, after elution, tested for hybridizability with rA105, r638, and r^+ DNA to determine how much of it is actually homologous with the rIIB cistron (Table 1). The RNA recovered from the bottom layer of column I (AI) represents 3 per cent of the total recovered from both layers: when applied after sonication to column II (AII), it is mostly retained on the upper layer, but 8 per cent of the recovered radioactivity was found in the bottom (r^+) DNA layer. Sixty-one per cent of this RNA hybridizes exclusively with rIIB DNA sequences, so that 0·15 per cent of the total RNA recovered from both columns is rIIB-specific. This result suggests that at least some rIIB messengers are synthesized contiguously with rIIA messengers. In a parallel experiment, half the original RNA was passed through a column (BI) containing rA105 DNA in the lower layer, allowing binding of B-specific RNA through overhang from both sides of rIIB (in case rIIB and D_1 messengers were contiguous). Here a larger amount of rIIB-specific RNA was retained, suggesting that most rIIB messengers are polycistronic with regard to D_1. It is possible, however, that the D_1 region is either not translated or is a dispensable part of the rIIB cistron, both possibilities being compatible with the fact that no mutations have been observed in it. Of the 0·76 per cent rIIB messengers found after column run BII, 0·47 per cent were found to be homologous with the right and 0·29 per cent with the left half of the rIIB cistron. This suggests that more rIIB-specific RNA was trapped on rA105 DNA in column BI by hybrid formation to D_1 than to rIIA. We interpret these data as indicating that transcription of only a fraction of the rIIB messengers originated at the same site which promotes transcription of the rIIA cistron.

Table 1. BINDING OF rIIB MESSENGERS TO DNA OF ADJACENT GENES

Column	DNA upper / DNA lower	RNA added to column (c.p.m. × 10^{-6})	Total RNA recovered on elution from both column layers (c.p.m. × 10^{-6})	RNA recovered from lower layer (per cent of total)
AI	r1272 / rPT8	15	10	3·0
BI	r1272 / rA105	15	12	4·1
		RNA recovered from r^+ layer (per cent of total)	Fraction of r^+ eluate which is rIIB-specific	rIIB-specific RNA (per cent of total hybridizable RNA, columns I and II)
AII	rA105 + r638 / r^+	8	0·61	0·15
BII	rA105 + r638 / r^+	21	0·88	0·76

An 80 ml. culture of *E. coli* B, grown to 5×10^8 cells/ml. at 37° C, was infected with 4 T4 r^+ phages per cell; after 2 min 1 mCi ^{3}H-uracil (specific activity 20 Ci/mmole) was added and the cells were harvested 5 min after infection. For subsequent steps, see text. DNA column runs and purity test were done as described[1], except that the total incubation time in the first column run was reduced to 8 h in order to minimize thermal degradation of the RNA.

Taking together the results of this and the previous section, it seems that part of the rIIB messengers are made by polymerases reading through from A to B, the rest being made by polymerases which initiate at the rIIB promoter. This suggests that a promoter which is in the path of transcribing polymerases does not necessarily have to act as a termination signal. The situation in which synthesis of messengers can apparently be initiated at more than one promoter site is not unique to T4-infected cells; an auxiliary weak promoter has been observed within the tryptophan operons of *S. typhimurium*[15] and *E. coli*[16]; an analogous observation was made with phage λ in that a low level of transcription of the S–R and A–J

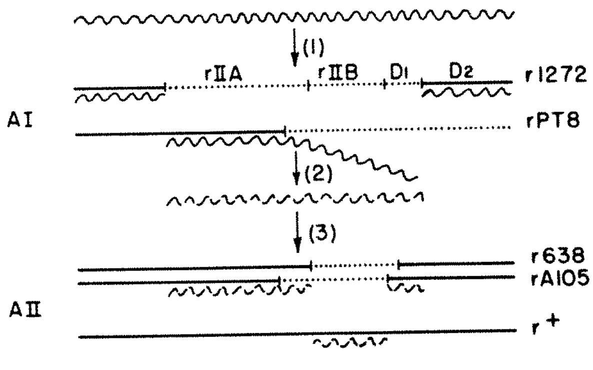


Fig. 3. Outline of experiment to test for polycistronic rIIB messengers. Experimental procedures are discussed in the text and Table 1.

Table 2. TRANSCRIPTION OF THE rII AND D REGIONS IN THE PRESENCE AND ABSENCE OF CHLORAMPHENICOL

Time of pulse (min)	CM	RNA added to column (c.p.m. × 10^{-6})	Total RNA recovered (c.p.m. × 10^{-6})	RNA recovered from T4W layer (per cent of total)	$\frac{\text{T4W}-222{\cdot}6}{\text{T4W}}.10^2$ (per cent purity)	P_t*	$\frac{1272-2226}{\text{T4W}-2226}.P_t$ (per cent D_2)	P_t–per cent D_2 (per cent rII + D_1)
1–2	–	20·7	10·4	7·28	95·5	6·9	5·2	1·7
3–4	–	29·3	11·8	9·25	95·9	8·9	1·8	7·1
1–2	+	14·7	4·0	8·89	97·6	8·7	8·7	0
3–4	+	12·9	3·4	6·28	97·8	6·1	6·1	0

* P_t = purity × per cent recovery from T4W layer.

The experiment was performed as described in the legend to Fig. 1, except that 100 μg/ml. chloramphenicol (CM, gift of Parke, Davis and Co.) was added to one-half of the culture 5 min before centrifuging the cells to prepare the infection mixture; these CM-treated cells were always in medium containing 100 μg CM/ml. during the experiment. The columns contained rNB2226 DNA in the upper layer and T4 wild DNA in the lower layer. DNA filters used to test the RNA were rNB2226, T4 wild, and r1272.

regions occurs in the absence of the Q gene product[17], which, if present, greatly enhances transcription of these late regions by recognizing at least one additional promoter between genes Q and S (I. Herskowitz and E. R. Signer, cited as personal communication in ref. 17). A similar situation seems to occur again in λ for the transcriptional process to the left of the N gene, where evidence for the functioning of auxiliary promoters superimposed upon the continued transcription from p_L leftward has been reported[17].

Are there more than Two Classes of Prereplicative Genes?

It was shown previously that maximal transcription is found between 0 and 2 min after infection for the D_2 region, between 1 and 3 min for the *e* gene, and between 4 and 6 min for the rII genes[1]. If chloramphenicol is added at or before the time of infection, transcription of only the pre-early D_2 region occurs and no rII or *e*-specific messengers are made (Tables 2 and 3). We could therefore argue that the D_2 region is under the transcriptional control of a pre-early promoter and that both rII and *e* genes are controlled by an early promoter which only responds to a phage-directed σ factor which is not made in the presence of chloramphenicol. It is difficult, however, to classify the rII genes and the *e* gene into one group because their patterns of transcription differ markedly. For example, the rII and *e* genes are similar in that neither is transcribed in the absence of protein synthesis and the first messengers of both regions appear at approximately the same time; but when the number of rII messages reaches a maximum level between 3 and 6 min, transcription of both the *e* and D_2 genes is approaching a minimum. Also, if we make adjustment for the difference in size between the D_2 and *e* genes, the number of messenger copies transcribed from these genes during this time interval is nearly the same but several-fold less than the number of rII messengers[1]. Finally, both *e* and D_2 regions are barely transcribed 20 min after infection with amBL292 (Table 3)—an amber mutant which fails to synthesize late messengers—whereas the early function rII genes are successfully transcribed in early amber mutants[1]. Thus while the transcription pattern of the typically early rII region differs markedly from that of the typically pre-early D_2 region, the pattern observed for the lysozyme gene is partly early and partly pre-early, depending on which criteria we apply for comparison.

Table 3. THE NUMBER OF LYSOZYME MESSENGERS PER CELL AT VARIOUS TIMES AFTER INFECTION

Infecting phage	Time of collection of cells (min after infection)	Number of *e* mRNA copies per cell – CM	+ CM*
T4 wild	3·5	12	0
	6·0	7	0
	15·0	20	0
amB22 (gene 43)	3·5	10	–
	8·0	3	–
	20·0	4	–
amBL292 (gene 55)	3·5	11	–
	8·0	4	–
	20·0	3	–

* 100 μg chloramphenicol/ml. of culture was added at time of infection.

Total RNA was prepared from infected cells collected at times indicated and increasing amounts were used for competition against a constant amount of ^{32}P-labelled lysozyme-specific RNA of high specific activity. The numbers of lysozyme messengers were calculated from the initial slopes of the competition curves. Experimental details will be published elsewhere (K. T. and E. K. F. B., in preparation).

To explain the peculiar transcription pattern of the *e* gene, we propose that the following mechanisms are involved in its early transcription. The *e* gene is located near a pre-early promoter. During the first minute after infection or at any time after infection in the absence of protein synthesis, the RNA polymerase which had initiated at this promoter fails to transcribe the *e* gene because of termination of transcription at a site located between the promoter and the *e* gene. This termination is probably performed by the host's ρ factor[9]. An accumulation of a phage-specified anti-terminator protein, about 1 min after infection, soon eliminates the termination signal and the RNA polymerase now reads into and through the *e* gene. While we have been able to show that there is a late promoter for the post-replicative synthesis of lysozyme messengers near the start of the *e* gene[1], we have no evidence for the existence of an early promoter specifically serving the *e* gene. Thus we propose that the indiscriminate action of the anti-terminator protein eliminates all transcriptional stops recognized by the ρ factor. Consequently, the early transcription of the *e* gene represents a "harmless accident" in the form of a continuation of transcription of a preceding pre-early gene. These early *e* messengers do not seem to be translated into functional lysozyme. We suggest that the initiating codon is not recognized by the translational machinery when the lysozyme message is contiguous with that of the preceding gene. This interpretation is in accord with the fact that an *in vitro* DNA-dependent protein synthesizing system produces functional lysozyme only in conditions of slight miscoding[18].

On the other hand, the fact that transcription of the rIIB cistron starts before the first rIIA messengers are completed can only be interpreted as proving the existence of an early promoter recognized by a σ factor different from the host σ factor. This result is not necessarily at variance with the finding of some contiguous rIIA and B messengers if we accept the existence of both mechanisms, that is, failure of chain termination plus new initiation within the rII region. The finding of substantially more rIIB messengers polycistronic with D_1 than with rIIA RNA (Table 1) actually indicates that not all rIIB messengers are linked with rIIA messengers. Termination between rIIB and D_2, however, must still occur with a high degree of efficiency, because no additional transcription of the pre-early D_2 region is observed at the time when the first polymerase molecules have finished transcribing the preceding rIIB and D_1 regions.

If indeed both the anti-terminator and the early T4 σ factor exist, we can expect to find, in addition to the pre-early genes, not one but two classes of early genes, namely those that are only transcribed together with pre-early genes through inhibition of termination and those which possess their own promoter sites for the T4 σ factor. The former class is expressed optimally within a very short time span (about 2 to 4 min, post infection), whereas expression of the latter class proceeds actively for a much longer time until it becomes gradually displaced by the transcription of late genes.

The postulated involvement of an anti-terminator and new promoter sites in the rigidly programmed expression of the T4 genome is not unique to this phage system; the N product of phage λ, for example, has been implicated as an anti-terminator to allow transcription to proceed further to the left and right of C_1 (ref. 17). In addition, there is evidence that the Q gene codes for a λ σ factor[19]. For T4 it is not yet possible to predict the relative contributions the anti-terminator and early T4 σ factor to the expression of all early genes. It is possible that their functions overlap almost completely at early times because no phage mutants have been reported to fail to express a significant part of their complement of early function genes.

This work was supported by a US Public Health Service career development award (to E. K. F. B.) and by research grants from the US Public Health Service and the US National Science Foundation. D. A. S. is a predoctoral trainee of the US Public Health Service.

Received February 2, 1970.

[1] Kasai, T., and Bautz, E. K. F., *J. Mol. Biol.*, **41**, 401 (1969).

[2] Salser, W., Bolle, A., and Epstein, R., *J. Mol. Biol.* (in the press).

[3] Burgess, R. R., Travers, A. A., Dunn, J. J., and Bautz, E. K. F., *Nature*, **221**, 43 (1969).

[4] Bautz, E. K. F., Bautz, F. A., and Dunn, J. J., *Nature*, **223**, 1022 (1969).

[5] Travers, A. A., *Nature*, **223**, 1107 (1969).

[6] Grasso, R. J., and Buchanan, J. M., *Nature*, **224**, 882 (1969).

[7] Bautz, E. K. F., Dunn, J. J., Bautz, F. A., Schmidt, D. A., and Mazaitis, A. J., *Proc. First Lepetit Colloquium on RNA Polymerase and Transcription* (North-Holland, Amsterdam, 1970).

[8] Milanesi, G., Brody, E. N., and Geiduschek, E. P., *Nature*, **221**, 1014 (1969).

[9] Roberts, J. W., *Nature*, **224**, 1168 (1969).

[10] Crick, F. H. C., Barnett, L., Brenner, S., and Watts-Tobin, R. J., *Nature*, **192**, 1227 (1961).

[11] Bautz, E. K. F., and Hall, B. D., *Proc. US Nat. Acad. Sci.*, **48**, 400 (1962).

[12] Bremer, H., and Yuan, D., *J. Mol. Biol.*, **34**, 527 (1968).

[13] Manor, H., Goodman, D., and Stent, G. S., *J. Mol. Biol.*, **39**, 1 (1969).

[14] Scherrer, K., and Darnell, J. E., *Biochem. Biophys. Res. Commun.*, **7**, 486 (1962).

[15] Margolin, P., and Bauerle, R. H., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 311 (1966).

[16] Morse, D., and Yanofsky, C., *J. Mol. Biol.*, **38**, 447 (1968).

[17] Szybalski, W., *Proc. First Lepetit Colloquium on RNA Polymerase and Transcription* (North-Holland, Amsterdam, 1970).

[18] Schweiger, M., and Gold, L. M., *Proc. US Nat. Acad. Sci.*, **63**, 1351 (1969).

[19] Naono, S., *Proc. First Lepetit Colloquium on RNA Polymerase and Transcription* (North-Holland, Amsterdam, 1970).

Fusion of Isolated Plant Protoplasts

by
J. B. POWER
S. E. CUMMINS
E. C. COCKING

Department of Botany,
University of Nottingham

A first step towards somatic hybridization in plants has been achieved. Protoplasts have been isolated and seen to fuse.

We have devised controlled conditions in which plant protoplasts isolated from various species and tissues can be induced to fuse reproducibly. The significance of this lies in the potentiality for producing hybrid cells which combine plant genotypes that cannot be crossed sexually. Hybrid cells could be cultured to develop into complete plants, giving otherwise unobtainable hybrids which might be of great interest and value to pure and applied plant geneticists.

Much discussion has centred recently on the processes by which plant cells might be hybridized in laboratory conditions[1] and the principal stages are clear. First, protoplasts—cells with the wall removed—must be isolated from suitable tissue or from cell suspension cultures. Protoplasts are essential for hybridization so that the membranes of different cells can be brought into contact. Second, protoplasts must be induced to fuse in controlled and repeatable conditions to give heterokaryons. Third, the wall must regenerate round the fused protoplast, either before or after nuclear division. Fourth, the nuclei of the heterokaryons must fuse, probably during the simultaneous mitotic divisions, to give a true hybrid nucleus. Fifth, the resulting hybrid cells must then be taken into culture, and generation of intact plants induced. Regeneration of cell cultures from single species has often been observed. Of course, even given complete stability of the chromosome constituents, which may not be easy to achieve in culture, the simplest products of cell hybridization between different species would be an allotetraploid. For practical plant breeding purposes an extended breeding programme initiated from the vegetative hybrid would be necessary. Protoplasts have been seen to fuse before[2,3], but in ill-defined conditions, and fusions were rare and non-reproducible[4]. In these cases protoplasts were freed from their walls mechanically[5] and not enzymatically[6,7] as now. Initially the procedure required the plasmolysis of cells of mature tissues in hypertonic solutions of various kinds, and these conditions indicated some of the factors that affect the likelihood of protoplast fusion. For example, the presence of sucrose in the solution apparently inhibited fusion[2], while deplasmolysis often favoured its occurrence[3].

Conditions for Fusion

With conditions for the enzymatic isolation of protoplasts from meristematic[6,8] and mature tissue[9-11] of various plant species known, our problem was to find the conditions favouring fusion of protoplasts. It seemed likely that they could be determined by studying sub-protoplasts within intact plasmolysed cells. In some conditions plasmolysis is known to result in the formation of two sub-protoplasts, of approximately equal size, within the same cell wall. This occurs without rupture of the plasmalemma, and, of course, one sub-protoplast is nucleate and the other enucleate. Yoshida[12] showed that the two sub-protoplasts could fuse in suitable conditions. Large vacuolated cells of onion epidermis were plasmolysed in several solutions. The sub-protoplasts fused when they touched and the resultant protoplast expanded to fill the cell (details in Table 1).

Table 1. BEHAVIOUR OF SUB-PROTOPLASTS ON DEPLASMOLYSIS

Solution	Behaviour of protoplasts
0·56 M sucrose	Rarely fused after 4 h plasmolysis
0·5 M calcium chloride	Rarely fused after 4 h plasmolysis
1 M sodium nitrate	Mostly fused after 4 h and even after 24 h plasmolysis
1 M potassium nitrate	Rarely fused after 4 h plasmolysis
1 M sodium nitrite	Some slow fusion after 5 h plasmolysis
1 M sodium chloride	Some slow fusion after 5 h plasmolysis

When plasmolysis was carried out in either 0·56 M sucrose or 1 M sodium nitrate with 10 per cent cellulase (Onozuka) and 5 per cent 'Macerozyme' also present[9], sub-protoplasts only fused on deplasmolysis when 1 M sodium nitrate had been present. And so sodium nitrate apparently favoured fusion, but we had to check whether this was also true of isolated protoplasts freed from the cell wall. To do this we isolated protoplasts mechanically from onion epidermal cells, after initial plasmolysis in appropriate solutions using Plowe's method[13]. Protoplasts were manipulated in a cavity slide, being pushed together with micro-needles made from 0·2 mm diameter glass capillary tubing. After this either protoplasts adhered to one another, apparently fusing, and could not be pulled apart, or they drifted apart when the slide was subjected to any slight movement.

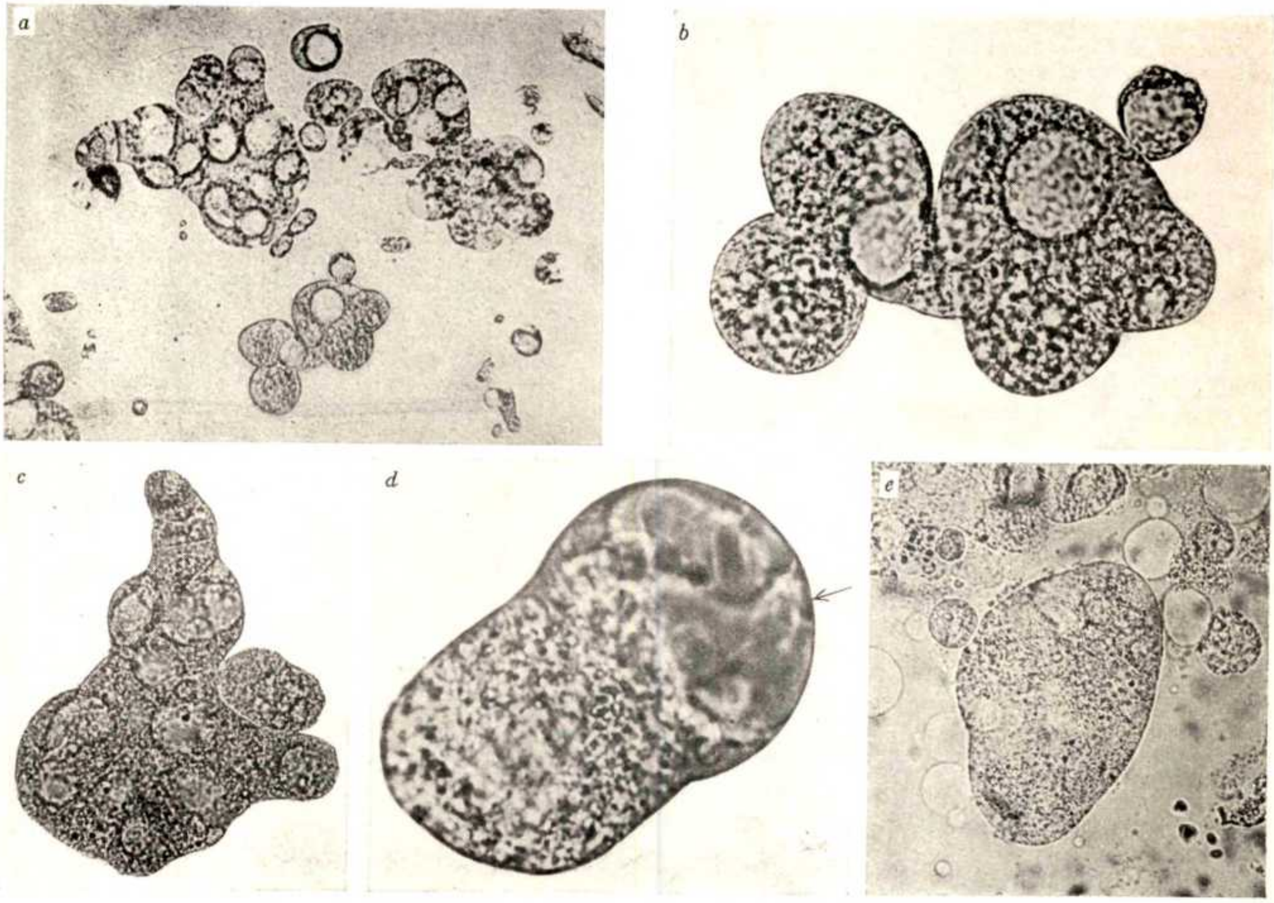


Fig. 1. *a*, Fusion of isolated protoplasts from oat root following treatment with 0·25 M sodium nitrate (×55); *b*, higher magnification of the fused protoplasts from *a* showing protoplast fusion, plasmalemma disappearance, and cytoplasm coalescence. Note presence of vacuoles within the cytoplasm (×175); *c*, another fused protoplast from *a* showing the more complete mergence of protoplasts. Note presence of vacuoles within the cytoplasm (×100). *d*, Fusion of a non-vacuolated meristematic protoplast from oat root with a vacuolated protoplast of a maize lateral root (arrowed) after treatment with 0·25 M sodium nitrate (×380). *e*, Spontaneous fusion body emerging from the oat root tip (×*c*. 117).

Fusion Achieved

When the plasmolyticum was 0·56 M sucrose and protoplasts had been in the sucrose solution for 1 h, no adhesion of the isolated protoplasts was observed. But when 0·25 M sodium nitrate was used as the plasmolyticum protoplasts of different sizes readily adhered and apparently fused. Over-plasmolysis, which resulted when 1 M sodium nitrate was the plasmolyticum, was detrimental to fusion.

When the cells were first plasmolysed in 0·56 M sucrose and the protoplasts were then isolated and placed in 0·25 M sodium nitrate, fusion occurred immediately. This resulted in the formation of several aggregates of fusing protoplasts, and there was a greatly enhanced tendency for protoplasts to adhere in these conditions.

Protoplasts have been isolated in this laboratory before, using pectinase, cellulases or mixtures of pectinase and cellulase in 0·56 M sucrose from such various plant tissues as mature tomato fruit[7], leaf tissue of tobacco[9] and radish storage root. These mature protoplasts are usually highly vacuolated, and only rarely has there been any semblance of fusion. Moreover, the peripheral alignment of chloroplasts or other plastids seems to be detrimental to fusion when sucrose is the plasmolyticum.

Thus it seemed that meristematic protoplasts isolated enzymatically from seedling tissues or tissue cultures would be most suitable for fusion studies. Surface sterilized seeds of oat, maize, wheat, barley and rice were germinated aseptically in the dark at 22° C. The excised tips of roots approximately 2–3 cm long were incubated for 24 h at room temperature on a glass slide with a few drops of the enzyme mixture (which had been filtered through 'Millipore' and contained 10 per cent (w/v) cellulase (Onozuka P1500) and 5 per cent (w/v) 'Macerozyme' in 0·56 M sucrose). Incubation was terminated by removing excess enzyme mixture and then twice washing each root tip with 0·25 M sodium nitrate. After the second wash 0·25 M sodium nitrate was added to cover each root tip and a cover slip was gently lowered onto the specimen. The pressure released numerous protoplasts from the meristematic region of the root tip and from the more vacuolated region. They floated free into the surrounding sodium nitrate solution; there was slight deplasmolysis at this molarity of sodium nitrate. During the next hour we observed fusion of the meristematic with vacuolated, meristematic with meristematic, and vacuolated with vacuolated protoplasts. Protoplasts first came into contact, adhered and subsequently were seen to fuse and gradually merge as a result of the disappearance of the two closely adpressed plasmalemmae. Initially, and particularly when several protoplasts were seen to fuse together, multi-lobed structures were evident (Fig. 1*a*, *b* and *c*); only later did these fused protoplasts round off. It was difficult to see nuclei during this progressive coalescence of the cytoplasm of adjacent protoplasts; sometimes fusion of the vacuoles was observed.

When root tips, after incubation with the enzyme mixture, were washed with 0·3 M sucrose, which was isotonic with 0·25 M sodium nitrate, and the cover slip was added, we could detect no fusion of the freely floating protoplasts from the meristematic region or the more vacuolated region.

Inter-species Fusion

Preliminary indications of the possibility of inter-species fusion were obtained using protoplasts from oat and maize. Portions of the meristematic region of oat roots and the vacuolated tissue from the root tip of maize (lateral roots) were incubated side by side with the enzyme mixture as described before. They were then washed twice with 0·25 M sodium nitrate, and a cover slip was gently lowered onto them. Contrasting protoplasts were released from the two tissues and fused with each other (Fig. 1*d*). More extensive and larger scale inter-species fusion of isolated protoplasts is being investigated using the culture chambers described by Pojnar and E. C. C.[14], which readily allow protoplasts to be pushed together while floating freely in sodium nitrate solution.

Similar fusion of protoplasts isolated from tobacco callus (*Nicotiana tabacum* var. P19) using a cellulase and 'Macerozyme' mixture has also been observed. It has also been found possible in this laboratory to isolate protoplasts from both cereal root tips and tobacco callus using a considerably purer enzyme preparation than that from Onozuka—purified *Trichoderma viride* cellulase (Miles Laboratories Inc.)—with the added 'Macerozyme' or pectinase; and it is known that it is possible to isolate tomato fruit protoplasts using a purified pectinase[15]. These findings should facilitate further studies of the cell wall regeneration and nuclear division of fused protoplasts, for, as discussed before[9], the use of such purified mixtures of cell wall degrading enzymes will probably be essential for cell wall regeneration and subsequent division. It should be noted that when mechanically isolated moss protoplasts are maintained in culture, cell wall readily regenerates, and whole plants readily form after protonemata have developed[16]. Eriksson[17] has reported limited nuclear division of enzymatically isolated protoplasts from *Haplopappus* and also some evidence of cell wall regeneration.

Protoplast fusion of the type we have described must not be compared with the development of the spontaneous fusion bodies formed after wall degradation within the cell symplasm of any tissue (Fig. 1*e*). These consist of two or more morphologically similar protoplasts, and, although they occur when isolation is carried out using an enzyme mixture in 0·56 M sucrose followed by washing with 0·3 M sucrose, they are much more frequent when enzyme treatment in 0·56 M sucrose is followed by washing with 0·25 M sodium nitrate. Similar fusion bodies have also been observed during the isolation of soybean protoplasts (O. Gamborg and R. Miller, personal communication). Such fusions are of little practical significance in that they always occur between protoplasts from a single tissue, whereas the fusion of isolated protoplasts from different tissues and species in the way we have described may provide entirely new genetic situations.

Value of Totipotency

Isolated meristematic protoplasts of plants seem closely to resemble cultured animal cells. By contrast with animal cells, however, the totipotent nature of plant cells[18] will now enable attempts to regenerate whole plants from inter-species heterokaryons and from the true hybrid cells that may be obtained ultimately. It is interesting that no added virus, such as inactivated Sendai virus, is required for the reproducible and controlled fusion of isolated plant protoplasts, although this has been necessary for the extensive fusion of animal cells in culture[19]. It is difficult to explain the action of sodium nitrate in inducing fusion, following plasmolysis of plant protoplasts in sugars such as sucrose. But changes in the electrical properties of the plasmalemma are probably involved, for there is a close parallel between the fusion phenomenon we have observed and the well known effect of sodium ions on the electrical excitability of frog muscle after treatment with sucrose, glucose or mannitol[20]. Furthermore, because freely isolated vacuoles are known to fuse[21] when maintained in sucrose, it may well be that the surface charge and fusion characteristics of the tonoplast differ from those of the plasmalemma.

Although continued examination of protoplast fusion is necessary, we now know that it can be achieved readily. The next stage in the development of procedures for the somatic hybridization of plants must be the study of the enzyme systems involved in cell wall degradation, and regeneration after protoplast fusion. It may, for example, be necessary to use competitive substrates for the effective removal of adsorbed enzymes from the protoplast. Whatever the outcome of future investigations it is nevertheless true that the two first steps towards the production of somatic plant hybrids can be taken. Protoplasts can be isolated in a state that allows fusion and interspecific fusions can take place.

This work was supported by a grant from the Agricultural Research Council. One of us (J. B. P.) is in receipt of an SRC research studentship. We thank Dr R. Riley for helpful discussions.

The contents of this article are covered by a UK patent application, the rights in which have been assigned to NRDC.

Received February 5, 1970.

[1] Torrey, J. G., and Nickell, L. G., *Science*, **166**, 1068 (1969).
[2] Küster, E., *Ber. Deutsch. Bot. Ges.*, **27**, 589 (1909).
[3] Michel, W., *Arch. Exp. Zellforsch.*, **20**, 230 (1937).
[4] Hofmeister, L., *Protoplasma*, **43**, 278 (1954).
[5] Klerker, J. A. F., *Ofvers. Vet.-Akad. Förhdl.*, **9**, 463 (1892).
[6] Cocking, E. C., *Nature*, **187**, 927 (1960).
[7] Gregory, D. W., and Cocking, E. C., *J. Cell Biol.*, **24**, 143 (1965).
[8] Schenk, R. U., and Hildebrant, A. C., *Crop. Sci.*, **9**, 629 (1969).
[9] Power, J. B., and Cocking, E. C., *J. Exp. Bot.*, **21**, 64 (1970).
[10] Pinto Da Silva, P. G., *Naturwissenschaften*, **56**, 41 (1969).
[11] Ruesink, A. W., and Thimann, K. V., *Science*, **154**, 280 (1966).
[12] Yoshida, Y., *Plant and Cell Physiol.*, **2**, 139 (1961).
[13] Plowe, J. Q., *Protoplasma*, **12**, 196 (1931).
[14] Pojnar, E., and Cocking, E. C., *Nature*, **218**, 289 (1968).
[15] Cocking, E. C., and Pojnar, E., *J. Gen. Virol.*, **4**, 305 (1969).
[16] Binding, H., *Z. Pflanzenphysiol. Bd.*, **55**, 305 (1966).
[17] Eriksson, T., and Jonasson, K., *Planta*, **89**, 85 (1969).
[18] Steward, F. C., *Plant Physiology*, 8 (Academic Press, 1969).
[19] Harris, H., *Proc. Roy. Soc.*, B, **166**, 358 (1966).
[20] Höber, R., *Physical Chemistry of Cells and Tissues*, 289 (Churchill, London, 1950).
[21] Gregory, D. W., and Cocking, E. C., *J. Exp. Bot.*, **17**, 68 (1966).

Ethylene, the Natural Regulator of Leaf Abscission

by
MICHAEL B. JACKSON
DAPHNE J. OSBORNE
Department of Agricultural Science
and ARC Unit of Experimental Agronomy,
University of Oxford

Natural leaf fall is triggered by increased ethylene production in senescing cells close to abscission zones.

ETHYLENE is increasingly thought of as an integral part of plant auto-regulation[1]; a concept originating from the discovery that ethylene was a natural product of plant metabolism[2,3]. More recently, the use of gas chromatography has shown that many plants can synthesize the gas, and a precise control mechanism involving ethylene has been described for plumular hook opening in the germinating pea seedling[4]. Although ethylene has been shown to be a potent accelerator of leaf abscission, there is no clear evidence correlating the pattern of ethylene production *in vivo* with the control of abscission. Indeed, Abeles[5] has concluded that the timing of abscission is determined not by changes in the rate of production, but rather by an increase in the sensitivity of the tissue to ethylene.

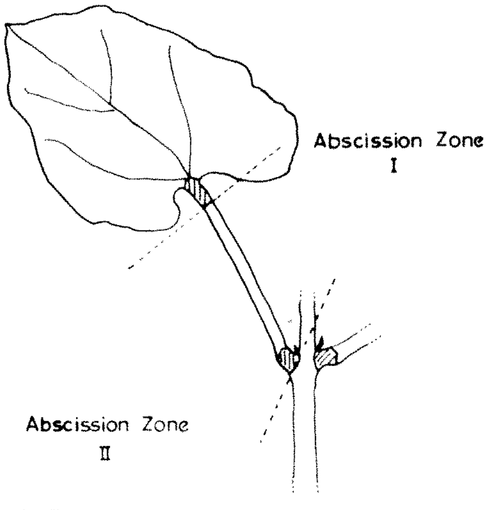


Fig. 1. The two abscission zones of a primary leaf of *Phaseolus vulgaris* (L).

Excised abscission zones (explants) are widely used in studies of abscission[5,6], and are usually kept in closed containers at high humidity. Our results show that unless precautions are taken to avoid accumulation of ethylene in the containers, the time of abscission is artificially accelerated and the relationship between ethylene production and abscission is obscured. Here we report experiments in which the ethylene produced was removed continuously by chemical absorption. They reveal a clear relationship between abscission and ethylene production. These and other experiments demonstrate that an increase in the rate of ethylene production after the onset of sensitivity to ethylene can be closely correlated with the time of abscission.

The plant material chosen for much of this work is similar to that used in previous studies of abscission[6]. Plants of *Phaseolus vulgaris* (L), variety 'Canadian Wonder', were grown for 14–18 days in a greenhouse with supplementary lighting. Tissue was taken from the first and second abscission zones of the primary leaves (Fig. 1) and the patterns of ethylene production and timing of abscission studied. Tissue was removed from the first abscission zone either as segments (explants) 1 cm in length and including the whole pulvinus, petiole tissue and the abscission zone, or as individual segments of pulvinus and petiolar tissue. Excised second abscission zone material consisted of stem, pulvinar and petiolar segments. We also studied the ethylene production in petioles from *Prunus serrulata senriko* (Lindl.) and *Parthenocissus quinquefolia* (Planch).

We first describe experiments with explants from abscission zone I. Twenty such explants were placed in 15 ml. Erlenmeyer flasks and sealed with plastic vaccine caps. They were used for (*a*) treating explants with ethylene, (*b*) following the visual progress of separation, and (*c*) collecting evolved ethylene. Concentrations of ethylene were estimated by flame-ionization gas chromatography every 12 h, or more frequently when required. The progress of abscission was followed in explants incubated in the presence or absence of an ethylene absorbant. For this purpose a tube containing 1 ml. of 0·2 M mercuric perchlorate in 0·2 M perchloric acid was inserted into each flask, obviating the artificial accumulation of gas in the collection flasks and within the explants. Flasks were kept in the dark at 25° C and, after each determination, ventilated with compressed air and resealed. After abscission, petiolar and pulvinar pieces were separated and their ethylene production determined independently. The patterns of abscission and ethylene production both before and after abscission are shown in Fig. 2*a*. The important features are: (1) a high rate of ethylene production confined to the first 12 h after excision; (2) a subsequent period of low ethylene production ($< 0{\cdot}25$ nl./g/h) lasting for some 90 h; towards the end of this period abscission takes place in explants incubated without mercuric perchlorate; (3) a later period of high ethylene production lasting about 90 h during which explants in the presence of mercuric perchlorate abscind at point X (Fig. 2*a*). Fig. 2*a* shows that most of this ethylene, produced between 100–190 h, is synthesized by the pulvinus while production by the petiole remains low. The petiole does, however, produce a peak of ethylene, at a later time (Fig. 2*b*). We suggest that the ethylene peaks shown in Fig. 2*b* are a feature of the tissues at a particular stage of senescence. It is already known that senescence in petiolar tissue is slower than that of the pulvinus. For example, the ability to synthesize new protein after excision falls more rapidly in the pulvinus than in the petiole[6].

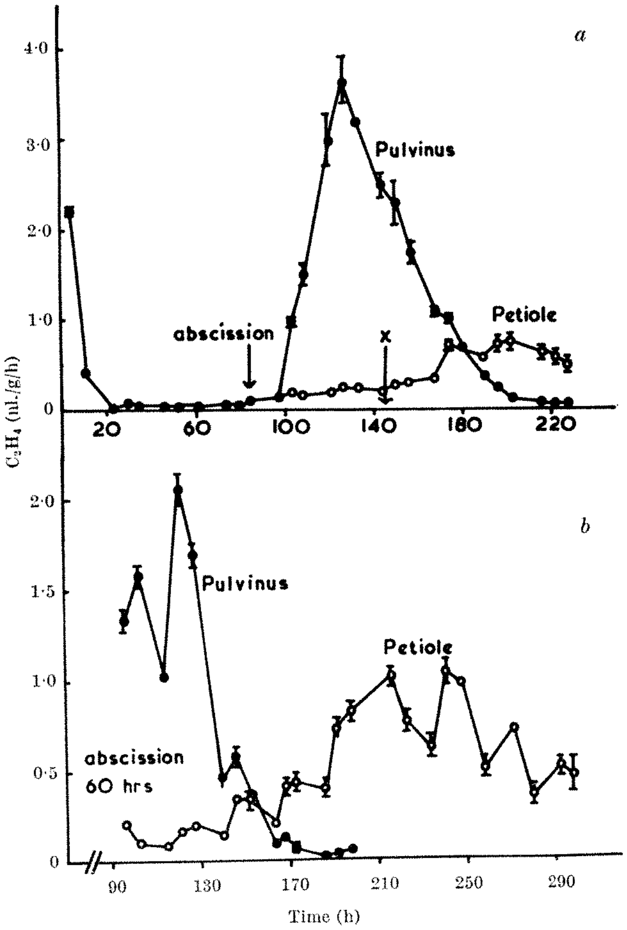


Fig. 2. *a*, Ethylene production by abscission zone I explants of *Phaseolus vulgaris* (L), before and after abscission. (Incubated in the absence of 1 ml. 0·2 M mercuric perchlorate.) Point X shows the time of abscission for explants incubated *with* 0·2 M mercuric perchlorate. *b*, Ethylene production by the petiole and pulvinar parts of explants during a later period (vertical lines represent *S.E.*). 1 ml. gas samples were withdrawn from collection flasks and injected into a Pye 'series 104' gas chromatograph fitted with a hydrogen flame ionization detector. The 61 cm glass column contained 7·5 g of 100–150 mesh activated silica gel and carried nitrogen flowing at 21·5 ml./min. Authenticity of the ethylene peak was determined using ethylene standards, retention times and mercuric perchlorate absorption. The concentrations of injected ethylene were proportional to peak height over the concentration ranges measured. At a column temperature of 132° C the retention time for 1 nl. of ethylene was 85 sec. Values above 0·04 nl./ml. could be accurately determined and lower levels detected. The results are expressed as nl./g (fresh) wt/h.

We interpret the results of Fig. 2 in the following way. The abscission time observed 80 h after excision is certainly an artefact resulting from the accumulation of ethylene in the flasks between ventilation times, for if ethylene is removed by mercuric perchlorate absorption, separation does not occur until 140–150 h (point X). Ethylene production by the pulvinus is then high (3·0 nl./g/h) and sufficient to raise the concentration of ethylene within the explant to abscission promoting levels. Results of experiments on the pattern of explant sensitivity to ethylene support and extend these interpretations.

The effects of supplying ethylene for precise intervals were determined as follows. Explants were incubated in the presence of mercuric perchlorate as described before. Every 12 or 24 h two flasks of twenty explants were selected at random and treated with 2 p.p.m. ethylene for two consecutive 12 h periods by removing the mercuric perchlorate and injecting through the vaccine cap. The results in Table 1 demonstrate the following points. Abscission is not accelerated by the added ethylene during the first 24 h after excision. After this period of insensitivity, explants become fully sensitive to ethylene and remain sensitive *throughout* the subsequent 132 h incubation with mercuric perchlorate. Abscission can be induced during the period of low ethylene production (24–100 h, Fig. 2*a*) by any 24 h period in 2 p.p.m. ethylene (Table 1). The low production between 24 and 100 h is alone insufficient to cause abscission, even though the tissue is sensitive to ethylene. Abscission can occur during this time only if ethylene is left to accumulate in the flasks or if additional ethylene is supplied. In the presence of mercuric perchlorate (Table 1), abscission occurs at 156 h; separation at this time can be correlated causally with the rise in ethylene production by the pulvinus commencing some hours before.

Table 1. EFFECTS OF SUPPLYING 2 P.P.M. ETHYLENE AT DIFFERENT TIMES ON THE SEPARATION OF EXPLANTS (*Phaseolus vulgaris* (L))

Ethylene treatment time (h)	Time to 50 per cent separation (h)
0– 12	144
12– 24	144
12– 36	48
24– 48	48
36– 60	60
48– 72	60
60– 84	71
72– 96	96
96–120	120
120–144	144
No ethylene	156

When not exposed to ethylene, the explants were enclosed with 0·2 M mercuric perchlorate in 0·2 M perchloric acid.

Ethylene produced during the first 12 h after excision was found to have no influence on the time of abscission, and therefore will be discussed only briefly. Precise measurements during the first 24 h show that ethylene production rises steeply after half an hour, is greatest by 1·5–2·0 h and then declines to a basal level after 12 h (Fig. 3). That the eighteen-fold maximum is reached only after 1·5–2·0 h shows that this is not a sudden release of accumulated ethylene from the tissue but is most probably a wound response. The amount of ethylene produced can be shown to be directly related to the extent of cutting (wounding). Pieces of 2 cm petiole, sliced into one, two or three pieces to expose two, four or six cut surfaces, synthesize amounts of ethylene proportional to the number of cuts made. In one experiment the rates of production of ethylene in the first 2–4 h period were

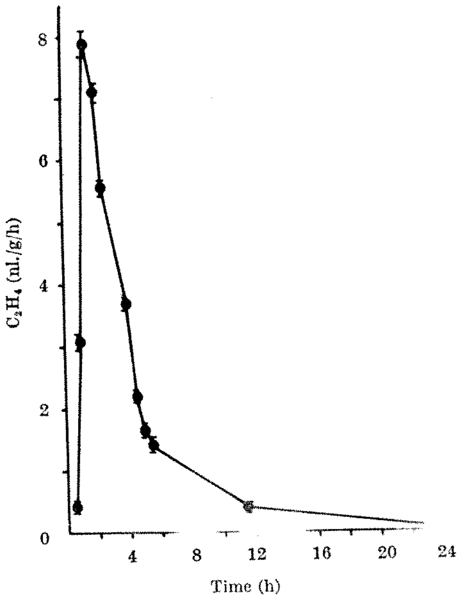


Fig. 3. Ethylene production during the first 24 h after excision of abscission zone I explants of *Phaseolus vulgaris* (L). (Vertical lines represent *S.E.*)

2·94 (±0·01), 6·85 (±0·63) and 8·38 (±0·78) nl./g/h respectively.

To determine whether the wound ethylene is involved in timing abscission in explants, the build-up of ethylene was permitted or reduced during the time of wound ethylene production. Explants were enclosed in collection flasks with 1 ml. vials of 0·2 M mercuric perchlorate. In this way, by securing the maximum possible diffusion gradient between the enclosed tissue and the surrounding atmosphere in the flasks, a reduction in the endogenous level of ethylene within the explant was possible. By omitting or supplying mercuric perchlorate for strategic intervals, it was possible to assess the activity of the wound ethylene. Treatments designed to reduce concentrations of ethylene in the tissue during the first 24 h after excision fail to delay separation when compared with control *A* (Table 2). Similarly, treatments that allow the maximum possible build-up of the wound ethylene in the tissue during the same period do not promote separation when compared with the appropriate control *B* (Table 2). We therefore conclude that the potentially interfering production of wound ethylene does not influence abscission in these explants. Further, applied ethylene does not promote separation unless administered 24 h after excision (Table 1), suggesting again that abscission of the explant is not regulated by any ethylene present before about 24 h have elapsed. In other words, the explant possesses an initial period of insensitivity to ethylene.

Table 2. EFFECTS OF ABSORBING WOUND ETHYLENE WITH 0·2 M MERCURIC PERCHLORATE IN 0·2 M PERCHLORIC ACID ON THE SEPARATION OF EXPLANTS (*Phaseolus vulgaris* (L.))

Treatment	Time to 25 per cent separation (h)
No absorption of ethylene (control *A*)	72
Absorption of ethylene 0–12 h	72
Absorption of ethylene 12–24 h	72
Absorption of ethylene 0–24 h	72
Continuous absorption of ethylene (control *B*)	144
No absorption of ethylene 12–24 h	> 144
No absorption of ethylene 0–24 h	144

We have obtained direct evidence that a rise in ethylene production precedes the normal shedding of leaves. Ethylene was collected from tissue excised from bean plants at three different ages: stage 1, plants entirely non-senescent; stage 2, plants with yellowing primary leaves but showing no sign of abscission; stage 3, plants with yellow primary leaves readily detached by light pressure. The whole pulvinus and a 1 cm piece of adjoining petiole were removed separately from abscission zone I. The whole pulvinus, 0·5 cm of adjoining stem and 1 cm of adjoining petiole were removed separately from abscission zone II. Ethylene production by these pieces was monitored in the usual way and the results are shown in Figs. 4 and 5. In first abscission zone tissue the non-senescent petiole and pulvinus produce only very small amounts of ethylene after the initial wounding effect. Ethylene production by these pieces from the yellowing but non-abscinding primary leaves was much greater and increased during the course of the experiment, while post-abscission material produced large but declining concentrations of ethylene. A rise in ethylene production by tissues surrounding the first abscission zone therefore occurs before abscission, and the rate declines only when separation is complete. Results for abscission zone II are very similar to those described for abscission zone I (Fig. 5). Ethylene production by petiolar and pulvinar tissue distal to the abscission zone rises only when the leaves are yellowing and before separation; production by these tissues falls only after separation is complete. There is no change in the rate of ethylene production by stem tissue proximal to the zone; the rate remains low at 0·07–0·11 nl./g/h throughout.

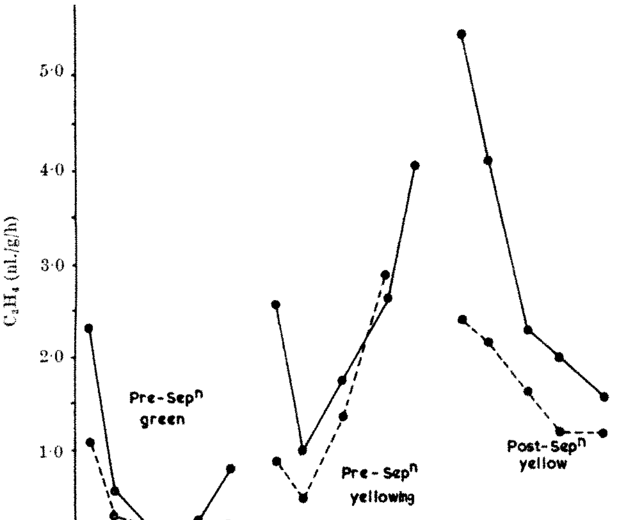


Fig. 4. Ethylene production by segments of petiole (● - - - ●) and pulvinus (●—●) adjacent to abscission zone I taken from primary eaves of *Phaseolus vulgaris* (L) at three ages: (1) pre-separation, non-senescent (green); (2) pre-separation senescing (yellowing); (3) post-separation, senescent (yellow).

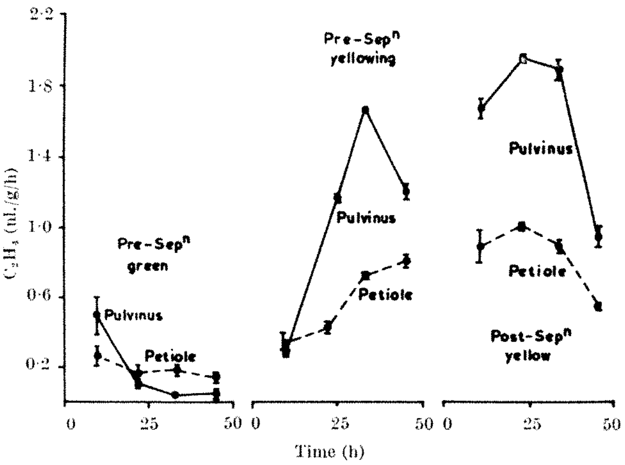


Fig. 5. Ethylene production by segments of petiole and pulvinus adjacent to abscission zone II taken from primary leaves of *Phaseolus vulgaris* (L) at three ages: (1) pre-separation, non-senescent (green); (2) pre-separation, senescing (yellowing); (3) post-separation, senescent (yellow).

The correlation between abscission and a rise in ethylene production has been extended to two deciduous woody species, *Prunus serrulata senriko* and *Parthenocissus quinquefolia*. Petiole segments from *Prunus* leaves in a green non-senescent state firmly attached to the tree, or in a yellow senescent state readily detached from the tree, were removed and enclosed in separate ethylene collection flasks. The quantities of ethylene produced were measured during a period of 135 h and are illustrated in Fig. 6. Ethylene production by yellow post-abscission petioles falls throughout the experiment. Production by the green pre-abscission petioles rises sharply after a period of low production, reaches a well defined maximum as the tissue becomes yellow and then declines. This situation is clearly very similar to that described for *Phaseolus*. Both species are readily defoliated by ethylene, both show a senescence related rise in ethylene production a short time before separation and a falling rate when separation is complete. Comparable but less extensive data are available from experiments with *Parthenocissus*. Concentrations of ethylene produced by petiole tissue adjacent to the proximal abscission zone in this species

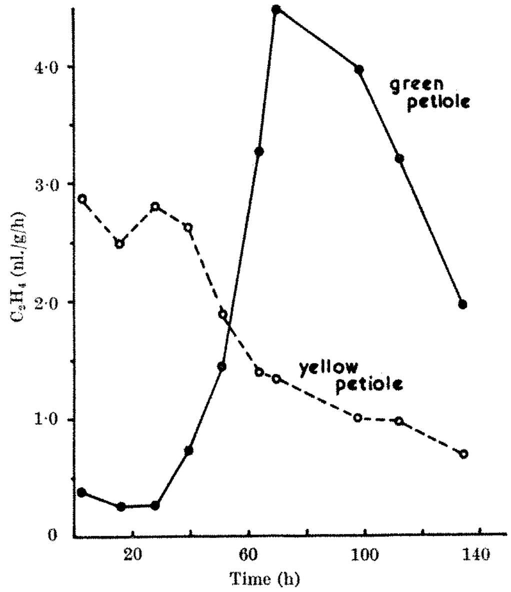


Fig. 6. Ethylene production by 2 cm petiole segments of *Prunus serrulata senriko* taken from leaves at two ages: (1) green—non abscinding; (2) yellow—abscinding. Sampled October 14, 1969.

are thirteen-fold higher at the time of abscission than some days before (Table 3). The results also show that most ethylene is produced by tissue close to the abscission zone, emphasizing again the importance of tissue adjacent and distal to the separating cells.

We present critical evidence that the timing of foliar abscission can be precisely related to the extent of ethylene production adjacent to the separation zone. In isolated abscission zones of *Phaseolus* (explants), so frequently used in abscission experiments, incubation techniques have hitherto obscured the role of endogenous ethylene production in the regulation of abscission. If accumulation of ethylene is precluded a clear relationship between increased ethylene production and abscission is obtained. No such accumulation occurs during natural leaf abscission in the open. In the three species studied, large quantities of ethylene are synthesized shortly before leaf fall in tissues adjacent to the zone of separation. We suggest that this ethylene production occurs when a particular stage of senescence is reached and that the ethylene initiates the biochemical sequences[6] responsible for separation.

Table 3. ETHYLENE PRODUCTION BY 1 CM SEGMENTS OF PETIOLE ADJACENT TO THE PROXIMAL ABSCISSION ZONE FROM THE LEAVES OF *Parthenocissus quinquefolia* (PLANCH) AT TWO STAGES: (1) PRE-ABSCISSION; (2) ABSCINDING

Time (hours)	Stage	Ethylene nl./g/h (± *S.E.*) Distance from the abscission zone		
		2–3 cm	1–2 cm	0–1 cm
0–12	Pre-abscission	0·08 ± 0·02	0·09 ± 0·02	0·06 ± 0·02
	Abscinding	0·47 ± 0·10	0·60 ± 0·13	0·82 ± 0·03

We thank Professor G. E. Blackman for his constant interest and advice, and one of us (M. B. J.) thanks the Ministry of Agriculture, Fisheries and Food for a postgraduate studentship.

Received December 8, 1969.

[1] Osborne, D. J., *Soc. Chem. Ind. Monograph*, **31**, 236 (1968).
[2] Cousins, H. H., *Jamaica Dept. Agric. Ann. Rep.*, **7**, 15 (1910).
[3] Gane, R., *Nature*, **134**, 1008 (1934).
[4] Goeschl, J. D., and Pratt, H. K., in *Biochemistry and Physiology of Plant Growth Substances* (edit. by Wightman, F., and Setterfield, G.), 1229 (Runge Press, Ottawa, 1968).
[5] Abeles, F. B., *Physiol. Plant.*, **20**, 442 (1967).
[6] Osborne, D. J., in *Biochemistry and Physiology of Plant Growth Substances* (edit. by Wightman, F., and Setterfield, G.), 815 (Runge Press, Ottawa, 1968).

Effects of Actinomycin D and Cycloheximide on RNA and Protein Synthesis in Cleavage Stage Mouse Embryos

by
RICHARD J. TASCA*
NINA HILLMAN
Department of Biology, Temple University, Philadelphia, Pennsylvania

In the earliest stages of the development of mouse embryos, RNA synthesis can be blocked without inhibition of protein synthesis; but stopping protein synthesis depresses RNA synthesis.

THERE have been several reports on the effects of actinomycin D on the morphological development of cleavage stage mouse embryos[1-4]. These studies show that low concentrations of actinomycin D ($1-2 \times 10^{-4}$ μg/ml.) have no effect on the rate of cleavage or on blastocyst development. Intermediate concentrations (6×10^{-4} to 1×10^{-3} μg/ml.) do not interfere with cell division during cleavage but prevent blastocyst differentiation (expansion, elongation, hatching) when the antibiotic treatment begins at the late four cell or older stages. Cells of embryos treated with higher concentrations ($> 0·08$ μg/ml.) undergo no more than one additional mitotic division before development is arrested.

Autoradiographic and cytochemical studies have shown that intermediate concentrations of actinomycin D cause a reduction of nucleolar RNA synthesis, while higher concentrations reduce, or block, all nuclear RNA synthesis[2]. There is only a slight reduction of protein synthesis as a result of these treatments. The sensitivity of cleavage stage embryos to a protein synthesis inhibitor (puromycin) has been described[1]. These studies show that there is a correlation between the dosage of puromycin and the stage at which development is blocked. There have been, however, no detailed biochemical analyses to determine the effects of antibiotics on RNA and protein syntheses during early mouse development. The present study reports the effects of actinomycin D and cycloheximide on the rates of RNA and protein synthesis in two cell to morula (sixteen to thirty-two cells) stage mouse embryos.

For this study, large numbers of synchronously developing, two cell embryos were obtained from 10–12 week old superovulated[5] albino females which had been mated to albino males. The embryos were placed in mouse embryo culture medium[6] in which they developed from the two cell stage to the blastocyst (more than thirty-two cells) stage in approximately two days. The two cell embryos were allowed to develop to specific cleavage stages and were then incubated in either ^{3}H-5-uridine (6 μCi/ml., 20–25 Ci/mmole, Schwartz) or in ^{14}C-leucine (0·6 μCi/ml., 170–200 mCi/mmole, Schwartz) for a 3 h period in the presence or absence of actinomycin D or cycloheximide. The embryonic stages treated were: late two cell (L2); late two cell–early four cell (L2E4); early four cell (E4); late four cell (L4); eight to sixteen cell; and late morula (LM, sixteen to thirty-two cells). After the 3 h incubation those embryos to be used for biochemical studies were counted, rinsed four times with culture medium, collected in 20 μl. of Earle's BSS, frozen in solid CO_2 and acetone and stored at −70° C. The remaining embryos were washed, placed into unlabelled culture medium and allowed to continue development so that the effects of the drugs on development could be determined. Each experiment was repeated at least three times.

---

* Present address: Department of Biological Sciences, University of Delaware, Newark, Delaware 19711.

A modified Schmidt–Thannhauser[7] technique was used for extracting the embryos. After thawing the embryos, 25 μl. of cold distilled water was added, and the embryos were ruptured by four freeze–thawings. Cold 1·6 M perchloric acid (PCA) was added to obtain a final concentration of 0·3 M PCA. This treatment was followed by ribonuclease (0·2 per cent) digestion of the acid-insoluble precipitate and then hyamine hydroxide digestion of the residual ribonuclease resistant pellet. All centrifugations were performed at 13,000 r.p.m. for 20 min and all precipitates were washed twice. These steps provided the following three fractions: acid soluble; acid insoluble, ribonuclease sensitive (RNA fraction); acid insoluble, ribonuclease resistant, hyamine hydroxide soluble (protein fraction). The amount of label contained in each fraction was determined by liquid scintillation counting. The total of the counts of the acid soluble fraction (unincorporated precursor), RNA fraction and protein fraction accounts for all of the radioactive label which enters the embryos and is designated as ^{3}H-5-uridine uptake or ^{14}C-leucine uptake.

In initial experiments it was found that the ^{3}H-5-uridine uptake (in c.p.m./100 embryos) increased by three-fold between the L4 stage and eight to sixteen cell stage, and by four-fold between the eight to sixteen cell stage and morula stage (Fig. 3). For this reason, the rates of RNA and protein synthesis are here expressed as "percentage incorporation", or the total number of counts in the RNA or protein fraction/total uptake of precursor × 100. The data are expressed as percentage incorporation so that syntheses of different stages may be compared despite permeability differences which may be reflexions of changes in the endogenous precursor pool sizes (not yet determined in mouse embryos). Treating the data in this way, it was found that the rates of synthesis are characteristic for each stage, for the percentage incorporation does not fluctuate significantly when the uptake of the precursors is changed by incubating the embryos with different amounts of radioactivity: ^{3}H-5-uridine (1·5–25 μCi/ml.) or ^{14}C-leucine (0·3 to 0·6 μCi/ml.).

The rate of RNA synthesis increases progressively in cleavage stage mouse embryos from 8 per cent at the late two cell stage to 30 per cent at the morula stage (RNA controls, Fig. 1). The largest increase (9 per cent) occurs between the eight to sixteen cell stage and the morula stage. Recently, it was reported that high molecular weight RNA synthesis is detectable as early as the two cell stage, and ribosomal RNA (rRNA) synthesis at the four cell stage[8]. Moreover, rRNA synthesis has been shown to increase at the eight cell and morula stages[9]. The increases can be correlated with a progressive hypertrophy of the pars granulosa of the nucleoli at each successive developmental stage[10]. Granular nucleoli are first present at the L2 stage, suggesting that part of the RNA which is synthesized at this stage is rRNA or rRNA precursor molecules.

Evidence which supports the above findings, that rRNA is synthesized in early mouse embryos, is provided by actinomycin D experiments (Fig. 1). Actinomycin D (0·0008 μg/ml.) reduces the rate of RNA synthesis at all stages. The rate of synthesis is reduced slightly at the L2–E4 stage (approximately 2 per cent). This reduction becomes greater through the cleavage stages until the morula stage when the percentage incorporation is about 14 per cent (16 per cent reduction from the normal value). Because low concentrations of actinomycin D preferentially inhibit rRNA synthesis[11], it is probable that most, if not all, of the mouse embryo RNA synthesis which is inhibited by 0·0008 μg/ml. actinomycin D is rRNA. Although this concentration of antibiotic allows embryos, regardless of stage of treatment, to form blastocysts, treatment at the eight to sixteen cell stage and at the morula stage prevents blastocyst differentiation (present work and others)[3]. A higher drug concentration (0·1 μg/ml.), which inhibits cell division, severely inhibits RNA synthesis at all stages (Fig. 1).

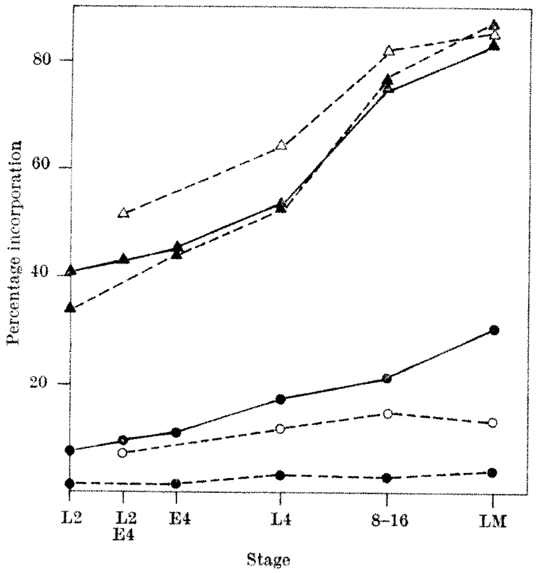


Fig. 1. Effects of actinomycin D on the rates of RNA and protein synthesis in cleavage stage mouse embryos. ^{14}C-leucine: ▲—▲, control; △---△, +0·0008 μg/ml. actinomycin D; ▲---▲, +0·1 μg/ml. actinomycin D. ^{3}H-5-uridine: ●—●, control; ○---○, +0·0008 μg/ml. actinomycin D; ●---●, +0·1 μg/ml. actinomycin D.

When rates of protein synthesis in mouse embryos are measured (controls, Fig. 1), it can be seen that 41 per cent of the ^{14}C-leucine which enters the two cell stage embryos is incorporated into PCA precipitable material during a 3 h treatment. This level of incorporation increases only slightly through the four cell stage. Between the L4 and eight to sixteen cell stages there is a major increase in the rate of protein synthesis (23 per cent higher than the rate at the L4 stage). This increase coincides with a great increase in the numbers of cytoplasmic polyribosomes noted in ultrastructural studies[10,12], and is in agreement with autoradiographic data[2]. The rate of protein synthesis increases to 83 per cent incorporation at the morula stage. These rates of protein synthesis are not altered during the exposure to actinomycin D (either 0·0008 μg/ml. or 0·1 μg/ml., Fig. 1), except for a slight reduction at the two cell stage (0·1 μg/ml.) and stimulation at the two cell and four cell stages (0·0008 μg/ml.). Actinomycin treatment of 0·1 μg/ml., which almost completely blocks RNA synthesis at each stage, does not reduce the rate of protein synthesis (Fig. 1).

Cycloheximide (0·5 μg/ml.) treatment decreases the rate of protein synthesis by at least 30 per cent at all stages (Fig. 2), but does not interfere with development. (Regardless of the cleavage stage at which treatment is applied, reincubated embryos continue to form blastocysts which differentiate normally.) The reduction in the rate of protein synthesis (absolute decrease in per-

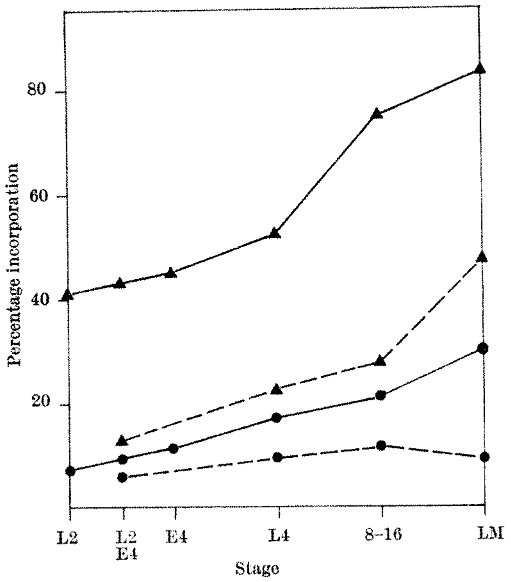


Fig. 2. Effects of cycloheximide (0·5 μg/ml.) on the rates of RNA and protein synthesis in cleavage stage mouse embryos. Embryos were incubated for 3 h at each stage in either 6 μCi/ml. ^{3}H-5-uridine (●) or 0·6 μCi/ml. ^{14}C-leucine (▲) in the presence (- - -) or absence (———) of cycloheximide.

centage incorporation) is approximately the same at each stage, except for a slightly greater inhibition at the eight to sixteen cell stage (Fig. 2). In addition to this effect on protein synthesis, cycloheximide reduces the rate of RNA synthesis, leucine uptake and, to a lesser extent, uridine uptake (Figs. 2 and 3). In each of these cases, the reductions are greater at the morula stage than at the earlier stages.

More detailed information concerning the effects of cycloheximide on RNA synthesis was obtained through sucrose density gradient analysis of drug-treated, ^{3}H-5-uridine labelled, morula stage embryos. Fig. 4 shows that the two major species of rRNA sediment at the same relative positions in both control and experimental gradient analyses. A quantitative analysis of the relative amount of 30–32S rRNA and 18S rRNA, in comparison with the total amount of new RNA synthesis (determined by perchloric acid (PCA) extraction), shows that although the cycloheximide treatment causes an 11 per cent reduction (15 per cent to 4·1 per cent) in rRNA synthesis, there is a reduction of RNA synthesis (PCA extraction) of 21 per cent. This latter reduction cannot be accounted for by the 11 per cent decrease in the rate of rRNA synthesis. Thus in cleavage stage mouse embryos, cycloheximide affects RNA synthesis in general, and is not specific for rRNA as opposed to reports on other systems[13].

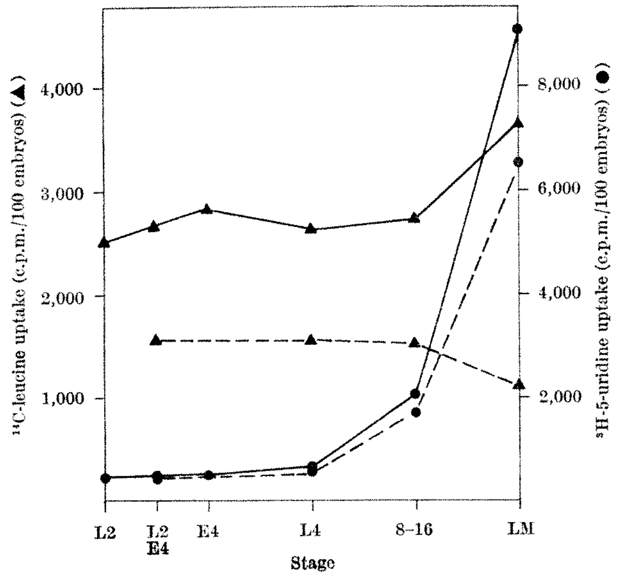
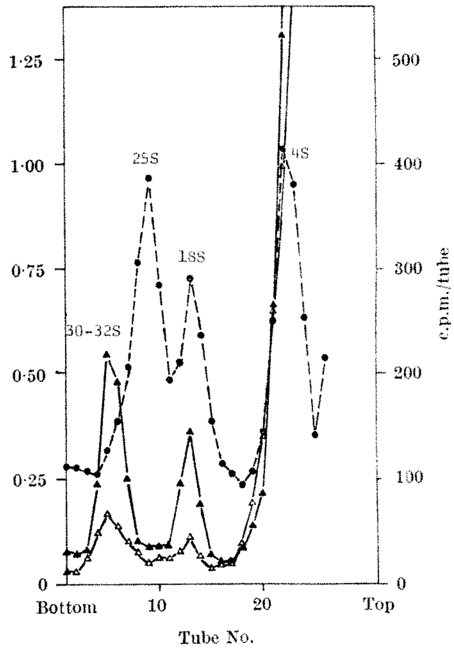


Fig. 3. Effects of cycloheximide (0·5 μg/ml.) on uptake of ^{3}H-5-uridine and ^{14}C-leucine by cleavage stage mouse embryos. The points on the graph represent the total uptake of precursors from the experiments shown in Fig. 2. The symbols are the same as those in Fig. 2.



	Percentage 30–32S rRNA	Percentage 18S rRNA	Total PCA extracted RNA (percentage incorporation)
Control (▲)	10	5	30
Cycloheximide (△)	3	1	10

Fig. 4. Inhibition of morula stage RNA synthesis by cycloheximide (0·5 μg/ml., 3 h). Sucrose density gradient analysis. Techniques for labelling, collecting and storing the embryos are the same as those described in the text. 25S, 18S (wheat germ rRNA, Calbiochem) and 4S (stripped calf liver sRNA, General Biochemicals) were used for the absorbance reference peaks (●). Thawed embryos were lysed in 0·5 ml., 0·01 M Tris-HCl (pH 7·4), 1 per cent sodium dodecyl sulphate, 100 μg/ml. polyvinyl sulphate. The lysate was placed on top of a 16·5 ml., 5–20 per cent sucrose gradient (prepared in 0·01 M sodium acetate, pH 5·0, 0·1 M NaCl, 0·1 mM EDTA) and the tubes were centrifuged at 24,000 r.p.m. for 18 h, 20° C, in a Spinco L2-65 ultracentrifuge (SW 25·3 rotor). Nine drop fractions were collected from the bottom of the gradient, the absorbance at 260 nm recorded for each fraction, and radioactivity assayed in a Packard 'Tri-Carb' liquid scintillation counter. The amount of radioactivity under each rRNA peak is summated and expressed as a percentage of the total uptake of ^{3}H-5-uridine by the embryos. The radioactivity at the top of the gradients is largely acid-soluble material. The data for total PCA extracted RNA, expressed as percentage incorporation, are derived from the experiments shown in Fig. 2.

In summary, the lower concentration of actinomycin D neither blocks cell division nor blastocyst differentiation when applied at the two cell or four cell stages. This is reasonable when one considers that rRNA synthesis is either non-detectable or at relatively low levels at these early stages[8,9,14], and may not be needed for early cleavage stage protein synthesis. The greater inhibition of RNA synthesis in the later stages probably reflects the normally increased rates of rRNA synthesis at these stages[8,9]. The inhibition of rRNA synthesis may be responsible for the blockage of blastocyst differentiation in embryos which were treated at the eight cell and morula stages.

The use of 0·1 μg/ml. of actinomycin D shows that concurrent RNA synthesis is not necessary for protein synthesis during mouse cleavage. This suggests that the messenger, transfer and ribosomal RNAs which are required for protein synthesis are stable for at least 3 h at each stage. This same concentration of actinomycin D, however, blocks cell division. Although this blockage may be the result of the almost total inhibition of RNA synthesis, high concentrations of the drug are known to damage chromatin material[4,15]. This effect could also be responsible for the cessation of cell division.

The cycloheximide experiments show that normal rates of protein synthesis are necessary for the maintenance of the normal rates of RNA synthesis and leucine and uridine uptake. The time between the inhibition of protein synthesis and the inhibition of transport and RNA synthesis may reflect the size of the available pool of proteins required for these processes. In the present instance, the rates of RNA synthesis and the transport of amino-acids and nucleosides are reduced in less than 3 h. Thus in normal conditions the required proteins must be newly synthesized within a 3 h period. The greater inhibition of both RNA synthesis and transport of precursors at the later cleavage stages may be caused by an increasing demand for certain proteins which are involved in these activities[16-20]. It is likely that newly synthesized ribosomal proteins are among those proteins required at the later cleavage stages, for these stages are characterized by large increases in the production of rRNA[8,9], the appearance of many cytoplasmic polyribosomes[10] and the incorporation of ^{3}H-leucine into the nucleolus[2]. Further studies should clarify some of the interrelationships between RNA and protein synthesis in cleaving mouse embryos.

This work was supported by a research grant and a training grant from the US National Institutes of Health.

Received August 18; revised December 4, 1969.

[1] Thomson, J. L., and Biggers, J. D., *Exp. Cell Res.*, **41**, 411 (1966).

[2] Mintz, B., *J. Exp. Zool.*, **157**, 85 (1964).

[3] Skalko, R. G., and Morse, J. M. D., *Teratology*, **2**, 47 (1969).

[4] Silagi, S., *Exp. Cell Res.*, **32**, 149 (1963).

[5] Edwards, R. G., and Gates, A. H., *J. Endocrinol.*, **18**, 292 (1959).

[6] Brinster, R. L., *Exp. Cell Res.*, **32**, 205 (1963).

[7] Schmidt, G., and Thannhauser, S., *J. Biol. Chem.*, **161**, 83 (1945).

[8] Woodland, H. R., and Graham, C. F., *Nature*, **221**, 327 (1969).

[9] Ellem, K. A. O., and Gwatkin, R. B. L., *Develop. Biol.*, **18**, 311 (1968).

[10] Hillman, N. W., and Tasca, R. J., *Amer. J. Anat.* (in the press).

[11] Perry, R. P., *Exp. Cell Res.*, **29**, 400 (1963).

[12] Hillman, N. W., and Tasca, R. J., *Proc. Twelfth Intern. Cong. Genetics, Tokyo*, Abstr. 7.5.1 (1968).

[13] Jackson, L. G., and Studzinski, G. P., *Exp. Cell Res.*, **52**, 408 (1968).

[14] Monesi, V., and Salfi, V., *Exp. Cell Res.*, **46**, 632 (1967).

[15] Fautrez-Firlefyn, N., and Fautrez, J., *Intern. Rev. Cytol.*, **22**, 171 (1967).

[16] Busch, H., *Histones and Other Nuclear Proteins* (Academic Press, New York, 1965).

[17] Chalkley, G. R., and Maurer, H. R., *Proc. US Nat. Acad. Sci.*, **54**, 498 (1965).

[18] Paul, J., and Gilmour, R. S., *J. Mol. Biol.*, **34**, 305 (1968).

[19] Mitchinson, J. M., and Cummins, J. E., *J. Cell Sci.*, **1**, 35 (1966).

[20] Pardee, A. B., *Nat. Cancer Inst. Monograph.*, **27**, 249 (1967).

Non-Darwinian Evolution: A Critique

by

ROLLIN C. RICHMOND

The Rockefeller University,
New York, NY 10021

The view that most evolutionary changes in DNA need not be attributed to natural selection rests on several lines of evidence. This article argues that the evidence leads more simply to a neo-Darwinian interpretation.

INVESTIGATING the genetic structure of natural populations of organisms, including man, has long been a goal of population geneticists. Understanding the genetic architecture of populations might make it possible to evaluate mathematical theories that predict the rates of frequency changes of genes within populations. Furthermore, such information might give at least a partial answer to a question that has interested man since the beginning of his interest in the biological world: what is the difference between species?

While biologists are a long way from such an achievement, molecular investigations of a number of proteins have begun to add to the data amassed by population geneticists during the past few decades[1]. The new molecular data[2-4] have brought into sharp focus the discrepancies between the predicted amounts of genetic variability, and postulated evolutionary rates on the one hand, and observed degrees of genetic variability on the other. Haldane[5], and later Kimura[6] and others, have argued that the substitution of one gene for another in a natural population places a burden or load on the population, because for the replacement to occur one individual must replace another (a genetic death, in Muller's terminology[7]) or leave relatively more progeny in a population of assumed constant size (substitutional load, in Kimura's terminology).

Data derived from studies of isozyme variability in *Drosophila*[2,3,8] show that natural populations of these species have polymorphisms at about 30 per cent of their structural gene loci, and an average individual can be expected to be heterozygous for some 12 per cent of his structural loci. Harris[9] and his colleagues have found similar degrees of variability after examining twelve enzyme systems present in human blood.

These data are incompatible with the model of evolutionary change involving the concepts of genetic death and substitutional load. If the observed genetic variability is maintained by natural selection, favouring individuals heterozygous for a number of loci, the maximum reproductive potential of a population would be reduced to an absurdly low value[2], even if one assumes only a 5 per cent loss in fitness of homozygous or nearly homozygous individuals. The model, of course, assumes that selection acts on each locus independently. It is clear that most natural populations are realizing a much higher reproductive potential than the theory would allow.

Several authors[10-14] have criticized these conclusions and pointed out that selection acts on whole organisms and populations of organisms rather than specific loci. Integrating this premise into Haldane's model makes it possible to account for the high amounts of genetic variability observed, and retain heterotic balance as a primary mechanism for maintaining the variability.

Evidence is rapidly accumulating on the primary structure of proteins in various classes of organisms. The *Atlas of Protein Sequence and Structure*[4] provides a valuable compilation of sequence data on many proteins and facilitates comparative studies of the evolution of proteins. As a result several authors have proposed that a new paradigm needs to be established to understand the observed variation in the presumed phylogenetic sequences of a number of proteins[15-17].

Their basic postulate is that most evolutionary changes in DNA need not be attributed to the action of natural selection. They claim that we know enough of the chemistry and physics of organic molecules to recognize parts of these structures that can be varied without altering their functional significance. In other words, some changes in DNA are adaptively neutral or undetectable by natural selection because the altered proteins or different tRNAs encoded have identical functions in the organism. Changes in DNA which are presumed to result in no selective advantage or disadvantage are the result of what has been termed "non-Darwinian" evolution[15].

Several lines of evidence have been brought together to uphold this conclusion. I intend to show here that the evidence can be interpreted more simply and validly within the framework of the neo-Darwinian or synthetic theory of evolution.

Synonymous Mutants

Because the genetic code is degenerate, some base pair changes in DNA do not result in the substitution of a different amino-acid into the encoded protein. This class of mutants, termed synonymous, has been cited as a prime example of neutral alterations in DNA[15,17]. Some recent findings make this idea questionable.

Several tRNAs are known to exist for some of the amino-acids, while the degeneracy of codons for other amino-acids is interpreted to mean that tRNAs are capable of recognizing several codons[18]. Suppose, for example, that the third position of a leucine codon has mutated from A to C. While still coding for leucine, another tRNA must be used in the synthesis of the encoded protein. For this synonymous mutation to be truly neutral, the various tRNAs bearing leucine molecules must be present within the cell, in equal supply, and the synthesis of each tRNA species must require approximately equal amounts of energy output by the cell. Caskey *et al.*[18], and others, have demonstrated that the first two of these three conditions do not hold at least for *E. coli*, yeast and mammalian liver (guinea-pig). Even if the conditions were met, one could not ignore the fact that regulatory molecules are known to bind to DNA[19] and alterations of the nucleotide sequences might affect the binding and subsequent function of these molecules. Such interference with a basic cellular mechanism can hardly result in a neutral effect for the organism.

The *mut-T* Gene of *E. coli*

Several authors[15,17] who postulate neutrality of many or most mutants have pointed out that a gene (*mut-T*) in *E. coli* has the effect of mediating AT to CG transversions within the bacterial DNA. Cox and Yanofsky[20] have investigated the effects of this gene continued for 1,200–1,600 generations on the GC content of *E. coli*. They found an increase of 0·4–0·7 per cent in the GC content, which corresponds well with that predicted from a knowledge of the mutation rates of known markers in strains bearing the *mut-T* gene. The authors note that the increased GC strains have apparently normal viability even though they are being grown on minimal medium.

These observations may be equally well or better interpreted on the basis of selection for those AT to CG transversions which are not deleterious to their carriers. A number of difficulties are, however, apparent in such an interpretation. First, the actual numbers of mutations accumulated over the course of the experiment cannot be estimated precisely, because the molecular weight of *E. coli* DNA is not exactly known. Second, as Cox and Yanofsky[20] point out, the reversion rate of *mut-T* mediated AT to CG transversion has not been taken into account. The presence of several chromosomes in an actively growing bacterial cell complicates the issue even further and would seem to make precise calculations difficult. While the increased GC strains are able to grow apparently normally, no attempt has been made to assess their ability to compete with strains that have a normal GC content and lack the *mut-T* gene. In conditions of competition, the frequency of the *mut-T* would give an indication of the fitness of the mutants induced in those bacteria carrying the gene. The adaptive value of the induced mutants could only be tested in a competitive situation.

Constancy of the Rate of Molecular Evolution

The initial observation that the rate of amino-acid substitution in cytochrome c was apparently linear with respect to time, and therefore apparently a result of some internal cellular process rather than representing the effects of a varying selection pressure, has been interpreted as evidence for the existence of neutral mutants[17,21]. The validity of this conclusion depends on estimates from palaeontological data of the times of divergence of various faunal groups. Although such data are available, they are subject to large errors and the conclusions based on them should not be taken as established[22]. Indeed, according to Simpson[23], the hypothesis of a constant rate of amino-acid substitution is not supported when the available data are plotted against the best estimates for the times of divergence of the species in question. Further, a comparison of the estimated number of amino-acid substitutions per codon for nine mammalian proteins (ref. 15, Table 1) reveals a wide range in the average number of substitutions. This would not be expected if a linear and constant rate of amino-acid substitution was the case.

Distribution of Changes in Proteins

A number of proteins contain amino-acid sites which have not apparently changed in phylogeny. These sites are assumed to be essential for the basic function of the molecule. Rare variants of these "invariant" sites which do occur in haemoglobin, for example, result in drastic alterations in the structure and function of the molecules[24]. Such "genetic diseases" are undoubtedly subject to severe selection. Many of the remaining sites in the haemoglobins and other proteins have changed in the course of evolution. It has been postulated[15] "that there is very little restriction on the type of amino-acid that can be accommodated at most variable sites". This conclusion is supported by the finding that the number of amino-acid changes per site agrees well with what is predicted if one assumes a Poisson distribution for the number of observed substitutions per site. Other interpretations of this finding are, however, possible.

Data compiled and analysed by Dayhoff[4] and others on the patterns of change in amino-acid substitutions in all the completely sequenced proteins show anything but a random pattern of substitution. Indeed, in a number of cases, the amino-acid most often substituted for the original amino-acid requires that more than one base of the original codon be altered. Such a pattern could be present only if natural selection has influenced the acceptance of amino-acid alterations.

For a substituted amino-acid to be of neutral selective value compared with the original amino-acid, a rather specific set of conditions must be met. The physiological functioning of the molecule must not be disturbed. Demonstration of apparent functional identity *in vitro* is not conclusive. The normal adult haemoglobin molecules of the gorilla and man bind oxygen with equal efficiency *in vitro*. Gorilla haemoglobin differs from human haemoglobin at two sites, one in the α chain, the other in the β chain[4]. Yet no man has ever been found with gorilla haemoglobin and at present no gorilla has been found with human haemoglobin (ref. 24, and personal communication from T. Dobzhansky).

Even if a substituted amino-acid were truly neutral in a functional sense, it is highly unlikely that factors involved in the synthesis of the protein would render the substitution neutral in the broader sense of the organism's integrated functioning. The substituted amino-acid must be present within the cell in equivalent quantity compared with the original amino-acid and, indeed, its synthesis or derivation from other molecules and transport into the cell require an equivalent amount of energy output. Amino-acids are not present within cells in equivalent quantities[25] and different mammalian organs show differences in concentration[26]. Investigation of the amino-acids present in human plasma shows a wide range of concentrations[27]. Further, the mechanisms for transport of amino-acids into cells further complicate the problem. The amino-acids vary in their ability to be transported across mucosal membranes and can be arranged into "transport families"[28]. Competition as well as facilitation of transport exist within and between such families.

Because mutations occur randomly, one would expect to find a Poisson distribution for any given set of genes or codons if the past history of mutation for each gene can be ascertained. The interesting and significant fact is not that the codons embodying the messages for globins, cytochromes c and immunoglobins G have mutated in a seemingly random fashion, but that the mutations that have been selected fall into non-random classes the composition of which can be understood from the physical and chemical properties of amino-acids.

Rates of Change of DNA and Protein

Measurement of the relative similarity in the nucleotide sequences of two sets of DNA by *in vitro* hybridization techniques has made it possible to estimate the divergence of two species based on DNA changes alone. If estimates of evolutionary rates are made from percentage changes in DNA sequences, values are obtained that are approximately one order of magnitude greater than those from estimates obtained from the rates of amino-acid substitutions in proteins[15]. This difference is interpreted as evidence for the presence of synonymous mutants which are neutral alterations in the DNA sequence. Considering the number of assumptions each estimate needs, the agreement would seem to be remarkably good.

But several other difficulties suggest themselves. Somewhat less than 50 per cent of the DNA of mammals studied occurs in the so-called repetitive sequences[29]. Further, parts of the genome are translated but never appear as cytoplasmic RNAs or structural protein. As Wallace[30], Stebbins[31] and, later, Britten and Davidson[29] have pointed out, much of the difference between higher species and faunal groups may reside in the dissimilarities of regulating mechanisms. Such differences are not represented in structural proteins from which estimates of evolutionary rates have been made.

Amino-acid Composition of Proteins

The hypothesis has been proposed that the amino-acid composition of protein is largely determined by the structure of the genetic code[15,32]. In other words, those amino-acids with the greatest number of codons (serine, leucine, arginine) are expected to be most frequent in proteins. If a plot of observed and expected amino-acid frequencies is made (expected frequencies are corrected for nucleotide frequencies in mRNA and for nonsense codons) for a large number of proteins, the correlation between predicted and observed values is very good (ref. 15, Fig. 1). This finding is interpreted as further evidence for the hypothesis " . . . that the average amino-acid composition of proteins reflects, more or less passively, the genetic code"[15]. The converse hypothesis, that natural selection influences the amino-acid composition of proteins, allegedly leads to the prediction that no relationship should exist between amino-acid frequencies and the structure of the code. Both of these expectations ignore the fact that the genetic code itself is a product of the evolutionary process.

A closer examination of the frequencies of occurrence of amino-acids yields the conclusion that the distribution is not simply linear, as has been proposed. Mackay[33] has shown that the amino-acids can be put into three natural groups based on their observed frequencies.

Dayhoff[4] has given the relative mutabilities of the amino-acids for all sequenced proteins. The relative mutability is a measure of the probability that an amino-acid at a given site in a protein will change in some small time interval. If most amino-acid residues in protein are not subject to selection, the relative mutabilities should also correlate with the number of codons for each amino-acid in the code. No correlation exists between relative mutabilities and the number of codons for each amino-acid.

The mechanisms of protein synthesis and the manner in which stored information is translated into protein structure are remarkably complex. Indeed, the very complexity of the code itself argues for a long history of evolutionary change which has produced the efficient and accurate system of modern organisms[34]. The patterns of degeneracy within the code have been explained as a mechanism to reduce the effects of mutation on protein structure[35]. Woese[34] and Crick[36] have argued that the degeneracy patterns are the result of the gradual evolution of a primitive system using a few amino-acids, each probably coded for by a larger number of codons than the maximum in the present code. New amino-acids could be incorporated into the evolving system if their accidental presence in a protein conferred a selective advantage on the primitive cell or replicating system. As the system evolved accuracy, protein structure became more precise until the insertion of any new amino-acid into the system disrupted the structure of too many proteins ever to confer a selective advantage. Only in this sense does the amino-acid composition of proteins reflect, as King and Jukes[15] and Kimura[6] have suggested, the genetic code. But the code has evolved as a response to the effects of selection on the molecule the synthesis of which it directed. The observed amino-acid composition of proteins is then a result of the effects of selection.

Allozyme Variation and Natural Selection

Zone electrophoresis followed by selective staining for enzyme or non-specific structural protein allows for the detection of functionally similar but physically or chemically different enzymes. Such variants, termed isozymes or allozymes[37], are similar in kind if not identical to those resulting from amino-acid substitutions in such proteins as haemoglobin and cytochromes c. Population genetic studies of all allozymes provide evidence that natural selection affects the frequencies of genes controlling allozyme structure.

Kojima and Yarbrough[38] demonstrated that two alleles at an esterase locus in *Drosophila melanogaster* reached a stable equilibrium in population conditions. Analysis of viability differences associated with changes in the frequencies of the alleles revealed a gene frequency-dependent selection system. At the point of genic equilibrium, no selective difference in viability was detected among the three possible genotypes. At frequencies other than the equilibrium value there were viability differences.

Dobzhansky[1] has theorized that genes contained within chromosome inversions in *Drosophila pseudoobscura* have been selected to produce phenotypes of high fitness when in heterozygous combinations with other inversions from the same population. Analysis of allozyme variants within the inversions of *D. pseudoobscura* has demonstrated that the genic content of third chromosome arrangements is differentiated to allow for the production of maximum amounts of heterozygosity[39]. Moreover, a given inversion usually contains a particular allele throughout the distribution of the species. Because occasional mutant gene arrangements occur, there is ample possibility for the effects of chance variation to substitute neutral alleles into a gene arrangement. This has not occurred. Prakash and Lewontin[39] estimate that the gene arrangements found within *D. pseudoobscura* are approximately one million years old. Natural selection has acted to maintain the genetic differentiation among these inversions for at least as long as this.

Demonstration of a cline in gene frequencies provides indirect evidence for the action of natural selection at the locus in question. O'Brien and MacIntyre[8] have analysed allozyme variability in natural populations of *D. melanogaster* and *simulans*. They report evidence for a cline in the gene frequencies for allozymes of alkaline phosphatase and 6-phosphogluconate dehydrogenase. A similar finding has been reported for an esterase locus in *D. pseudoobscura*[37]. Burns and Johnson (ref. 40, and personal communication) have studied esterase allozyme variation in natural populations of the sulphur butterfly *Colias eurytheme*. Clinal variation in the frequencies of several of the many alleles present at one esterase locus in this species has been demonstrated.

Perhaps the most convincing demonstration of the action of natural selection in maintaining clinal variation has been provided in a study by Koehn[41] of the freshwater fish, *Catostomes clarkii*. The two alleles at a serum esterase locus show clinal variation correlated with latitude. Koehn has been able to relate the variation to differences in temperature by assaying the activity of the esterase allozyme associated with each of the three genotypes. The two homozygous genotypes produce esterases which have maximum activity at opposite ends of the temperature range encountered by the species. The serum esterase produced by the heterozygous genotype has maximum activity at intermediate temperature. Koehn has identified not only the primary selective force acting on the three allozymes but also the physiological difference which allows selection to act.

The mechanisms of the action of natural selection are often misunderstood, and indeed there remains much to be elucidated about its effects. Dobzhansky[42], Simpson[23] and others have pointed out that natural selection does not act on separate genes or traits but on whole genotypes and phenotypes. Selection for single genes must be a rare phenomenon; even in cases such as industrial melanism studied by Kettlewell[43] and others, the pleiotropic effects of the gene in question and the genetic background within which the gene must act all influence the outcome of the selective process. Molecular data of the types discussed here may add an invaluable dimension to our understanding of the evolutionary process, but such information should be considered in the light of what is already understood of the nature of evolution.

Some of the ideas and approaches presented here resulted from discussions with T. Dobzhansky and Francisco J. Ayala, who also criticized the manuscript. My work was supported by the US National Institutes of Health and the US Atomic Energy Commission.

Received October 7; revised December 31, 1969.

[1] Dobzhansky, Th., *Genetics and the Origin of Species* (Columbia University Press, New York, 1951).

[2] Lewontin, R. C., and Hubby, J. L., *Genetics*, **54**, 595 (1966).

[3] Johnson, F. M., Kanapi, C. G., Richardson, R. H., Wheeler, M. R., and Stone, W. S., *Proc. US Nat. Acad. Sci.*, **56**, 119 (1966).

[4] Dayhoff, M. O. (editor), *Atlas of Protein Sequence and Structure* (National Biomedical Research Foundation, Maryland, 1969).

[5] Haldane, J. B. S., *J. Genet.*, **57**, 511 (1957).

[6] Kimura, M., *J. Genet.*, **57**, 21 (1960).

[7] Muller, H. J., *Amer. J. Hum. Genet.*, **2**, 111 (1950).

[8] O'Brien, S. J., and MacIntyre, R. J., *Amer. Nat.*, **103**, 97 (1969).

[9] Harris, H., Hopkinson, D. A., and Luffman, J., *Ann. NY Acad. Sci.*, **151**, 232 (1968).

[10] Sved, J. A., Reed, T. E., and Bodmer, W. F., *Genetics*, **55**, 469 (1967).

[11] Sved, J. A., *Amer. Nat.*, **102**, 283 (1968).

[12] King, J. L., *Genetics*, **55**, 483 (1967).

[13] Milkman, R. D., *Genetics*, **55**, 493 (1967).

[14] Smith, J. M., *Nature*, **219**, 1114 (1968).

[15] King, J. L., and Jukes, T. H., *Science*, **164**, 788 (1969).

[16] Kimura, M., *Nature*, **217**, 624 (1968).

[17] Arnheim, N., and Taylor, C. E., *Nature*, **223**, 900 (1969).

[18] Caskey, C. T., Beaudet, A., and Nirenberg, M., *J. Mol. Biol.*, **37**, 99 (1968).

[19] Maurer, H. R., and Chalkey, G. R., *J. Mol. Biol.*, **27**, 431 (1967).

[20] Cox, E. C., and Yanofsky, C., *Proc. US Nat. Acad. Sci.*, **58**, 1895 (1967).

[21] Margoliash, E., *Proc. US Nat. Acad. Sci.*, **50**, 672 (1963).

[22] Smith, E. L., and Margoliash, E., *Fed. Proc.*, **23**, 1243 (1964).

[23] Simpson, G. G., *Science*, **146**, 1535 (1964).

[24] Perutz, M. F., and Lehman, H., *Nature*, **219**, 902 (1968).

[25] Holden, J. T., in *Amino Acid Pools* (edit. by Holden, J. T.), 73 (Elsevier, New York, 1962).

[26] Roberts, E., and Simonsen, D. G., in *Amino Acid Pools* (edit. by Holden, J. T.), 284 (Elsevier, New York, 1962).

[27] Soupart, P., in *Amino Acid Pools* (edit. by Holden, J. T.), 220 (Elsevier, New York, 1962).

[28] Christensen, H. N., and Oxender, D. L., *Amer. J. Clin. Nutrit.*, **8**, 131 (1960).

[29] Britten, R. J., and Davidson, E. H., *Science*, **165**, 349 (1969).

[30] Wallace, B., *Canad. J. Genet. Cytol.*, **5**, 239 (1963).

[31] Stebbins, G. L., *The Basis of Progressive Evolution* (University of North Carolina Press, 1969).

[32] Kimura, M., *Genet. Res.*, **11**, 247 (1968).

[33] Mackay, A. L., *Nature*, **216**, 159 (1967).

[34] Woese, C. R., *The Genetic Code* (Harper and Row, New York, 1967).

[35] Wittes, R. E., and Goldberg, A. L., *Science*, **153**, 420 (1966).

[36] Crick, F. H. C., *J. Mol. Biol.*, **38**, 367 (1968).

[37] Prakash, S., Lewontin, R. C., and Hubby, J. L., *Genetics*, **61**, 841 (1969).

[38] Kojima, K., and Yarbrough, K. M., *Proc. US Nat. Acad. Sci.*, **57**, 645 (1967).

[39] Prakash, S., and Lewontin, R. C., *Proc. US Nat. Acad. Sci.*, **59**, 398 (1968).

[40] Burns, J. M., and Johnson, F. M., *Science*, **156**, 93 (1967).

[41] Koehn, R. K., *Science*, **163**, 943 (1969).

[42] Dobzhansky, T., *Amer. Nat.*, **100**, 541 (1966).

[43] Kettlewell, H. B. D., *Science*, **148**, 1290 (1965).

Carbon Compounds in Apollo 11 Lunar Samples

by

BARTHOLOMEW NAGY
WARD M. SCOTT
VINCENT MODZELESKI

Department of Geochronology,
University of Arizona,
Tucson, Arizona 85721

LOIS ANNE NAGY

Department of Geology,
University of Arizona,
Tucson, Arizona 85721

CHARLES M. DREW
WILLIAM S. McEWAN
JOSEPH E. THOMAS

Naval Weapons Center,
China Lake, California 93555

PAUL B. HAMILTON

Alfred I. Du Pont Institute,
Wilmington, Delaware 19899

HAROLD C. UREY

University of California
at San Diego, La Jolla,
California 92037

Are they entrapped solar wind particles, meteoritic or cometary contributions, remnants of a primordial atmosphere or products of the degassing of the Moon ?

THE Apollo 11 lunar samples contain traces of carbon compounds[1–8]. A number of the investigators regard some of the low molecular weight carbon compounds, such as CH_4, CO and CO_2, to be indigenous to the Moon. It is difficult to explain the origin of CH_4, CO and CO_2 by terrestrial contaminations. If the lunar fines and rocks had been contaminated by these gases, other gaseous products—such as those common in the environments to which the samples were exposed on Earth (N_2, O_2 and so on)—should also be present in significant quantities. These have not been found, however. Contaminations from exhaust gases of the lunar descent module engine are more likely, yet the samples are apparently relatively free from these contaminants[9]. Furthermore, particles of the lunar fines (10086) and the rocks (10002, 54) contain small cavities[1], some of which seem to be filled with seemingly undisturbed fluid substances.

Higher molecular weight compounds were also detected in the lunar samples. A few ninhydrin positive substances, possibly amino-acids[1,8], were obtained. Lunar fines (32·66 g) were extracted with water in a Soxhlet apparatus.

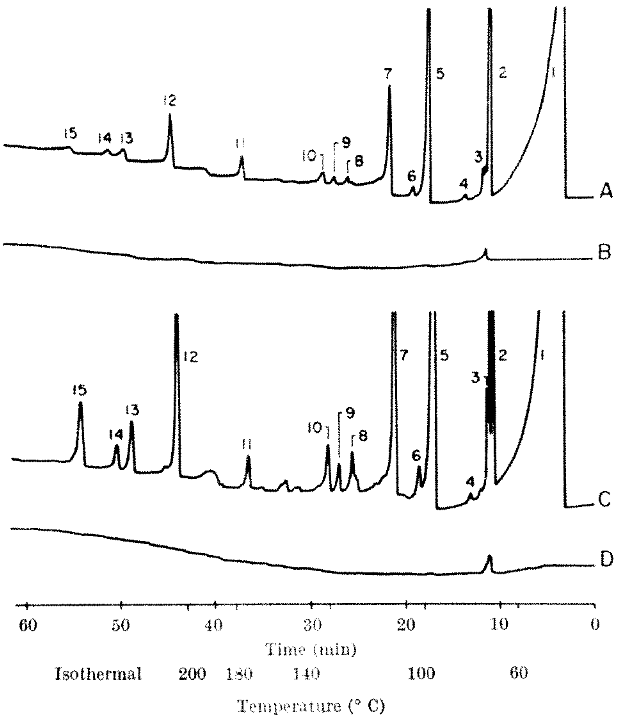


Fig. 1. Gas chromatograms of the pyrolysis products of, *A*, untreated lunar fines (sample 10086) and lunar fines, *C*, treated with benzene–methanol (4 : 1) which was used to remove soluble organic substances such as common contaminations; traces of solvent were removed by heating the solvent extracted sample in a vacuum oven at 134° C for 6 h at 35 mm Hg pressure. Peak 1, H_2 and CH_4; 2, CO_2, C_2H_4 and C_2H_6; 3, C_5H_8; 4, C_5H_{10}; 5, benzene; 6, thiophene; 7, toluene; 8, 9, *m* and *o*-xylenes; 10, styrene; 11, indene; 12, naphthalene; 13, 14, 2 and 1-methyl naphthalenes; 15, diphenyl. Some of the smaller peaks were also identified and are referred to in the text. Curves *B* and *D*: blank procedure runs for *A* and *C*, respectively. The two small peaks shown on curves *B* and *D* are reproducible instrumental background.

The individual components of the extract were separated by a high resolution ion exchange column[10] and analysed at the 5700 Å wavelength absorption of the ninhydrin positive species. What seems to be glycine, alanine, ethanolamine and urea were present in 32, 36, 22 and 65 p.p.b. quantities, respectively[1]. Utmost care was exercised to exclude laboratory contaminations and, in fact, procedure blanks showed a different amino-acid pattern (at lower concentrations) than the lunar fines. It should be noted that glycine and alanine are amino-acids most commonly synthesized in simulated primitive Earth (abiological) environments (Miller–Urey and related syntheses, refs. 11–13). A sample of the terrestrial rock, dunite, subjected by NASA in an initial vacuum to the exhaust of the lunar descent module engine, did not exhibit the same amino-acid distribution as the lunar samples; nor did the biological controls[1].

Pyrolysis experiments of both lunar fines and the interior of a lunar breccia showed CH_4, C_2–C_5 hydrocarbons, CO_2, as well as aromatic compounds: benzene, toluene, *o*, *m* and *p*-xylenes, ethylbenzene, styrene, indene, naphthalene, biphenyl, thiophene and several methyl substituted isomers. A peak of $m/e = 28$ in the low resolution mass spectra may be CO (ref. 1). CO has been described as the major component by high resolution mass spectrometry[2]. It has also been reported[2] that CO was the major component liberated when the fines were dissolved with HF. Straight-chain alkanes of high molecular weight have been found by mass spectrometry in one extract of lunar fines[1], but there is some reason to believe that they may be contaminations.

Pyrolysis Experiments

The pyrolysis products were obtained in the Organic Geochemistry Laboratory of the University of Arizona using a modified Hamilton pyrolysis unit, consisting of an externally heated fused quartz tube (43 cm × 4 mm internal diameter) connected through a heated transfer line and a liquid N_2 trap to a Perkin–Elmer model 226 gas chromatograph, containing a 50 feet × 0·02 inch internal diameter polyphenyl ether capillary column and by hydrogen flame detector. The column was first run isothermally at 60° C and also after 200° C. The gas chromatograph was in turn connected through a Watson–Biemann molecular separator to a Hitachi 'RMU-6E' single focusing mass spectrometer. Samples of fines, 623·4 mg and 717·2 mg, untreated and sonically extracted with benzene–methanol (4 : 1), respectively, were pyrolysed for 7·5 min in a stream of He (3–5 ml./min). The solvent extracted sample (717·2 mg) had been heated for 6 h at 134° C in a vacuum oven at 35 mm Hg pressure before pyrolysis to remove traces of the benzene and methanol. The pyrolysis products condensable at − 196° C temperature were collected in the liquid N_2 trap which was next heated rapidly to 250°–300° C with an adjustable oven to release the trapped products instantaneously into the gas chromatograph.

Fig. 1 illustrates the pyrolysis curves of the untreated and solvent extracted fines together with the corresponding blanks. The two curves show close agreement, suggesting that the pyrolysis products are not common terrestrial contaminations which would have been removed by solvent extraction. At least some of the aromatic hydrocarbon products could have been synthesized by pyrolysis. It has been reported[14] that basically the same aromatic hydrocarbons are obtained when methane is heated at higher temperatures (1,000° C) over silica gel.

Examination by Microscopy at Elevated Temperatures

Lunar fines were examined in Permount preparations with a Leitz 'Ortholux' microscope before and after pyrolysis at 700° C. In addition, lunar fines on sapphire disks were examined with a Leitz water cooled heating stage, model 1350, mounted on a Leitz 'Ortholux' microscope, during continuous heating in air from room temperature to 1,250° C.

The untreated lunar fines showed mineral debris and relatively few glass spheres and dumbbell shaped particles; this was also noted by several other investigators. A number of the glass beads seem to contain cavities, presumably consisting of enclosed matter. A few of the glass beads which were pyrolysed at 700° C had openings connected with hollow interiors; others still showed unopened bubble-like enclosures.

Heating of the lunar fines while the temperature was gradually raised led to the disappearance of transparent central spots in glass beads around 800°–850° C. These spots may represent optical effects or hollow interiors in the beads. Their disappearance suggests chemical changes in the glassy material or in the encapsulated substances. On further heating to about 1,250° C the irregular particles melted and the resultant silicate melt showed slight bubbling. Glass beads generally did not display sharp melting points, and at least one particle was observed at approximately 1,150° C to spread apart instantaneously in an irregular, radiating shape as if it had been disrupted by increasing internal pressure; this disruption seems to be rare. Our observations raise the possibility that some of the lunar fine particles contain gaseous or low molecular weight components in enclosures.

Morphological Features revealed by Scanning Electron Microscopy

Both the lunar fines and the lunar rock chip (breccia, both the outside and the freshly broken inside surfaces) were examined with a 'Stereoscan Mark II' electron microscope (Cambridge Instruments). In a special clean room, the fines were placed on precleaned sample pedestals with a drop of absolute ethanol which was then allowed to evaporate. The rock chip was secured to the pedestal with a double adhesive tape. The samples were placed in a bell-jar chamber which was next evacuated and the pedestals were rotated at 120 r.p.m. under a grid of Au–Pd (90 : 10) alloy wires heated to incandescence. An approximately 500 Å thick layer of Au–Pd, containing no coating artefacts such as granular textures, was deposited on the surfaces of the samples.

Glass beads were either spherical, nearly spherical or dumbbell shaped (Figs. 2*a*, *c*, *d*). They exhibited various surface features, the most prominent of which was the small, irregular shaped particles partially embedded in the bead surfaces (Figs. 2*a*, *b*, *c*, *d*). Some broken glass beads had hollow interiors[1]. The fine particles usually showed "felted" surfaces (Figs. 2*a*, *b*, *d*) whereas broken surfaces revealing the interiors of particles or of rocks did not manifest this "felted" effect (Fig. 3*d*). Broken interior parts of the rock (Figs. 3*b*, *d*) had an abundance of submicroscopic sized spherical cavities. The interiors

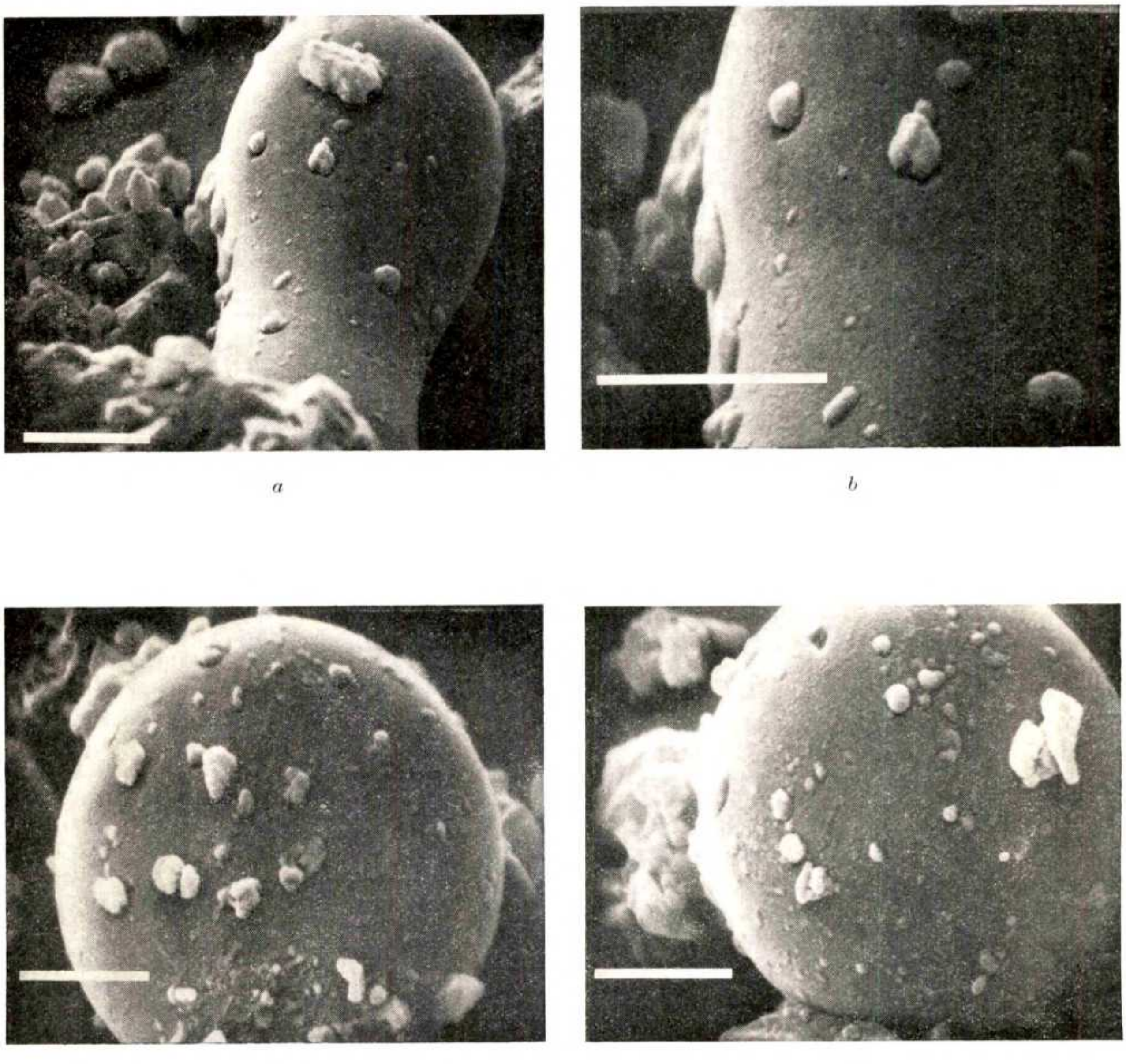


Fig. 2. *a*, Scanning electron micrographs of part of a dumbbell shaped glass bead. *b*, Same particle at higher magnification. Note that small particles are partially embedded in the bead surface. *c*, Spherical bead showing cratering at the lower edge of the photograph. *d*, Spherical bead with partially embedded particles. The horizontal white lines represent 5 μm.

of impact craters (Fig. 3*a*) were lined with a thin fused crust which resembled a frozen, once frothy and boiling melt with cavities behind the shells of the bubbles (Figs. 3*a*, *c*). Freshly broken surfaces of the breccia demonstrated that glass beads also occurred embedded in the fine grained matrix.

Possible Origin(s) of Carbon Compounds on the Moon

The nature and quantity of the carbon compounds as well as the morphology of the lunar fines and rocks may help to elucidate the origin of the carbonaceous substances on the Moon. In this study CH_4, CO_2, CO and the aromatic compounds were primarily considered. These compounds were found and independently confirmed by most investigators[1-4,7].

(1) The accumulation of carbon and hydrogen from the solar wind is one possible explanation for the origin of the carbon compounds. An experiment on Tranquillity Base measured the influx of solar wind He, the results showed $6{\cdot}3 \pm 1{\cdot}2 \times 10^6$ He atoms/cm²/s (ref. 15). Using the solar proportions of the elements of Suess and Urey[16] the influx of carbon on the Moon during geologic time ($4{\cdot}6 \times 10^9$ yr) is 0·011125 g/cm². The solar wind derived carbon content of the lunar surface was estimated by Moore *et al.*[17] to approximate the experimentally determined values of carbon. These estimates were based on calculations of the relative retention of the rare gases on lunar materials which the authors pointed out is a major unknown

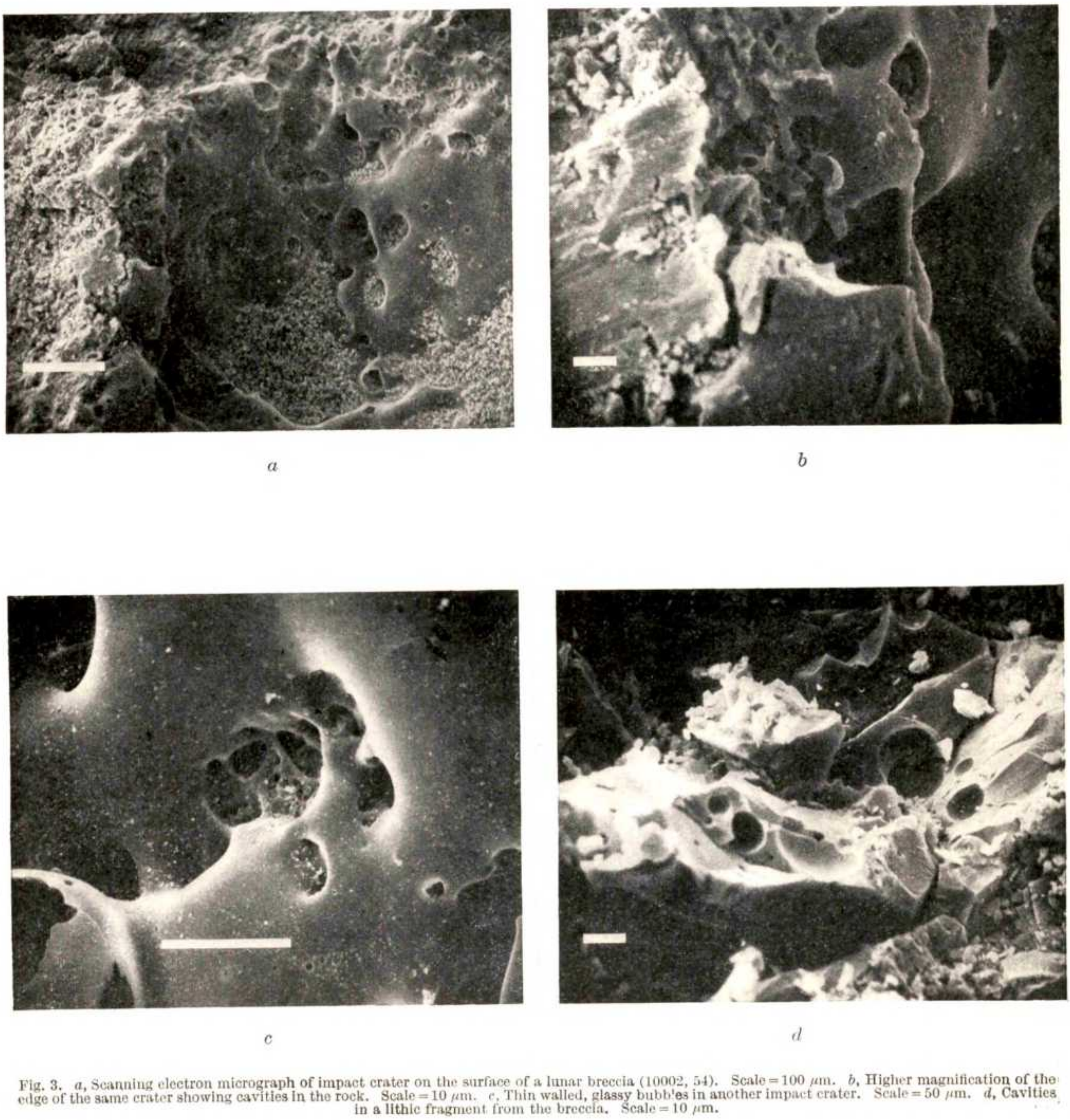


Fig. 3. *a*, Scanning electron micrograph of impact crater on the surface of a lunar breccia (10002, 54). Scale = 100 μm. *b*, Higher magnification of the edge of the same crater showing cavities in the rock. Scale = 10 μm. *c*, Thin walled, glassy bubbles in another impact crater. Scale = 50 μm. *d*, Cavities in a lithic fragment from the breccia. Scale = 10 μm.

factor. Because carbon from the solar wind would penetrate only the uppermost layers of mineral matter and because the lunar fines on the lunar surface were repeatedly churned up and mixed, one could visualize an average carbon content in the top lunar surface of approximately 150–250 p.p.m. Yet it seems from the Apollo 12 measurements, according to preliminary data, that the carbon content increases with depth (personal communication from D. A. Flory); this makes it difficult to account for the carbon content from the solar wind alone. It could be argued that the solar wind carbon and hydrogen formed volatile carbon compounds that were more easily lost near the lunar surface than from deeper layers. Yet these very surface samples still contain significant but small (about 10–150 p.p.m.) quantities of CH_4, CO, CO_2, and so on, which should have been lost long ago if they were not securely entrapped in cavities and other suitable locations inside the rocks and in the glass beads. The cavities in the fine particles attest to a reasonably high vapour (or gas) concentration at early lunar times because these fines, with the cavities, seem to be[18] very old ($4{\cdot}5 \times 10^9$ yr). It is difficult to visualize how a high concentration of carbon could have already accumulated throughout a relatively thick lunar surface layer from the solar wind right at the beginning of lunar history. It therefore seems that the solar wind origin of the carbon content must await confirmation by further experimental data.

Accumulation from Meteorites, Comets and a Primordial Atmosphere

(2) The accumulation of carbon from carbonaceous meteorite and cometary impacts on the Moon is another possibility. Nothing is known, of course, about cometary carbon contribution to the surface of the Moon, but it has been estimated[19] that impacted carbonaceous meteorites account for not more than approximately 1·5–2 per cent of the lunar surface matter. Carbonaceous meteorites contain up to 7 per cent carbon compounds (in type I; in type III approximately 0·5 per cent). Using an average meteoritic carbon content of 2 per cent approximately 400 p.p.m. meteoritic carbon could be present in the fines and breccias. This again approximates the carbon content of the Apollo 11 samples. Certain organic components of carbonaceous meteorites, such as the higher molecular weight branched and cycloalkanes, are, however, missing from the lunar samples. Basically the same argument seems to hold true for the meteoritic carbon as for the solar wind carbon. Carbonaceous meteorites are relatively frequent arrivals on Earth in present times and their cosmic ray exposure ages are low. Is it possible that there was already enough meteoritic carbon present very early in lunar history to account for all the carbonaceous gases forming the cavities? The larger particles containing cavities—some of which could have formed at a later time from melts—would particularly be expected to show some morphological deformation if they were scattered, while still molten, through relatively dense carbonaceous vapours.

(3) It is also possible that the primitive Moon may have had an atmosphere. Ammonia, water[2,20] and methane, which would likely form such an atmosphere based on theoretical considerations, have been reported in trace quantities in the lunar samples. As noted previously, glass beads and dumbbells were once molten, which is demonstrated not only by their shapes but also by the numerous irregular mineral grains adhering to or partially embedded in their surfaces. Yet these particles, which were formed and splattered at an early stage when an atmosphere still would have been present, show no signs of the deformation in shape which molten particles, particularly the large ones, would have acquired from atmospheric friction during flight. In other words, they do not have aerodynamic shapes. Considering that the apparent impact hole and splash marks on the glass bead (shown on the bottom edge of Fig. 2c) were caused by a particle of diameter less than 0·1 of the diameter of the glass bead, the mass of the particle must have been less than 10^{-3} of the glass bead. The conclusion, and observations of the cratering effects of particles impacting from detonation and shaped charge studies, lead to a particle velocity of the order of 6–9 km/s. Velocities of this magnitude are not maintained by such small particles in a gaseous atmosphere of any appreciable density for any length of time, which argues strongly against a relatively dense atmosphere at the early times when the glass beads were formed.

Degassing from the Moon

(4) Finally, the possibility that the carbon compounds originate from the degassing of the Moon has to be considered. Carbon was a relatively common element in the solar nebula, so that the accreting primitive Moon must have included significant quantities of carbon. While part of the light elements would have been readily lost near the lunar surface, some must have been left behind incorporated in the melts that formed the crystalline rocks, glass beads and the breccias. This could explain the increasing carbon content with depth, the cavities and bubbles, and the lack of aerodynamic shapes of the glass beads. We suggest that the lunar carbon compounds may originate from the degassing of the Moon with possibly lesser contributions from solar wind and perhaps meteoritic sources.

We thank Mrs Alice Lumpkins, Judith Modzeleski, Sister Mary Ellen Murphy and Dr Maria Young from the University of Arizona; Mrs Reba Ward of the Naval Weapons Center, China Lake; Mrs Roberta De Fiore, Johanne C. Dickinson, Janet Greenquist, Drs Marjorie Lou and T. Timothy Myoda of the Alfred I. Du Pont Institute, Wilmington, for their assistance. One of us (B. N.) thanks Dr G. P. Kuiper of the University of Arizona for discussions on the origin of lunar carbon compounds. This investigation was supported by the US National Aeronautics and Space Administration with contributions from the Naval Weapons Center, China Lake, and the Alfred I. Du Pont Institute.

Received February 17, 1970.

[1] Nagy, B., Drew, C. M., Hamilton, P. B., Modzeleski, V. E., Murphy, Sr., M. E., Scott, W. M., Urey, H. C., and Young, M., *Science*, **167**, 770 (1970).

[2] Burlingame, A. L., Calvin, M., Han, J., Henderson, W., Reed, W., and Simoneit, B. R., *Science*, **167**, 751 (1970).

[3] Murphy, R. C., Preti, G., Nafissi-V, M. M., and Biemann, K., *Science*, **167**, 755 (1970).

[4] Oro, J., Updegrove, W. S., Gilbert, J., McReynolds, J., Gil-Av, E., Ibanez, J., Zlatkis, A., Flory, D. A., Levy, R. L., and Wolf, C., *Science*, **167**, 765 (1970).

[5] Kaplan, I. R., and Smith, J. W., *Science*, **167**, 541 (1970).

[6] Ponnamperuma, C., Kvenvolden, K., Chang, S., Johnson, R., Pollock, G., Philpott, D., Kaplan, I., Smith, J., Schopf, J. W., Gehrke, C., Hodgson, G., Breger, I. A., Halpern, B., Duffield, A., Krauskopf, K., Barghoorn, E., Holland, H., and Keil, K., *Science*, **167**, 760 (1970).

[7] Abell, P. I., Draffan, G. H., Eglinton, G., Hayes, J. M., Maxwell, J. R., and Pillinger, C. T., *Science*, **167**, 757 (1970).

[8] Fox, S. W., Harada, K., Hare, P. E., Hinsch, G., and Mueller, G., *Science*, **167**, 767 (1970).

[9] Flory, D. A., and Simoneit, B. R., *A Summary of the Apollo 11 Organic Contamination History* (NASA Technical Report, 1969).

[10] Hamilton, P. B., *Anal. Chem.*, **35**, 2055 (1963).

[11] Miller, S. L., *J. Amer. Chem. Soc.*, **77**, 2351 (1955).

[12] Miller, S. L., and Urey, H. C., *Science*, **130**, 245 (1959).

[13] Oro, J., in *The Origins of Prebiological Systems* (edit. by Fox, S. W.), 146 (Academic Press, New York, 1965).

[14] Oro, J., and Han, J., *J. Gas Chromatog.*, **5**, 480 (1967).

[15] Bühler, F., Eberhardt, P., Geiss, J., Meister, J., and Singer, P., *Science*, **166**, 1502 (1969).

[16] Suess, H. E., and Urey, H. C., *Rev. Mod. Phys.*, **28**, 53 (1956).

[17] Moore, C. B., Lewis, C. F., Gibson, E. K., and Nichiporuk, W., *Science*, **167**, 495 (1970).

[18] Albee, A. L., Burnett, D. S., Chodos, A. A., Eugster, O. J., Huneke, J. C., Papanastassiou, D. A., Podosek, F. A., Russ, G. P., Sanz, H. G., Tera, F., and Wasserburg, G. J., *Science*, **167**, 463 (1970).

[19] Keays, R. R., Ganapathy, R., Laul, J. C., Anders, E., Herzog, G. F., and Jeffery, P. M., *Science*, **167**, 490 (1970).

[20] Friedman, I., O'Neil, J. R., Adami, L. H., Gleason, J. D., and Hardcastle, K., *Science*, **167**, 538 (1970).

LETTERS TO THE EDITOR

GENERAL

Statistical Methods in Scientific Inference

It is regrettable that Edwards's interesting article[1], supporting the likelihood and prior likelihood concepts, did not point out the specific criticisms of likelihood (and Bayesian) concepts that seem to dissuade most theoretical and applied statisticians from adopting them. As one whom Edwards particularly credits with having "analysed in depth . . . some attractive properties" of the likelihood concept, I must point out that I am not now among the "modern exponents" of the likelihood concept. Further, after suggesting that the notion of prior likelihood was plausible as an extension or analogue of the usual likelihood concept (ref. 2, p. 200), I have pursued the matter through further consideration and rejection of both the likelihood concept and various proposed formalizations of prior information and opinion (including prior likelihood). I regret not having expressed my developing views in any formal publication between 1962 and late 1969 (just after ref. 1 appeared). My present views have now, however, been published in an expository but critical article (ref. 3, see also ref. 4), and so my comments here will be restricted to several specific points that Edwards raised.

If there has been "one rock in a shifting scene" or general statistical thinking and practice in recent decades, it has not been the likelihood concept, as Edwards suggests, but rather the concept by which confidence limits and hypothesis tests are usually interpreted, which we may call the confidence concept of statistical evidence. This concept is not part of the Neyman–Pearson theory of tests and confidence region estimation, which denies any role to concepts of statistical evidence, as Neyman consistently insists. The confidence concept takes from the Neyman–Pearson approach techniques for systematically appraising and bounding the probabilities (under respective hypotheses) of seriously misleading interpretations of data. (The absence of a comparable property in the likelihood and Bayesian approaches is widely regarded as a decisive inadequacy.) The confidence concept also incorporates important but limited aspects of the likelihood concept: the sufficiency concept, expressed in the general refusal to use randomized tests and confidence limits when they are recommended by the Neyman–Pearson approach; and some applications of the conditionality concept. It is remarkable that this concept, an incompletely formalized synthesis of ingredients borrowed from mutually incompatible theoretical approaches, is evidently useful continuously in much critically informed statistical thinking and practice.

While inferences of many sorts are evident everywhere in scientific work, the existence of precise, general and accurate schemas of scientific inference remains a problem. Mendelian examples like those of Edwards and my 1969 paper seem particularly appropriate as case-study material for clarifying issues and facilitating effective communication among interested statisticians, scientific workers and philosophers and historians of science.

Allan Birnbaum

New York University,
Courant Institute of Mathematical Sciences,
251 Mercer Street,
New York, NY 10012.

Received November 28, 1969: revised January 19, 1970.

[1] Edwards, A. W. F., *Nature*, **222**, 1233 (1969).

[2] Birnbaum, A., *J. Amer. Stat. Assoc.*, **57**, 269 (1962).

[3] Birnbaum, A., in *Philosophy, Science and Method: Essays in Honor of Ernest Nagel* (edited by Morgenbesser, S., Suppes, P., and White, M.) (St Martin's Press, NY, 1969).

[4] *Likelihood* in *International Encyclopaedia of the Social Sciences* (Crowell-Collier, NY, 1968).

PHYSICAL SCIENCES

Redshifts of Companion Galaxies

Since 1966 (ref. 1) there has been observational evidence[2-4] that quasi-stellar radio sources (QSRs) and compact radio galaxies are ejected from galaxies. Whatever distance scale one uses, one of the characteristics of QSRs and compact radio galaxies is that a great deal of energy is compressed into very small dimensions. Unless very special conditions prevail, such concentrated sources would be expected to expand with time. Indeed, the short lifetimes which are inferred from their rapid rate of energy expenditure, the associated jets of material and the prevalence of ejected radio pairs across the sources all attest to expansion and eruption.

We would therefore expect that when a compact object which is initially not optically resolvable (that is, a QSR or a QSO) expands sufficiently it eventually must become resolvable (a compact galaxy). As it continues to expand we would expect the surface brightness to diminish and eventually the object should look only slightly more compact than a galaxy of normal surface brightness. I have recently reported results[5] which lead to the conclusion that compact galaxies and companion galaxies in general have also been ejected from large, central galaxies. The observations also indicate that the ejections are initially very compact and rapidly expand. The model which suggests itself is one where matter is ejected in a compact, QSR-like form, which evolves into a compact radio galaxy and finally into a less and less compact companion galaxy. (This is, of course, an evolutionary interpretation. As far as the observational evidence goes, one may simply wish to consider that smaller bodies, with a continuity of properties that reaches to the extremes of QSRs, are associated with large, normal galaxies.)

It is usually assumed in astronomy that galaxy redshifts are only due to Doppler, velocity shifts. Objects which differ greatly in redshift are always presumed to be at different distances so as not to violate the (expansion) velocity–distance relation. The only way such an assumption could be shown to be incorrect would be to demonstrate that objects of greatly different redshifts cluster together on the sky. The observational evidence on the QSRs and radio compact galaxies referred to simply argues that they do in fact cluster on the sky around lower redshift galaxies and tend to be aligned and paired across these galaxies.

The proposed continuity of characteristics of the ejected compact bodies, however, suggests another kind of test on the nature of their redshifts. We can investigate smaller companions around galaxies, companions which astronomers have long accepted as belonging to the central galaxy and being at essentially the same distance as the larger galaxy. If the intrinsic redshift evolves from high to low as ejected objects evolve toward brighter and less compact companions, then we would expect even the normally accepted companions to have at least some small residual, excess redshift left. Even if there is no evolution involved and it is simply the case that objects of various redshifts are ejected, we would still expect the companion galaxies to have somewhat higher redshifts, on the average, than the parent galaxy.

In order to test this prediction I have listed in Table 1 all the galaxies having companions which are unquestionably accepted as belonging to the larger galaxies. The Andromeda Nebula and the four well known companions that fall within 15 arc degrees are listed, followed by the M81 and NGC 5128 groups with the members as defined by de Vaucouleurs[12]. In eleven out of these thirteen cases the companion galaxies have higher redshifts than the central galaxies.

The data for the first three groups are taken from redshifts, corrected for galactic rotation, listed by de Vaucouleurs and de Vaucouleurs[6]. These redshifts represent, for the most part, means of two or more optical determinations. The independent determinations usually agree within about 20 km s^{-1}. In addition, there are redshifts from radio (21 cm) observations for five of the objects which confirm the optical redshifts to within about this same accuracy. Mean errors of the redshifts in the first part of Table 1 may be obtained from the weights listed in column 23 of the de Vaucouleurs Reference Catalogue. Of course, the errors of measurements, like orbital or ejection velocities around the central galaxy, will simply spread the distribution of redshifts around their mean value. The important point, as Fig. 1 indicates, is whether the mean residual redshift is significantly different from zero in terms of the dispersion regardless of the cause of that dispersion.

Also listed in Table 1 are six spiral galaxies with companions on the ends of arms[5]. These can be included because the connexion to the companion galaxy ensures that the companion is at the same distance as the main galaxy. The listed redshifts represent all that I know of in this class of galaxies.

Altogether the redshift of the companion galaxy is higher than that of the main galaxy in sixteen out of nineteen cases in Table 1. This could occur by accident in less than two out of a thousand chances. If we were to continue to interpret the entire redshift of each companion as a velocity shift, we would have an absurd situation where the companions were systematically receding from the main galaxy along our line of sight to that main galaxy.

It should be emphasized that Table 1 represents a conservative assignment of companion galaxies. For example, NGC 404 (not listed in Table 1) is situated on a line between M31 and M33, and is believed to be a companion of M31 (refs. 4 and 12). Its redshift is +237 km s^{-1} greater than M31.

Table 1. GALAXIES KNOWN TO BE COMPANIONS OF LARGER GALAXIES

Dominant galaxy	Companion galaxy	Differential redshift (km s^{-1})
M31	M32	+85
	NGC 205	+62
	NGC 185	+58
	M33	+57
M81	M82	+234
	NGC 2976	+81
	NGC 3077	−104
	IC 2574	+91
	HO II	+215
NGC 5128	NGC 5102	+77
	NGC 5236	+64
	NGC 5253	−42
	NGC 5068	+139
M51	NGC 5195	+109
Atlas 48	Companion	−120
Atlas 58	Companion	+60
Atlas 82	Companion	+90
Atlas 86	Companion	+23
Atlas 87	Companion	+180

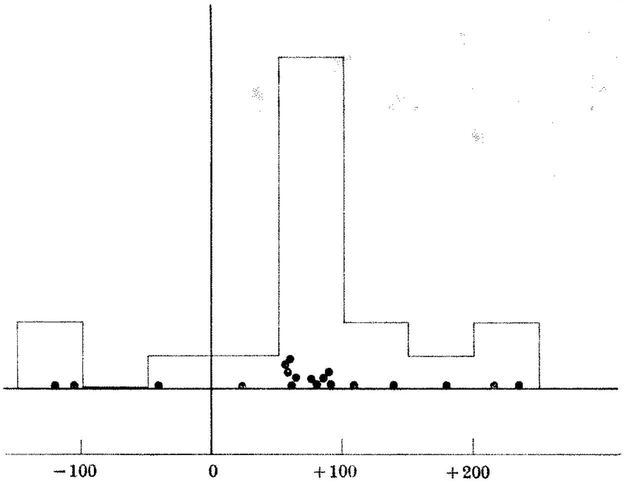


Fig. 1. Residual redshifts of nineteen galaxies known to be companions of larger galaxies.

Of the three negative redshifts, the two most negative are questionable for the following reasons. (1) The companion to Atlas 49 registers only emission lines which may well come from material in the disk of the parent galaxy excited by the object which appears to be in the process of ejection. There is therefore no assurance that the measured redshift really represents the intrinsic redshift of the compact companion. (2) NGC 3077 forms a pair with M82 on either side of M81. If we consider them to be an ejected pair, the mean of their redshifts is +65 km s^{-1}. This is just about the expected redshift of these kind of companions as judged from Fig. 1. The remaining redshift would then represent a separation of about 170 km s^{-1} in mutually opposite directions from M81.

Table 1 essentially exhausts those cases where redshifts are known in groups where we are certain that the smaller companions belong to the large central galaxies. If we try to extend this list we encounter groups where there are a number of galaxies of comparable size and therefore it is not certain which are the companions and which are the main galaxies. We also encounter looser groups in which membership is less certain. Of the five nearest groups which de Vaucouleurs[12] lists, three are represented in Table 1. Of the remaining two, the Sculptor Cloud is a loose group of comparable Sc to Sm nebulae. (Nevertheless the faintest member of the group, NGC 45, has a redshift about 400 km s^{-1} greater than the group mean.) The other group, the Canes Venatici I Cloud, is very heterogeneous and overlaps with various, presumably more distant, groups in that general direction in the sky.

Although, as I have mentioned, clusters of galaxies represent a somewhat different situation than dominant galaxies with companions, if we investigate some of the fainter clusters we find further evidence for fainter galaxies in the clusters tending to have higher redshifts. Holmberg[7] demonstrated that there is an increasing redshift with increasing faintness among galaxies belonging to the Virgo cluster. It is well known that late type (young population) spiral galaxies in Virgo tend to have considerably higher redshifts than the mean of the cluster[13]. Even when we exclude Sc spirals, however, the remaining Virgo cluster galaxies still show a correlation of redshift with apparent magnitude (unpublished results).

The same situation is true with respect to the four largest groups listed in Humason, Mayall and Sandage[8]

(G383, Leo, G5846 and G7619). The first of these groups in particular, the galaxies involved in the Markarian chain centred on NGC 383, shows a marked correlation of higher redshifts with fainter magnitudes. Recently Sargent[9] reported one of the fainter galaxies in another chain to have an excess redshift of considerable magnitude, and earlier I argued[10] that IC4296 was a member of the NGC 5128 chain despite a considerably higher redshift.

The slope of the excess redshift–magnitude relation looks steeper (in km s^{-1} per magnitude) for clusters than it does for the dominant-galaxy-with-companions case treated in Table 1. It might have something to do with the possibility of larger galaxies fragmenting[11,14], which would make each original, low redshift fragment fainter and thus steepen the relation. It may also be related to the much higher dispersion of redshifts which is encountered in the more distant clusters. Much higher redshift objects are therefore included as members of clusters and the slope can thus be much steeper. The higher dispersion of velocities, of course, makes the cluster membership criterion generally more dependent on areal projection on the cluster area. The generally small differences in redshift between the main galaxies and companions in Table 1, on the other hand, are added confirmation of their accepted group membership.

In conclusion, the systematically higher redshifts for the companion galaxies supports the conclusion that compact, intrinsically higher redshift objects are ejected from nearby galaxies. At the same time it contradicts the current assumption that galaxy redshifts are only due to Doppler velocities. That is, it observationally contradicts exactly that principle which has been invoked to reject the previous observational evidence for association of high and low redshift objects.

HALTON ARP

Mount Wilson and Palomar Observatories,
Carnegie Institution of Washington,
California Institute of Technology,
Pasadena, California 91106.

Received December 11, 1969.

[1] Arp, H., *Science*, **151**, 1214 (1966).
[2] Arp, H., *Astrophys. J.*, **148**, 321 (1967).
[3] Arp, H., *Astrophyzika*, **4**, 59 (1968).
[4] Arp, H., *Astron. J.* (in the press).
[5] Arp, H., *Astron. Astrophys.* (in the press).
[6] de Vaucouleurs, G., and de Vaucouleurs, A., *Reference Catalogue of Bright Galaxies* (Univ. Texas Press, Austin, 1964).
[7] Holmberg, E., *Astron. J.*, **66**, 120 (1961); *Uppsala Astr. Obs. Medd. No. 138.*
[8] Humason, M. L., Mayall, N. U., and Sandage, A. R., *Astron. J.*, **61**, 97 (1956).
[9] Sargent, W. L. W., *Astrophys. Lett.*, **153**, 135 (1968).
[10] Arp, H., *Pub. Astron. Soc. Pacific*, **80**, 129 (1968).
[11] Sersic, J. L., *Astron. Soc. Pacific Leaflet No. 453* (1967); *Astrophyzika*, **4**, 105 (1968).
[12] de Vaucouleurs, G., *Stars and Stellar Systems* (edit. by Kuiper, G., and Middlehurst, B.), **9** (1969).
[13] de Vaucouleurs, G., and de Vaucouleurs, A., *Astron. J.*, **68**, 96 (1963).
[14] Vorontsov-Velyaminov, B., *Astron. Soc. Pacific Leaflet No. 458* (1969).

Low-frequency Spectrum of the Crab Nebula

THE compact low-frequency radio source[1] in the Crab nebula is exceptional in most of its properties. Its spectral index[2] of ~1·75 between 26·5 and 1,407 MHz is the highest known for a galactic continuum source, and its brightness temperature of ~10^{14} K at 38 MHz implies an unusual emission mechanism with a high volume emissivity. The high spectral index suggests that only a small fraction of the total radio luminosity of the source may have been observed in the frequency range studied so far. The error field of its radio position[3] contains the pulsar NP 0532, and these objects may therefore be related; if the relationship were substantiated, it would be the only continuum source of small angular size known to coincide with a pulsar. This report presents evidence that the high spectral index is maintained to 10 MHz.

Observations of the flux density of radiation from the Crab nebula at frequencies below ~40 MHz must be corrected for absorption by free-free transitions of electrons in the intervening interstellar medium to determine the spectrum of the emitted radiation. The interstellar absorption along the 2,020 pc line of sight[4] to the nebula ($l^{\text{II}}=184^\circ\cdot6$, $b^{\text{II}}=-5^\circ\cdot8$) can be estimated from low-frequency studies of other continuum radio sources at low galactic latitudes, and also from 21 cm line observations of the interstellar medium on this line of sight.

Observations[5] of discrete radio sources at 10 MHz have recently been used to define models of the low frequency absorption by the interstellar medium. The 10 MHz optical depth of the line of sight to the Crab nebula predicted by a uniform-disk model[6] of the interstellar electron gas is $\tau(10)=0\cdot68\pm0\cdot17$. A non-uniform model[7] which allows for differences in the absorption coefficient $\varkappa(10)$ between the local spiral arm and the inter-arm region predicts $\tau(10)=0\cdot62\pm0\cdot17$ on this line of sight. These predictions are derived from the same 10 MHz data and are not independent; the agreement between them indicates that the expected value of $\tau(10)$ on this line of sight is only weakly dependent on the model used to interpret the 10 MHz data.

The optical depth $\tau(10)$ of the line of sight to IC443, which is 1,500 pc (ref. 8) from the Sun in the direction $l^{\text{II}}=189^\circ\cdot0$, $b^{\text{II}}=+3^\circ\cdot2$, should be similar to that of the line of sight to the Crab nebula and the value observed[7], $\tau(10)=0\cdot65\pm0\cdot25$, agrees with the other estimates.

Hjellming *et al.*[9] have estimated the electron density and temperature within interstellar clouds and the inter-cloud medium on the line of sight to the Crab nebula from the 21 cm absorption of the nebular continuum. The optical depth $\tau(10)$ calculated from their data on this line of sight is $0\cdot42\pm0\cdot10$, with approximately equal contributions from clouds at ~50 K and from the inter-cloud medium at ~1,000 K.

Table 1. CORRECTIONS FOR INTERSTELLAR ABSORPTION AT LOW FREQUENCIES

Frequency (MHz)	Measured intensities multiplied by
10	1·82
12·5	1·46
14·7	1·31
16·7	1·23
20	1·15
22·25	1·12
25	1·09
26·3	1·08
35	1·04
38	1·04
81·5	1·01

Observations of the integrated flux density of the Crab nebula at low frequencies[5,10–15] have been corrected for interstellar free-free absorption by assuming that $\tau(10)=0\cdot6$ on the line of sight, and calculating the corresponding optical depths at higher frequencies from the relation $\tau(\nu)=k\nu^{-2}[17\cdot7+\ln(T^{3/2}\nu^{-1})]$. The corrections at each frequency are given in Table 1. Fig. 1 shows the estimated spectrum of the radiation leaving the nebula between 10 and 15,500 MHz. The spectrum shows an enhancement of the total radiation at low frequencies which is consistent with a power-law extrapolation of the spectrum of the compact source to 10 MHz; a marginally significant enhancement is noticeable in the uncorrected data.

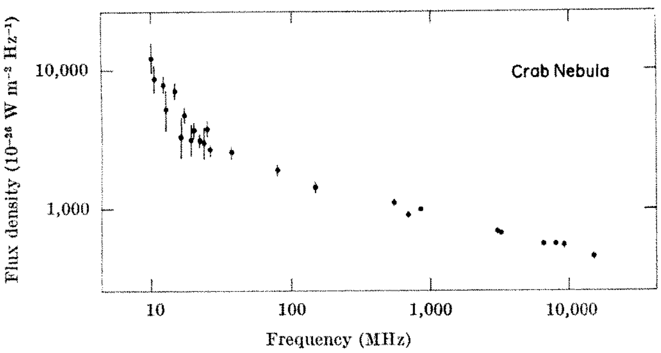


Fig. 1. The emission spectrum of the Crab nebula between 10 and 15,500 MHz, corrected for interstellar absorption. Some pairs of measurements at similar frequencies have been slightly separated along the frequency axis to clarify the figure.

The contribution of the compact source at the lowest frequencies can be estimated from Fig. 1 if it is assumed that the diffuse nebular emission maintains its high-frequency power-law spectrum throughout the low frequency range. The most probable departure from this spectrum would be reduction of the spectral index at low frequencies due to free-free absorption or electron energy losses within the nebula, in which case this analysis would underestimate the low-frequency emission from the compact source, and hence its spectral index, luminosity and brightness temperature.

The spectrum of the nebula at frequencies above 80 MHz was fitted with a power law using a least-squares procedure. The 10 and 15 MHz intensities extrapolated from this power law were subtracted from the mean spectrum defined by the low-frequency measurements to estimate the contributions of the compact source at these frequencies. Intensities so estimated would be unreliable at frequencies above 20 MHz, but the spectrum of the compact source is defined at these frequencies by observations[1,2] at 26·5, 38, 81·5 and 1,407 MHz. Bell and Hewish[2] have suggested that the intensity of the compact source derived from the 26·5 MHz occultation observation of Andrew *et al.*[1] is invalid, for the position of the source given by this observation disagrees with the subsequent measurement by Gower[3]. This discrepancy may be resolved by rejecting the 26·5 MHz emersion record, which was seriously affected by terrestrial interference[1]. The position of the limb of the Moon at the immersion was consistent with the later result, and the 26·5 MHz intensity derived from the interference-free immersion record should be valid. This interpretation of the 26·5 MHz occultation data suggests that the compact source is feature "2" in the nomenclature proposed by Matveenko[16].

Table 2. FLUX DENSITIES OF THE COMPACT SOURCE IN THE CRAB NEBULA

Frequency (MHz)	Flux density (10^{-26} W m^{-2} Hz^{-1})	Reference
10	6,900 ± 2,200	This paper
15	2,120 ± 700	This paper
26·5	1,230 ± 150	1
38	486 ± 50	2
81·5	190 ± 20	2
1,407	< 0·9	2

The spectrum derived for the compact source (Table 2) can be represented by a power law of index $\alpha = 1{\cdot}76 \pm 0{\cdot}15$ from 10 to 1,407 MHz. The luminosity of the source between 10 and 1,407 MHz is $\sim 3 \times 10^{32}$ erg s^{-1}, which is about ten times that of the pulsar NP 0532 at radio frequencies, but only one-tenth of its luminosity at optical frequencies. The angular diameter of the compact source is known to be $\sim 0{\cdot}2$ arc s from studies of its interplanetary scintillation[2] at 81·5 MHz. Its brightness temperature at 10 MHz would be as high as $\sim 3 \times 10^{15}$ K if the diameter were the same at this frequency, which leaves little doubt that the source cannot radiate by the electron synchrotron mechanism: the magnetic field strength within the source would be $< 6 \times 10^{-8}$ G and the total energy of relativistic electrons alone would be $> 4 \times 10^{51}$ erg, assuming the source to be homogeneous.

Rees[17] has suggested that the emission may be proton synchrotron radiation emitted at a frequency below the electron gyrofrequency. The 10 MHz parameters then imply a magnetic field strength $< 0{\cdot}4$ G, a total magnetic field energy $< 8 \times 10^{44}$ erg, and a total proton energy $> 1{\cdot}5 \times 10^{47}$ erg, again assuming a homogeneous source. The proton synchrotron model is attractive, for it requires field and particle energies which are modest in comparison with the total energy supply of the nebula, and which could be stabilized by the gravitation of an object of mass $\sim 1\ M_{\odot}$, as pointed out by Burbidge and Hoyle[18]. The magnetic field and proton flux required on this model of the compact source may be associated with the outer magnetosphere of the pulsar. A magnetic field of strength $< 0{\cdot}4$ G gives an electron gyrofrequency $< 1{\cdot}2$ MHz, however, so Rees's interpretation[17] cannot be maintained if there are electrons present in the region of the source, as these would absorb the proton radiation.

Alternatively, the high brightness temperature of the compact continuum source might be attributed to a coherent plasma oscillation[19] or to radiation from a synchrotron maser[20]. In either case the characteristic frequency associated with the mechanism must be < 10 MHz, and the total energy output must therefore appreciably exceed the 3×10^{32} erg s^{-1} detected by the present observations, unless the emission were anisotropic.

It is possibly significant that the spectral index of the mean radio pulse[21] from NP 0532 is ~ 2 at 196 MHz, comparable with the spectral index of the continuum source at lower frequencies. The equivalent continuum flux density of the pulsar is ~ 20 per cent of that expected for the compact source at 196 MHz. The intrinsic pulse width from NP 0532 increases with decreasing frequency below 150 MHz (reports by Drake and by Sutton *et al.*, at the 131st meeting of the American Astronomical Society, New York, 1969), and furthermore the high dispersion of the pulses results in large numbers being instantaneously superimposed in the bandwidths of most low-frequency instruments. It would therefore be difficult to distinguish between NP 0532 and a continuum source at low frequencies.

The apparent angular diameter of 0·2 arc s observed for the compact source at 81·5 MHz may exceed its true diameter due to scattering of its radiation by density irregularities in the interstellar electron gas if the typical density fluctuations ΔN(cm^{-3}) and their scale a (cm) were related[22] as $\Delta N \sim 2 \times 10^{-9}\ \sqrt{a}$. This condition is quite likely to be satisfied in the interstellar medium. The true diameter of the source might therefore be $< 0{\cdot}2$ arc s and the brightness temperature at 10 MHz $> 3 \times 10^{15}$ K, which would increase the difficulties associated with a proton synchrotron model of the source. This could be investigated by long-baseline interferometry or interplanetary scintillation studies of the compact source at very low frequencies; if the apparent diameter were simply that imposed by interstellar scattering, it would be found to increase as the square of the observing wavelength. Such observations, and an improved position for the compact source, will be important for theories of the source and of its relationship to NP 0532.

This research was supported by an operating grant from the National Research Council of Canada.

A. H. BRIDLE

Astronomy Group,
Department of Physics,
Queen's University at Kingston,
Ontario, Canada.

Received January 22, 1970.

[1] Andrew, B. H., Branson, N. J. B. A., and Wills, D., *Nature*, **203**, 171 (1964).
[2] Bell, S. J., and Hewish, A., *Nature*, **213**, 1214 (1967).

[3] Gower, J. F. R., *Nature*, **213**, 1213 (1967).
[4] Trimble, V. L., *Astron. J.*, **73**, 535 (1968).
[5] Bridle, A. H., and Purton, C. R., *Astron. J.*, **73**, 717 (1968).
[6] Bridle, A. H., and Venugopal, V. R., *Nature*, **224**, 545 (1969).
[7] Bridle, A. H., *Nature*, **221**, 648 (1969).
[8] Sharpless, S., in *Stars and Stellar Systems* (edit. by Blaauw, A., and Schmidt, M.), **5**, 316 (University of Chicago Press, 1965).
[9] Hjellming, R. M., Gordon, C. P., and Gordon, K. J., *Astron. Astrophys.*, **2**, 202 (1969).
[10] Bazelyan, L. L., Braude, S. Ya., Bruck, Yu. M., Zhouck, I. N., Megn, A. V., Ryabov, B. P., Sodin, L. G., and Sharykin, N. K., *Radiofizika*, **6**, 897 (1963).
[11] Braude, S. Ya., Lebedeva, O. M., Megn, A. V., Ryabov, B. P., and Zhouck, I. N., *Mon. Not. Roy. Astron. Soc.*, **143**, 289 (1969).
[12] Roger, R. S., Costain, C. H., and Lacey, J. D., *Astron. J.*, **74**, 366 (1969).
[13] Erickson, W. C., and Cronyn, W. M., *Astrophys. J.*, **142**, 1156 (1965).
[14] Williams, P. J. S., Kenderdine, S., and Baldwin, J. E., *Mem. Roy. Astron. Soc.*, **70**, 53 (1966).
[15] Parker, E. A., *Mon. Not. Roy. Astron. Soc.*, **138**, 407 (1968).
[16] Matveenko, L. I., *Astron. Zh.*, **45**, 695 (1968), trans. in *Sov. Astron. A J*, **12**, 552 (1968).
[17] Rees, M. J., *Astrophys. Lett.*, **2**, 1 (1968).
[18] Burbidge, G. R., and Hoyle, F., *Nature*, **221**, 847 (1969).
[19] Ginzburg, V. L., and Ozernoi, L. M., *Radiofizika*, **9**, 221 (1966).
[20] Kaplan, S. A., *Astrofizika*, **2**, 409 (1966).
[21] Comella, J. M., Craft, H. D., Lovelace, R. V. E., Sutton, J. M., and Tyler, G. L., *Nature*, **221**, 453 (1969).
[22] Scheuer, P. A. G., *Nature*, **218**, 920 (1968).

Anomalous Rhenium Isotopic Ratio in the Solar Wind: Detection at the Nanogram Level

CLAYTON[1] recently considered an apparent anomaly in the ^{187}Re–^{187}Os cosmochronology. He showed that this could be resolved if the half life of ^{187}Re was significantly shortened in a stellar environment, and considered the solution to lie in the bound beta decay process, which is energetically feasible for the weak ^{187}Re transition[2]. The ionization of Re in the outer solar convective zone increases the number of vacant bound states available, and Clayton suggests a reduction in the total ^{187}Re half life from 43×10^9 yr to about 3×10^9 yr. It might therefore be expected that the ^{187}Re/^{185}Re ratio in the solar wind would be between one-half and one-third that observed terrestrially. Clayton proposed that the hypothesis could be tested by measuring the isotopic ratio of Re in the surface layer of an old part of the lunar regolith, and stressed that "this measurement should eventually be undertaken if there is any reasonable chance of detection". In this article I suggest a simple sensitive method to detect the expected isotopic anomaly.

The solar wind flux of Re is given[1] as 10^{-3} atoms cm^{-2} s^{-1}, which deposits approximately 10^{-8} (g Re) cm^{-2} in 10^9 yr. To estimate the likelihood of detection the probable Re abundance of the lunar surface must first be considered. The preliminary examination of Apollo 11 samples[3] shows only that Re is below the spectrographic detection limit of 10^{-4} g g^{-1}. For the present discussion it is supposed that the Re content of lunar material might resemble that of terrestrial basic rocks on the one hand, or the achondrites on the other. Re has been measured in some basic and ultrabasic rocks and abundances lie generally between 10^{-9} and 10^{-10} g Re g^{-1} (ref. 4). Very few Re abundances are known for achondrites; however, some hitherto unpublished analyses are given in Table 1. These abundances lie within a range similar to that found for terrestrial rocks. Lunar rocks examined to date[3] are unusual for a very high Ti abundance. No achondrite is enriched in this element to quite the same extent, but a reasonably high abundance is found in the Angra dos Reis achondrite. The enrichment of Ti in this meteorite does not seem to be accompanied by a similar Re enrichment. It is reasonable to suppose, therefore, that an upper limit for the Re content of lunar material would be about 10^{-9} g g^{-1}.

Table 1. RHENIUM ABUNDANCES IN SOME ACHONDRITIC METEORITES

Achondrite	Re 10^{-10} g g^{-1}	
Bishopville (aubrite)	$2 \cdot 2 \pm 0 \cdot 1$ $2 \cdot 8 \pm 0 \cdot 1$	2·5
Johnstown (diogenite)	$12 \cdot 8 \pm 0 \cdot 2$ $2 \cdot 6 \pm 0 \cdot 2$	7·7
Ellemeet (diogenite)	$0 \cdot 6 \pm 0 \cdot 1$ $1 \cdot 3 \pm 0 \cdot 1$	0·9
Moore County (eucrite)	$0 \cdot 3 \pm 0 \cdot 1$ $0 \cdot 9 \pm 0 \cdot 1$	0·6
Binda (howardite)	$0 \cdot 8 \pm 0 \cdot 4$ $\leqslant 0 \cdot 7$	0·7
Nakhla (nakhlite)	$\leqslant 0 \cdot 6$ $0 \cdot 9 \pm 0 \cdot 1$	0·8
Angra dos Reis (angrite)	$0 \cdot 6 \pm 0 \cdot 1$ $0 \cdot 8 \pm 0 \cdot 1$	0·7

Clayton feared that the Re abundance of the lunar surface might be prohibitively enhanced from contamination by chondritic material, which has an average abundance of roughly 5×10^{-8} g Re g^{-1} (refs. 5 and 6). In most common chondrite groups, Re is predominantly concentrated in the Ni–Fe phase[5] and Clayton apparently interprets the high Fe abundance in lunar material as indicating Re enrichment. This is unlikely, for Fe in the lunar rocks occurs chiefly as ilmenite, and in the major silicates. Troilite and native Fe have been tentatively identified but are present in very small amounts. In any event, a simple magnetic separation could be used to remove metal or troilite if present in significant quantities.

Estimates of the concentration of solar wind Re depend on the model used. In crystalline rocks, solar wind enrichment will be largely a surface effect. If the rocks are continuously ablated, the Re concentration will depend on the erosion rate, and equilibrium could be reached in considerably less than 10^9 yr. Conservatively estimating an equilibrium exposure time of 10^6 yr, and assuming quantitative Re retention, 10^{-11} g Re cm^{-2} will be implanted. If a surface layer 0·1 cm thick is sampled, the solar wind contribution will be 10^{-10} g Re cm^{-3}, or approximately 3×10^{-11} g Re g^{-1}. Thus, even for this very short exposure time, an Re isotopic anomaly of 1 per cent more could be expected. It is possible that the rounded shape of many lunar rocks is the result of discrete ablation events rather than continuous erosion, and in this case the solar wind exposure time could well be very long. The surface of these rocks may then contain large amounts of solar wind Re, and an isotopic anomaly of 10 per cent or more might be present.

Rather different arguments apply to the lunar soil, and these may be extended to include the lunar breccias. The exposure of the soil is not limited to the surface, and a lower limit to the implanted Re can be estimated from the lunar soil Xe abundance, which is largely of solar wind origin[3]. Assuming that Xe is quantitatively retained, a concentration of approximately 2×10^{-11} g Re g^{-1} can be obtained by simply applying the ratio of Re to Xe in solar material[7]. This estimate is pessimistic, for the retention of Re will be better than that for the rare gases under reducing lunar conditions. Solar wind Re will be more abundant relative to normal Re in the fine-grained soil fraction because of the greater surface to volume ratio.

Riley[8] has discussed the mass spectrometric determination of isotopic ratios of Re, and the difficulties resulting from the high ionization potential of this element. Some increase in sensitivity can be attained by pulse counting techniques, but the procedures detailed by Riley do not seem easily applicable to Re at the nanogram level.

The nuclear characteristics of Re are particularly favourable for its determination by neutron activation and both naturally occurring isotopes yield radionuclides of convenient half-life. The relevant nuclear properties are summarized in Table 2. For Re of normal isotopic composition, the saturation activity of the (n,γ) products of ^{185}Re and ^{187}Re are approximately $1 \cdot 3 \times 10^4$ and $1 \cdot 5 \times 10^4$ disintegrations s^{-1} for 10^{-8} g Re and a thermal flux of 10^{13} neutrons cm^{-2} s^{-1}. It is possible, therefore, to determine the isotopic ratio of Re with good sensitivity. In principle the gamma rays can be used for the ratio

Table 2. NUCLEAR DATA FOR THE NEUTRON ACTIVATION DETERMINATION OF RHENIUM ISOTOPIC RATIOS

Target	Isotopic abundance (%)	(n,γ) cross section (barns)	Product nuclide	Half lif	Beta energy (MeV)	Gamma energy (MeV)
^{185}Re	37·07	110	^{186}Re	88·9 h	1·07 (95%)	Os X-ray 0·137 (9%)
^{187}Re	62·93	1·3	^{188m}Re	18·7 min	—	
		70	^{188}Re	16·7 h	2·12	Os X-ray 0·155 (10%)

determination, but have rather small branching ratios. Fortunately the beta rays are sufficiently energetic to be counted with little interference from source self-absorption. The isotopic ratio can then be derived by comparison of the decay curves of a radiochemically purified Re source from a lunar specimen, with that of a standard of normal, or known, isotopic ratio.

If the expected isotopic anomaly can be demonstrated it will not only verify Clayton's hypothesis but would be of considerable value in determining relative deposition rates of the lunar regolith and ablation rates of lunar rocks.

I thank an anonymous referee for his suggestions. This work was supported in part by a NASA contract.

J. W. MORGAN

Department of Chemistry,
University of Kentucky,
Lexington, Kentucky 40506.

Received November 17, 1969; revised January 15, 1970.

[1] Clayton, D. C., *Nature*, **224**, 56 (1969).
[2] Gilbert, N., *Compt. Rend.*, **247**, 868 (1958).
[3] Lunar Sample Preliminary Examination Team, *Science*, **165**, 1211 (1969).
[4] Morgan, J. W., and Lovering, J. F., *Earth Planet. Sci. Lett.*, **3**, 219 (1967).
[5] Fouché, K. F., and Smales, A. A., *Chem. Geol.*, **2**, 105 (1967).
[6] Morgan, J. W., and Lovering, J. F., *Geochim. Cosmochim. Acta*, **31**, 1893 (1967).
[7] Cameron, A. G. W., in *Origin and Distribution of the Elements* (edit. by Ahrens, L. H.) (Pergamon, Oxford, 1968).
[8] Riley, G. H., *J. Sci. Instrum.*, **44**, 769 (1967).

Longitudinal Structure in the Plasmapause: VLF Goniometer Observations of Knee-whistlers

A PRELIMINARY study of knee-whistlers observed in the late morning of a quiet day, July 26, 1967, 1206–1236 UT ($K_p = 2-$), by means of a wide-band (0·5 to 11 kHz) VLF goniometer receiver at Halley Bay (333·4° E of N, 75·52° S, generalized invariant latitude at 100 km: $\Lambda' = 61 \cdot 02°$) has revealed the existence of marked longitudinal variation ($\sim 3:1$) in electron tube content on an L-shell ($L \simeq 4 \cdot 7$–$4 \cdot 8$) just outside the plasmapause.

The VLF goniometer receiver installed at Halley Bay, Antarctica, in 1967 consisted of two vertical diamond (30 foot × 30 foot) loops mounted at right angles to each other. From these two loops an equivalent vertical loop, rotating at a frequency of 25 cycles s^{-1}, was simulated electronically. If the electromagnetic wave detected by the equipment has travelled some distance in the Earth–ionosphere waveguide, so that the transverse magnetic mode is dominant, then the modulation of the signal amplitude by the rotating loop (see Fig. 1*a*) may be used to determine the azimuth of the source. The crossed loop technique of determining the azimuth of a whistler exit point is imprecise, chiefly because of bearing errors introduced by multiple rays (J. H. Crary, unpublished) and signal fading. Nevertheless, provided the whistler duct exists long enough to yield a number of bearings determined over a range of frequencies, it is possible to distinguish vetween latitudinal and longitudinal structure in the magnetosphere.

During the period under study the whistlers in ducts near the plasmapause fell into eight groups (Table 1), two of which (N_1, N_2) occurred just inside the plasmasphere at $L \simeq 4 \cdot 2$; the remainder, knee-whistlers, occurred just outside at $L \simeq 4 \cdot 7 - 4 \cdot 8$. The analysis was on sonagrams as illustrated in Fig. 1. In Fig. 1*a* the sonagraph bandwidth ($\Delta f = 300$ Hz) is optimized for bearing determination whereas in Fig. 1*b* a narrower bandwidth ($\Delta f = 45$ Hz) is used to help determine nose frequency and whistler dispersion. For a short period (1222–1224 UT) the whistler groups occurred simultaneously, so it was possible to determine the relative travel times at the nose frequencies (f_n) rather precisely. In the absence of a positive identification of the associated sferic the absolute travel times (t_n) at the nose frequencies were obtained by curve-fitting. The latter calculations were carried out using the "diffusive equilibrium" and "collisionless (R^{-4})" models of the magnetosphere for the normal and knee-whistlers respectively. The treatment followed closely that outlined by Angerami and Carpenter[1] and Angerami (unpublished), except that a more up-to-date model of ion and temperature distribution in the magnetosphere (presented by Rycroft and Alexander at the Prague COSPAR meeting, 1969) was used.

A mean azimuth was determined for each rotation of the loop, up to four individual measures (two minima, two maxima) being made in each rotation. Each azimuth thus determined was weighted according to the number (1 to 4) of individual measures in the rotation and an overall weighted mean for the group calculated. Histograms of the measurements are shown in Fig. 2. The standard deviation (Table 1) as indicated by the histograms is large; nevertheless, there are significant differences in the azimuthal bearings of the exit points of the knee-whistlers.

The generalized invariant latitude (Λ') for an altitude of 100 km was calculated for each group. It is clear that all the knee-whistlers occurred in ducts lying very close (within $\pm 0 \cdot 3°$) to the same L latitude. The contours of $\Lambda' = 60°$, $65°$ shown in Fig. 3 were obtained from tables provided by Jacka[2] and the values of Λ' in Table 1 plotted by interpolation. The points plotted in Fig. 3 indicate the intersection of the mean bearing of a group with its appropriate contour of L latitude. The azimuthal bearing obtained for K_4 did not intersect the appropriate L latitude, but considerations of signal attenuation with distance travelled in the Earth–ionosphere waveguide make

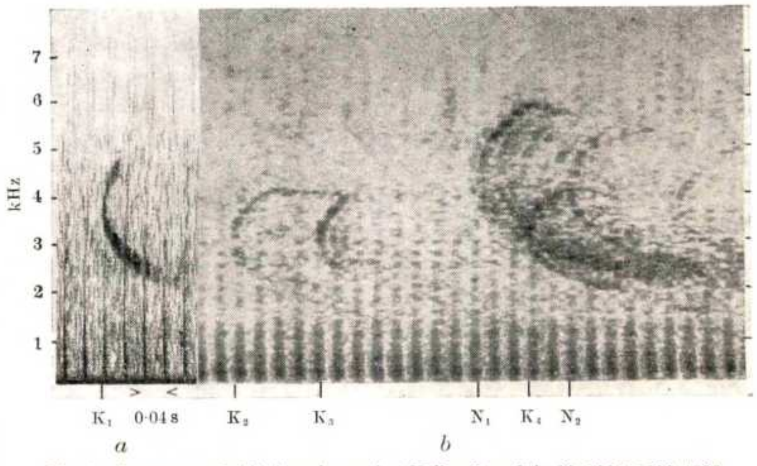


Fig. 1. Sonagrams of whistlers observed at Halley Bay, July 26, 1967, 1206–1236 UT. For a plane electromagnetic wave (transverse magnetic) propagating along the N–S meridian the leading edge of the calibration marker is coincident with a minimum in the signal intensity. *a*, Knee-whistler K_1; sonagraph bandwidth, 300 Hz. *b*, Knee-whistlers K_2, K_3, K_4 and normal whistlers N_1, N_2; sonagraph bandwidth, 45 Hz.

Table 1. GROUPING OF THE WHISTLERS

Whistler group	Period of activity (UT)	No. of whistlers	Mean t_n (s)	Mean f_n (kHz)	Mean L	Mean Λ' (deg.)	No. of measurements of azimuth	Weighted mean azimuth °E of N	R.m.s. deviation (deg.)	N_{eq} ($\times 10^2$ cm^{-3})	Tube content* N_T (electrons cm^{-2}-tube)
N_1	1,210–26	19	2·05	4·55	4·20	60·5	175	66·1	± 17	3	3×10^{14}
N_2	27–35	4	2·21	4·37	4·23	60·7	10	57·2	15	2	3×10^{14}
K_1	10–24	12	1·25	3·54	4·66	62·2	43	− 50·7	13	0·1	6×10^{12}
K_2	22–35	11	1·53	3·24	4·79	62·6	9	20·7	8	0·1	7×10^{12}
K_2'	32–34	3	1·61	3·14	4·83	62·7	—	—	—	0·1	8×10^{12}
K_3	22–35	10	1·70	3·21	4·80	62·6	10	34·4	22	0·15	9×10^{12}
K_3'	22–23 34–35	2	1·755	3·18	4·82	62·7	—	—	—	0·15	1×10^{13}
K_4	11–15 25–34	13	2·13	3·30	4·75	62·4	12	64·6	17	0·3	2×10^{13}

* Above 1,000 km and referred to 1,000 km level.

it likely that the exit point did not lie at a range much in excess of that where the dashed line (K_4'), corresponding to one standard deviation (17°), intersected the L latitude, $\Lambda' = 62{\cdot}45°$. Measurement of the bearing of K_4 was difficult because the whistler was superposed on that of N_1 (Fig. 1*b*). The bearing of N_1 lay rather close to a tangent to the L latitude contour, so there was ambiguity and it could have occurred to the west of Halley Bay at N_1'. The fact that the most intense knee-whistlers were those of K_1 is consistent with its being the nearer duct.

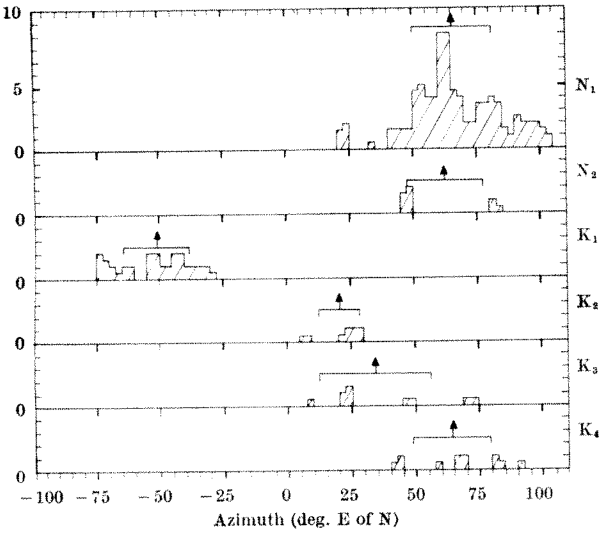


Fig. 2. Histograms of measurements of the azimuth of whistler exit points. The arrows show the weighted mean azimuth.

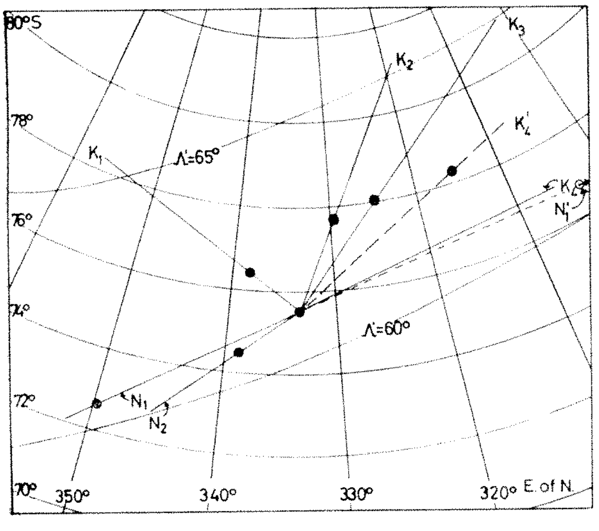


Fig. 3. Map showing probable location of the whistler exit points relative to Halley Bay and the contours, $\Lambda' = 60$, 65°, of generalized invariant latitude for an altitude of 100 km

The values of equatorial electron density and tube content (electrons cm^{-2}-tube) in Table 1 were obtained from the graphs of Angerami for the values of t_n, f_n shown. It can be seen that the equatorial electron density decreased by an order of magnitude across the plasmapause while the tube content dropped ten to fifty times. These values are typical of those obtained by Carpenter and his co-workers. The position of the plasmapause, as might be expected, is slightly to the south of its mean location ($L = 3{\cdot}7$) at this local time (1020 − 1050 LT) on days of moderately steady agitation ($K_p = 2$ to 4) (ref. 3). Especially interesting is the marked longitudinal variation of 3 : 1 in the tube content as we move westward along the same L shell from K_1 to K_4' on Fig. 3. Clearly, the existence of such structure must be borne in mind in the interpretation of knee-whistlers and merits the use of a goniometer to distinguish such features from the latitudinal multilevel structure which has been observed by satellites during magnetic storms.

A more comprehensive study of these and related observations on the same day will be published elsewhere.

We thank the personnel of the British Antarctic Survey, especially Messrs J. Jamieson and W. Laidlaw, for the installation and operation of the equipment at Halley Bay, and Professor T. R. Kaiser for encouragement and advice. One of us (J. L. S.) received a fellowship from ESRO.

K. BULLOUGH
J. L. SAGREDO

Department of Physics,
University of Sheffield,
Sheffield.

Received December 29, 1969.

[1] Angerami, J. J., and Carpenter, D. L., *J. Geophys. Res.*, **71**, 711 (1966).
[2] Kilfoyle, B. P., and Jacka, F., *Nature*, **220**, 773 (1968).
[3] Carpenter, D. L., *Radio Sci.*, **3**, 719 (1968).

Strength–Modulus Relation for Carbonized Acrylic Fibres

THE Young's modulus of carbon fibres based on a single commercial acrylic steadily increases with increasing process temperature[1-3], and reaches 30×10^6 pounds/inch^2 at a final processing temperature of 1,000° C. A corresponding relation for the breaking strength of the fibres shows a peak in strength at either 1,500° C (ref. 1) or 1,200° C (refs. 2 and 3), giving the high breaking strain (1·3 per cent) type of fibre, followed by a decrease in strength at higher processing temperatures. Tests of strength versus gauge length[1,4] have shown that weak spots or flaws influence the breaking strength of the fibres, and this is illustrated, at least for the high strain type of fibres, by microscopy and fractography[2,3,5]. Any relationship between strength and modulus, which has a positive slope, seems to be limited to the processing temperature range 500°–1,000° C. In this temperature range particularly, mechanical properties of the fibres are influenced by the processing conditions and by the nature of the starting material. Any material or processing condition which allows fusion or gross disruption of the pyrolysing

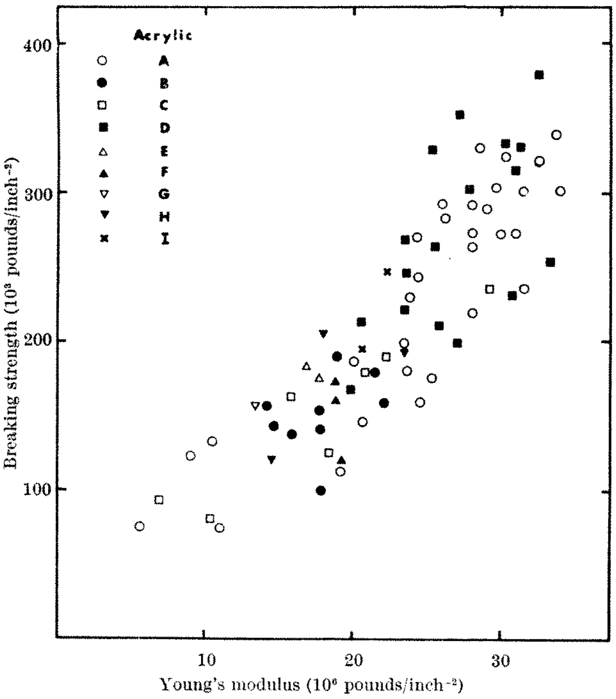


Fig. 1. Breaking strength versus Young's modulus of carbon fibres from various acrylics, variously processed to 1,000° C.

polymer will lead to incoherent carbon fibre with poor mechanical properties.

We show here a positive relationship between breaking strength and Young's modulus of carbon fibres, which is illustrated for a variety of acrylic precursors processed to 1,000° C using a variety of processing conditions. Nine acrylics, one experimentally produced and eight which are commercially available, were processed by the well known techniques of pre-oxidation at 200°–300° C followed by pyrolysis in an inert atmosphere to 1,000° C. The rates of processing during the two stages were varied to optimize the mechanical properties of the resultant carbon fibres. The fibres were submitted to a tensile test at room temperature, using a gauge length of 2·3 cm, to determine both Young's modulus and breaking strength. Usually twelve or more fibres were tested to obtain mean values of modulus and strength. Cross-sectional areas of round fibres were determined from diameters measured by optical microscopy and those of irregularly shaped fibres[5] from optical or scanning electron micrographic enlargements of the fractured ends.

Mean Young's moduli and strengths for the carbonized acrylics are shown in Fig. 1. Although there is a large scatter, a positive relationship between strength and modulus is shown, with a correlation coefficient of 0·86. The relationship applies to a wide range of commercial acrylics, containing a variety of co-polymers and spun by a variety of methods[6]. The main conclusion is that, over the modulus range shown, the fibres have a fairly constant breaking strain of approximately 1·0 per cent, which is not likely to be increased substantially, except by improving strength independently of modulus.

The scatter in the correlation largely results from the influence of flaw mechanisms of fracture on the breaking strength. Flaws operative at this stage of processing have previously been described as surface flaws of obscure identity and internal flaws of known identity[2,3,5,7]. The level of Young's modulus must be specified before the effect of such flaws on the strength of carbon fibres processed to around 1,000° C can be assessed. Moreover, if two or more samples of fibre with different flaw concentrations[7] or simply with different precursor identities are compared on the basis of breaking strength, they must be processed to a similar level of Young's modulus.

A discussion of the complex effect of processing conditions (time, temperature, environment, and so on) on the level of modulus and strength achieved by the various carbonized acrylics is not within the scope of this communication. Usually, however, any processing which leads to physical disruption, as distinct from controlled chemical decomposition, of the pyrolysing polymer structure results in low strength and modulus of the carbonized fibres.

D. J. THORNE

Department of Fibre Research,
Rolls-Royce Limited,
The Old Hall,
Littleover, Derby.

Received February 2, 1970.

[1] Moreton, R., Watt, W., and Johnson, W., *Nature*, **213**, 690 (1967).
[2] Johnson, J. W., *ACS Polymer Preprints*, **9**, 1316 (1968).
[3] Johnson, J. W., *J. Appl. Polymer Symp.* (9), 229 (1969).
[4] Moreton, R., *Fibre Sci. Tech.*, **1**, 273 (1969).
[5] Johnson, J. W., and Thorne, D. J., *Carbon*, **7**, 659 (1969).
[6] Sanders, R. E., *Chem. Process Eng.*, **49**, 100 (1968).
[7] Thorne, D. J., *J. Appl. Polymer Sci.*, **14**, 103 (1970).

Five-fold Pseudosymmetry

IN a previous article[1] I described the properties of a non-crystallographic five-fold pseudosymmetric structure with a density of 0·7236 (compared with 0·7405 for close packing). This structure is generated by the simple process of continued packing on the close packed faces of a pentagonal pyramid (or dipyramid) nucleus (just as the continued packing on the close packed faces of a square pyramid or dipyramid nucleus results in cubic close packing[2]), and it was suggested[1] that the growth of such a pentagonal nucleus was a simple explanation for the origin of observed five-fold pseudosymmetric structures. Further discussion on the origin of pseudosymmetry is presented elsewhere[3].

There have been a number of observations of five-fold pseudosymmetry in many diverse materials[3]. In one of these observations, Allpress and Sanders[4] have tested the model by examination of the distortions in a small five-fold pseudosymmetric gold "crystal". Whereas the model[1] predicted an increase in (011) spacing (compared with face centred cubic packing), careful measurements from diffraction patterns showed a decrease of about 0·2 per cent. The authors conclude[4]: "Hence Bagley's model does not apply to the pentagonal crystals of gold, and the strain must be accommodated in some other way".

In the original model[1] the parameters were chosen so as to minimize the surface area; that is, the surfaces of the pentagonal dipyramid were assumed to be close packed. This note describes the properties of a five-fold pseudosymmetric structure which results from an alternative assumption, that of minimum volume (maximum density).

The pentagonal dipyramid consists of five distorted tetrahedra with one edge of each tetrahedron in common along the dipyramid axis (Fig. 1; see also Fig. 1 of ref. 1). Within each tetrahedron the structure is body centred orthorhombic with cell dimensions now chosen such that the volume will be a minimum (consistent with the non-overlap of the spheres) and such that two adjacent tetrahedra will be joined by a coincidence boundary. These conditions yield a body centred orthorhombic cell with dimensions (diameter of sphere = 1·000), $a = \sqrt{3}\sin 36° = 1·0181$, $b = \sqrt{3}\cos 36° = 1·4013$, $c = 1·000$). The assignment of axes is the same as in the previous

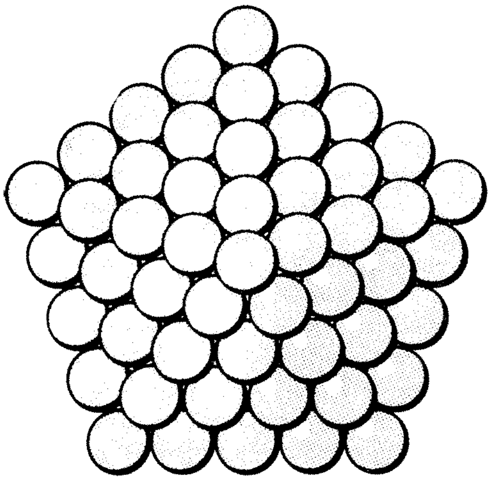


Fig. 1. View along the five-fold axis of a pentagonal structure obtained by the continued packing of hard spheres on the faces of a pentagonal dipyramid.

article[1] and the *c* axis is parallel to the five-fold pseudosymmetric axis. Thus this pentagonal structure has a density of 0·73406, independent of position. This density is slightly lower than that for close packing (0·74048), but higher than that for body centred cubic (0·68017), icosahedral shell packing (0·68818)[5] or the previous five-fold pseudosymmetric structure (0·72357)[1]. The coordination is 10 at a distance of 1·000 and 2 at a distance of 1·018. As with the previous structure, this structure is an example of G_3^1 type symmetry and its symmetry group is 5mP2ml (Niggli's nomenclature[6]).

This new structure predicts a decrease in the (011) spacing (compared with face centred cubic packing) of 0·2 per cent, in agreement with the results of Allpress and Sanders[4]. Thus we conclude that the five-fold gold structure observed by these authors resulted from the growth of a pentagonal dipyramid nucleus, and during this growth the density was maximized.

If a pentagonal dipyramid nucleus forms, either by chance or because of energy considerations[3], its continued growth will lead to a structure with five-fold pseudosymmetry. Depending on the material and growth conditions the surface area or volume may be minimized, or some balance obtained in between. Thus observed structure parameters can vary between those described previously[1] and those described here.

B. G. Bagley

Bell Telephone Laboratories, Incorporated,
Murray Hill, New Jersey 07974.

Received November 3, 1969.

[1] Bagley, B. G., *Nature*, **208**, 674 (1965).
[2] Coxeter, H. S. M., *Illinois J. Math.*, **2**, 746 (1958).
[3] Bagley, B. G., *J. Crystal Growth* (in the press).
[4] Allpress, J. G., and Sanders, J. V., *Surface Sci.*, **7**, 1 (1967).
[5] Mackay, A. L., *Acta Cryst.*, **15**, 916 (1962).
[6] Niggli, A., *Zeit. Krist.*, **111**, 288 (1959).

BIOLOGICAL SCIENCES

Effect of Phosphate and Non-phosphate Buffers on Thermolability of Unstable Haemoglobins

A GROUP of hereditary non-spherocytic anaemias have been shown to be associated with unstable haemoglobins through the application of a simple screening test for thermolability involving incubation of haemolysate at 50° C in phosphate buffer (*p*H 6·5–7·4; 0·1 M)[1-3]. Unstable haemoglobins form a precipitate within a few hours but normal haemoglobin remains in solution. We have encountered two families with this type of anaemia whose haemoglobins demonstrated little or no precipitate in these conditions, but formed copious precipitate in the presence of non-phosphate buffers—observations which led us to wonder whether the recently described effects of phosphates on the oxygen-binding properties of haemoglobin[4-7] might not also modify the heat stability of the molecule.

Most unstable haemoglobins subjected to structural analysis have revealed non-polar amino-acid substitutions at sites which would seem to affect haem–globin association adversely[8]. Access of water to a relatively loose haem–globin binding site in the presence of oxygen would promote the formation of methaemoglobin[9,10], weakening the haem–globin linkage even further[11]. Methaemoglobin formation as a precondition to heat precipitation has been an early and frequent observation in the unstable haemoglobins[1-3,12]. The developing precipitate is, for the most part, free globin, which is known to be more vulnerable to conditions of mild heating than intact haemoglobin[7].

We tested the heat stability of haemoglobin solutions prepared from affected subjects and from controls in different buffer systems following the method of Dacie *et al.*[3]. Buffers included sodium phosphate, NaCl, *tris*-(hydroxymethyl)aminomethane (Tris), Tris–maleate, sodium barbital and inositol hexaphosphate (IHP) at *p*H 7·4, in the molar range 0·001 to 1·0. Like 2,3-diphosphoglycerate (DPG), IHP is an organic polyphosphate, the chief organic phosphate in avian erythrocytes[14,15], and exerts a profound effect on the oxygen affinity of haemoglobin[4-7]. Studies were done on freshly prepared haemolysates, haemolysates dialysed exhaustively against deionized water, and haemolysates that had been allowed to age in the cold (4° C) for varying lengths of time. Incubation took place in a 50° C oven and observations were made every 30 min until visible precipitate appeared.

Table 1. PRESENCE (+) OR ABSENCE (–) OF GROSS TURBIDITY AND/OR PELLET IN BUFFERS (*p*H 7·4) CONTAINING 1 G PER CENT HAEMOGLOBIN FOLLOWING INCUBATION FOR VARYING LENGTHS OF TIME AT 50° C. HAEMOGLOBIN WAS PREVIOUSLY DIALYSED AGAINST DEIONIZED WATER

D.T. (patient)

	NaCl	Tris	Tris-maleate	Barbital	Sodium	Phosphate	IHP
Molarity:	0·1	0·1	0·1	0·1	0·1	0·05	0·1
Ionic strength:	0·1	0·1	0·1	0·1	0·2	0·1	
0·5 h	–	–	–	–	–	–	–
1 h	+	+	+	+	–	–	–
1·5 h	+	+	+	+	–	–	–
2 h	+	+	+	+	–	–	–
2·5 h	+	+	+	+	+	+	–
3 h	+	+	+	+	+	+	+
4 h	+	+	+	+	+	+	+

I. J. (control)

	NaCl	Tris	Tris-maleate	Barbital	Sodium	Phosphate	IHP
Molarity:	0·1	0·1	0·1	0·1	0·1	0·5	0·1
Ionic strength:	0·1	0·1	0·1	0·1	0·2	0·1	
0·5 h	–	–	–	–	–	–	–
1 h	–	–	–	–	–	–	–
1·5 h	–	–	–	–	–	–	–
2 h	+	–	–	–	–	–	–
2·5 h	+	–	–	–	–	–	–
3 h	+	+	+	+	–	–	–
4 h	+	+	+	+	+	+	+

Both normal and unstable haemoglobins displayed a tendency to form precipitate more readily and more copiously in non-phosphate buffers. Whether the appearance of precipitate was recorded qualitatively (Table 1) or quantitated by the Lowry method, this phenomenon was clearly evident. In all our studies, haemoglobins from patients were more heat labile than normal haemoglobin. Phosphate buffer, however, particularly IHP, strikingly limited and retarded precipitate formation. The effect of IHP could not be attributed merely to the absence of inorganic salt, for it occurred even in the presence of varying concentrations of NaCl. Cold storage of normal and abnormal haemoglobins, both dialysed and undialysed, resulted in increased thermolability, the pattern of responses reflecting that seen in the fresh state. Like other observers, we noted the appearance of methaemoglobin coincident with precipitate formation.

If the events leading to heat precipitation involve conversion of oxyhaemoglobin to methaemoglobin with subsequent precipitation of released globin, then it is likely that the presence of phosphates interrupts this sequence by displacing oxygen from the haemoglobin molecule. In the patients studied by us, the haem–globin defect, albeit sufficient to cause haemolytic anaemia, seemed subtle enough to elude detection by the conventional procedure for screening of thermolability using phosphate buffer.

It is tempting to hope that such *in vitro* effects of organic phosphates might have some beneficial therapeutic application. Obviously, oxygenation of haemoglobin is vital for survival; it is, however, conceivable that some shift in the oxygen saturation curve might be tolerated by a person with unstable haemoglobin, particularly if it resulted in a significant reduction in haemoglobin precipitation and haemolysis. The most immediate application of our observations lies in the recommendation that non-phosphate buffers be used as a more sensitive and suitable system for the thermolabile screening test.

This work was supported in part by grants from the US National Institutes of Health and the Muscular Dystrophy Associations of America. We thank Wallace I. Sampson and Theodore Cohen for helping obtain blood samples. B. Brimhall and R. T. Jones have identified the haemoglobin in one family as Lepore (Washington–Augusta–Boston). Structural study of the other haemoglobin is in progress.

LAWRENCE J. SCHNEIDERMAN
IRENE G. JUNGA
DOROTHEA E. FAWLEY

Stanford University School of Medicine,
Department of Medicine,
Stanford, California 94305.

Received October 16; revised December 16, 1969.

[1] Grimes, A. J., and Meisler, A., *Nature*, **194**, 190 (1962).
[2] Grimes, A. J., Meisler, A., and Dacie, J. V., *Brit. J. Haemat.*, **10**, 281 (1964).
[3] Dacie, J. V., Grimes, A. J., Meisler, A., Steingold, L., Hemsted, E. H., Beaven, G. H., and White, J. C., *Brit. J. Haemat.*, **10**, 388 (1964).
[4] Chanutin, A., and Curnish, R. R., *Arch. Biochem. Biophys.*, **121**, 96 (1967).
[5] Benesch, R., and Benesch, R. E., *Biochem. Biophys. Res. Commun.*, **26**, 162 (1967).
[6] Benesch, R., Benesch, R. E., and Yu, C. I., *Proc. US Nat. Acad. Sci.*, **59**, 526 (1968).
[7] Benesch, R., Benesch, R. E., and Enoki, Y., *Proc. US Nat. Acad. Sci.*, **61**, 1102 (1968).
[8] Lehmann, H., and Carrell, R. W., *Brit. Med. Bull.*, **25**, 14 (1969).
[9] Carrell, R. W., and Lehmann, H., *Seminars in Hematology*, **6**, 116 (1969).
[10] Weiss, J. J., *Nature*, **202**, 83 (1964).
[11] Bunn, H. F., and Jandl, J. H., *Proc. US Nat. Acad. Sci.*, **56**, 974 (1966).
[12] Jones, R. V., Grimes, A. J., Carrell, R. W., and Lehmann, H., *Brit. J. Haemat.*, **13**, 394 (1967).
[13] Fanelli, A. Rossi, and Antonini, E., *Biochim. Biephys. Acta*, **30**, 608 (1958).
[14] Rapoport, S., and Guest, G. M., *J. Biol. Chem.*, **138**, 269 (1941).
[15] Oshima, M., Taylor, T. G., and Williams, A., *Biochem. J.*, **92**, 42 (1964).

High Resolution Nuclear Magnetic Resonance Studies of Haemoglobin J Capetown

HAEMOGLOBIN J CAPETOWN[1] is a Hb variant in which one amino-acid in the α-chain, arginine α92 (FG4), is replaced by glutamine. In Hb Chesapeake[2] the same arginine is replaced by leucine, and the properties of the two variants are seen to be similar[2,3]. Compared with normal Hb A, both have increased oxygen affinities, smaller "haem-haem" interactions, and alkaline Bohr effects which are approximately normal. The X-ray crystallography of oxyhaemoglobin at 2·8 Å resolution shows that the residue α92 (FG4) is at the α1β2 interface[4]. Perutz and H. L.[5] have pointed out that all known haemoglobin variants with alterations at the α1β2 interface have reduced "haem-haem" interaction.

The NMR spectra of paramagnetic haem proteins indicate that the protons of the haem group are shifted by hyperfine interactions with the unpaired electrons which are delocalized from the iron[6-8]. These shifted resonances are well resolved and for the cyanide complexes of myoglobin[9] and haemoglobin (S. O., M. Fujiwara, T. Yamane and R. G. S., to be published) have been identified with specific haem protons. The positions of these resonances can be directly related to the spin densities in the porphyrin ring at the points of contact of these protons. Hence they are very sensitive to changes in the electron distribution of the haem group. Recently it has been shown by nuclear magnetic resonance (NMR) measurements on a mixed state haemoglobin consisting of methaemoglobin α and deoxyhaemoglobin β-chains that the electronic structures of the α and β-haems were the same as in the corresponding homogeneous tetramers[10]. Because this mixed state haemoglobin has a higher oxygen affinity than deoxy Hb A, it was concluded that this higher affinity was caused by changes at the interfaces between the haemoglobin subunits and not by changes at the haem.

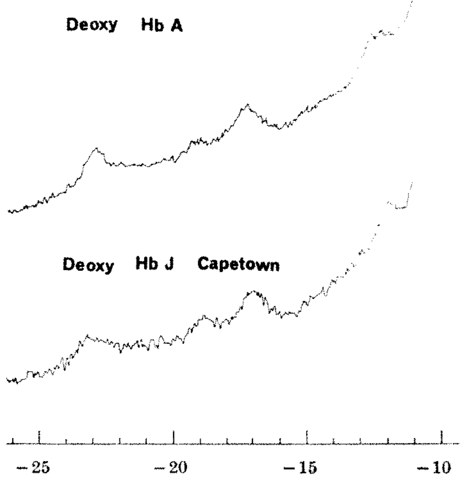


Fig. 1. Low field shifted resonances of Hb J Capetown and Hb A in the deoxy form at 20° C, *p*D 7·0.

It was already known that in the deoxy form of single Hb A chains and several high oxygen affinity haemoglobins, the Soret band of the optical absorption spectrum is broadened and shifted as compared with that of Hb A[11,12]. This correlation between optical spectrum and raised oxygen affinity was not found in Hb Chesapeake[2].

It is the purpose of the present experiment to see if the small change at the α1β2 interface in Hb J Capetown leaves the electronic structure of the haem unaltered, while reducing the "haem-haem" interaction. If these two effects can be separated, then the changes of oxygen affinity are not necessarily related to haem changes. We have therefore compared the electron distribution of Hb J Capetown and of normal Hb A before and after ligation. The separation of the two effects was observed in the previous NMR experiments on a mixed state partially ligated haemoglobin which represented a possible intermediate state of oxygenation[10], but it is desirable to carry out these experiments on two natural haemoglobins with different oxygen affinities.

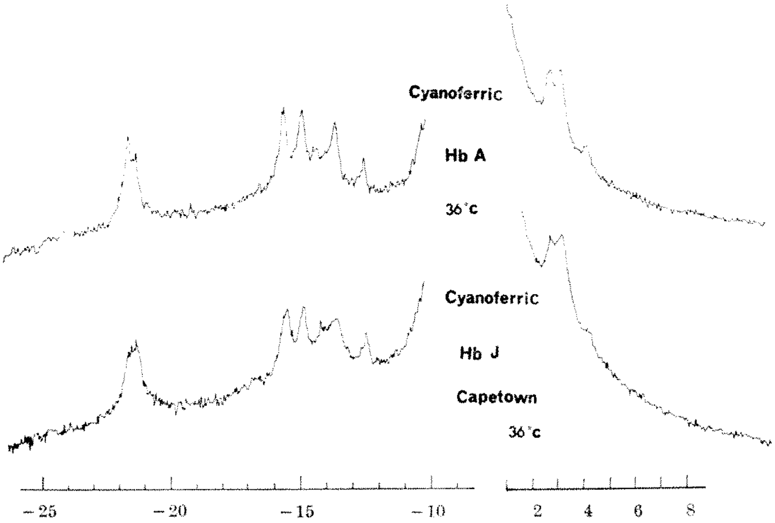


Fig. 2. Shifted resonance lines of Hb J Capetown and Hb A in the cyanoferric form at 36° C, pD 7·0

Human haemoglobin A was prepared from freshly drawn blood using the procedure of Bunn and Jandl[13]. Cyanoferric (Cyan-met) haemoglobin was obtained by oxidation of oxyhaemoglobin with 1·3 equivalents of ferricyanide in the presence of KCN and pH 7·0 phosphate buffer. Hb J Capetown was prepared from the haemolysate of a heterozygote containing 32 per cent of the Hb variant by repeated chromatography on 'DEAE Sephadex' using a pH gradient according to Huisman and Dozy[14] followed by paper electrophoresis at pH 8·9 (ref. 15) until there was no demonstrable Hb A.

The NMR spectra were measured at 220 MHz in a 'Varian *HR*-220' spectrometer. Peak positions were measured with respect to 2,2 dimethyl-2-silapentane-5-sulphonate (DSS). Low field shifts have been assigned negative signs. Because the signals were weak it was necessary to accumulate the spectra for several hours in a 1024 channel analyser.

The NMR spectra of the deoxy and cyanoferric forms of haemoglobin J Capetown and haemoglobin A are compared in Figs. 1 and 2. The spectra are the same in both forms. For deoxyhaemoglobin there are five identifiable resonances at low fields and all five in both haemoglobins have the same shifts within the experimental uncertainties of ±30 Hz. For the lowest field line, which from its measured intensity of three protons per haem is a methyl group, the total shift is more than 4,000 Hz to lower field from its position in a diamagnetic compound. Thus the fractional difference between these shifts in the two haemoglobins is less than 0·5 of 1 per cent. Slightly larger fractional values of the upper limits would be obtained from the other resonances because their total shifts are smaller.

The similarity between the Hb J Capetown spectrum and that of Hb A is established within narrower limits in the cyanoferric haemoglobins than in the deoxyhaemoglobins because the resonances in the former are narrower and more intense. Quantum mechanical considerations indicate that for the cyanoferric haem proteins the spin densities are approximately proportional to the ligand binding energy (S. O. *et al.*, to be published). On the other hand, deoxyhaemoglobin is not understood quantum mechanically, and shifts may arise from other mechanisms such as pseudocontact interaction[16]. Regardless of the mechanism, however, the shifts in deoxyhaemoglobin should be very sensitive to the electronic structure of the haem. Wyman[17] has shown that there must be an approximately 10 per cent change in the oxygen binding energy during oxygenation to account for the observed "haem-haem" interaction in normal haemoglobin. Our experiments prove that in ligated cyanoferric haemoglobin the energy difference between Hb J Capetown and Hb A is at least ten times smaller. In the deoxy form no differences were observed between the NMR spectra of the two haemoglobins. This suggests that in the unligated form also, energy differences are negligible. Thus the difference in oxygen affinity between the two haemoglobins cannot be caused by differences at the haem. Similar results in the mixed state haemoglobin could be explained by a sequential model[18] but not by the symmetric allosteric model[19]. A consequence of the sequential model is that the amino-acid change in Hb J Capetown reduces co-operation between subunits during oxygenation by affecting the binding energies at their interfaces.

Thus in the strict sense the phrase "haem-haem interaction" is a misnomer when used to describe the cooperative oxygenation of haemoglobin.

We thank M. F. Perutz and J. Greer for valuable discussions.

S. Ogawa
R. G. Shulman

Bell Telephone Laboratories, Inc.,
Murray Hill,
New Jersey.

P. A. M. Kynoch
H. Lehmann

MRC Abnormal Haemoglobin Unit,
University of Cambridge.

Received December 23, 1969; revised January 12, 1970.

[1] Botha, M. C., Beale, D., Isaacs, W. A., and Lehmann, H., *Nature*, **212**, 792 (1966).
[2] Nagel, R. L., Gibson, Q. H., and Charache, S., *Biochemistry*, **6**, 2395 (1967).
[3] Lines, J. G., and McIntosh, R., *Nature*, **215**, 297 (1967).
[4] Perutz, M. F., Muirhead, H., Cox, J. M., and Goaman, L. C. G., *Nature*, **219**, 131 (1968).
[5] Perutz, M. F., and Lehmann, H., *Nature*, **219**, 29 (1968).
[6] Kowalsky, A., *Biochemistry*, **4**, 2382 (1965).
[7] Wüthrich, K., Shulman, R. G., and Peisach, J., *Proc. US Nat. Acad. Sci.*, **60**, 373 (1968).
[8] Kurland, R. J., Davis, D. G., and Ho, C., *J. Amer. Chem. Soc.*, **90**, 2700 (1968).
[9] Shulman, R. G., Wüthrich, K., Yamane, T., Antonini, E., and Brunori, M., *Proc. US Nat. Acad. Sci.*, **63**, 623 (1969).
[10] Shulman, R. G., Ogawa, S., Wüthrich, K., Yamane, T., Peisach, J., and Blumberg, W. E., *Science*, **165**, 251 (1969).
[11] Benesch, R., Gibson, Q. H., and Benesch, R. E., *J. Biol. Chem.*, **239**, 1668 (1964).
[12] Brunori, M., Antonini, E., Wyman, J., and Anderson, S. R., *J. Mol. Biol.*, **34**, 357 (1968).
[13] Bunn, H. F., and Jandl, J. H., *J. Biol. Chem.*, **243**, 465 (1968).
[14] Huisman, T. H. J., and Dozy, A. M., *J. Chromat.*, **19**, 160 (1965).
[15] Cradock-Watson, J. E., Fenton, J. C. B., and Lehmann, H., *J. Clin. Path.*, **12**, 372 (1959).
[16] McConnell, H. M., and Robertson, R. E., *J. Chem. Phys.*, **29**, 1361 (1958).
[17] Wyman, J., *Adv. Protein Chem.*, **19**, 223 (1964).
[18] Koshlan, D. E., Némethy, G., and Filmer, D., *Biochemistry*, **5**, 365 (1966).
[19] Monod, J., Wyman, J., and Changeux, J. P., *J. Mol. Biol.*, **12**, 88 (1965).

Exchange of Bound Tritium between DNA and Water at Different Temperatures

A CLUE to the structure of macromolecules in solution is given by the exchange of protons between the macromolecules and the surrounding solvent. We have investigated the exchange of tritium between DNA and water at different temperatures. We have used the technique

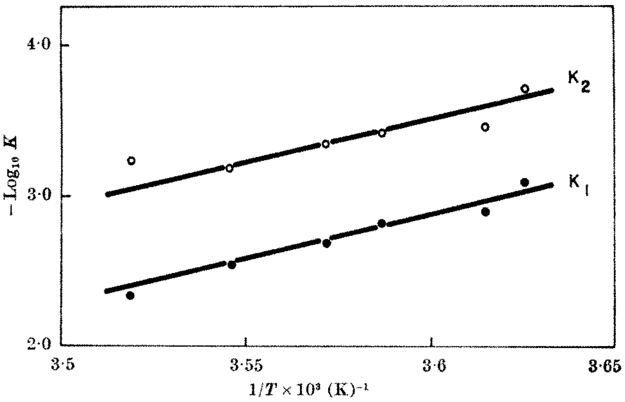


Fig. 1. Loss of ^{3}H from DNA previously equilibrated with ^3H_2O. DNA samples were equilibrated with ^3H_2O and passed through a 'Sephadex *G*-50' column to remove the ^3H_2O. The eluted DNA was incubated at the indicated temperature and at intervals samples were passed through a '*G*-25' column to separate the DNA from ^{3}H which had been exchanged. The results are presented as the residual ^{3}H bound per nucleotide pair, calculated from the specific activity of the ^3H_2O and from the amount of DNA in the DNA peak eluting from the 'Sephadex *G*-25' column (see text). The radioactivity per sample varied from 7,000 to 60 c.p.m. during the course of the exchange reaction, depending on the loss of bound ^{3}H and the yield of DNA in each sample. The solid curves were calculated as a best fit to the six sets of data using the equation

$$R = \bar{A}\ 10^{-K_1^*(T)t} + \bar{B}\ 10^{-K_2^*(T)t}$$

(see text) where $\bar{A} = 1{\cdot}962$ and $\bar{B} = 0{\cdot}410$. The fit should be compared with the experimental points.

of 'Sephadex' chromatography described by Englander[1] and used by Printz and von Hippel[2] to study tritium exchange between DNA and water.

Calf thymus DNA (Sigma) was sheared by sonication and equilibrated with ^3H_2O (500 mCi/ml.). In all experiments the concentration of thymus DNA was between 2 and 3 mg/ml. A small amount (less than 1 µg/ml.) of ^{14}C-labelled DNA from *Escherichia coli* was added as a marker. A 2 ml. aliquot of the tritiated DNA was equilibrated at the desired temperature and passed through a jacketed column (44 cm × 5 mm) of 'Sephadex *G*-50'. The emerging ^{14}C–DNA peak contained less than 1 part in 10^6 of the original ^{3}H radioactivity which could be eluted subsequently from the column. Samples from this DNA solution were passed through a second jacketed column (44 cm × 5 mm) of 'Sephadex *G*-25' to remove the free tritium. The bound tritium emerging with the ^{14}C–DNA was counted in an aqueous scintillation fluid[3].

Throughout the experiments the solvent used to equilibrate the DNA and ^3H_2O and to wash the columns was 0·1 M NaCl in 0·01 M sodium cacodylate (*p*H 7·5). The temperature of the columns and DNA solution was maintained to within ±0·2° C. The time allowed for exchange was taken as the difference between the time when the DNA solution was put on to the 'Sephadex *G*-50' column and the time when a sample emerged from the '*G*-25' column.

Fig. 1 shows the loss of ^{3}H from DNA at different temperatures. The experimental points are given as residual ^{3}H bound per nucleotide pair. Printz and von Hippel[2] observed that the loss of tritium from DNA followed a biphasic curve which corresponded to two possible tritium populations, each exchanging independently of the other. We have fitted our data with curves corresponding to the equation $R = \bar{A}\ 10^{-K_1^*(T)t} + \bar{B}\ 10^{-K_2^*(T)t}$, where R is the residual bound tritium, $\bar{A}$ and $\bar{B}$ are independent but the same for all temperatures (T), and $K_1^*(T)$ and $K_2^*(T)$ are independent constants that vary with the temperature.

The following hand computational technique has been used to find a family of functions $\bar{A}\ 10^{-K_1^*(T)t} + \bar{B}\ 10^{-K_2^*(T)t}$ to approximate f(t,T), so that $\bar{A}$ and $\bar{B}$ are independent and K_1^* and K_2^* are dependent on T. (A mathematically more sophisticated program for optimal fit by high speed computer is being prepared by R. G. Selfridge at the Computing Center, University of Florida, Gainesville.)

If $K_1^* \ll K_2^*$ the effect of K_2^* will become negligible for large t. For each T value, therefore, choose (t_1,f(t_1,T)) such that f(t_1,T) is the last datum in which the experimenter has confidence. Choose (t_2,f(t_2,T)) such that f(t_2,T) is eight or nine times greater than f(t_1,T). Solve the equations

$$\mathrm{f}(t_1,T) = A(T)\ 10^{-K_1(T)t_1}$$
$$\mathrm{f}(t_2T) = A(T)\ 10^{-K_1(T)t_2}$$

for $A(T)$ and $K(T)$ at each T. To impose the condition that $\bar{A}$ be independent of T, calculate $\bar{A}$, the arithmetic mean of the $A(T)$. Recalculate $K_1^*(T)$ for each T by solving $\mathrm{f}(t_3,T) = \bar{A}\ 10^{-K_1^*(T)_3}$ such that $t_1 \geq t_3 \geq t_2$ to give an optimal fit to the data $f(t,T)$; $t_1 \geq t \geq t_2$. Calculate $\mathrm{g}(t,T) = \mathrm{f}(t,T) - \bar{A}\ 10^{-K_1^*(T)t}$ (again choose the largest value of t, that is, t_4, for which g(t,T) has experimental validity). Choose t_5, the next to smallest t value. t_5 is not the least t, because of the possibility of contamination by another exponential $\bar{C}\ 10^{-K_3^*t}$ where $K_3^* > K_2^*$. Calculate $B(T)$ and $K_2(T)$ from $\mathrm{g}(t_4,T) = B(T)\ 10^{-K_2(T)t_4}$ and $\mathrm{g}(t_5,T) = B(T)\ 10^{-K_2(T)t_5}$. Calculate $\bar{B}$, the arithmetic mean of $B(T)$, and recalculate $K_2^*(T)$ from $\mathrm{g}(t_6,T) = \bar{B}\ 10^{-K_2^*(T)t_6}$ where $t_4 \geq t_6 \geq t_5$ and t_6 is chosen to optimize the fit to g(t,T). Then $\bar{A}\ 10^{-K_1^*(T)t} + \bar{B}\ 10^{-K_2^*(T)t}$ approximates f(t,T) with the desired properties.

Table 1. VALUES OF K_1^* AND K_2^* OBTAINED AT DIFFERENT TEMPERATURES BY FITTING THE DATA IN FIG. 1 WITH THE EQUATION $R = 1{\cdot}962 \times 10^{-K_1^*(T)t} + 0{\cdot}410 \times 10^{-K_2^*(T)t}$

T	K_1^*	K_2^*
11·2° C	$4{\cdot}6 \times 10^{-3}$	$5{\cdot}82 \times 10^{-4}$
9·0°	2·86	6·39
7·0°	2·02	4·95
5·8° C	1·51	3·92
3·6° C	1·26	3·49
2·8° C	0·818	1·98

It has been possible to fit all of the data with a value of $\bar{A}$ (1·962) and of $\bar{B}$ (0·410) both of which correspond well with the values determined previously by Printz and von Hippel[2]. Evidence for a more rapidly exchanging component can be observed at early times in the 2·8° and 3·6° samples. This component has been ignored in our analysis.

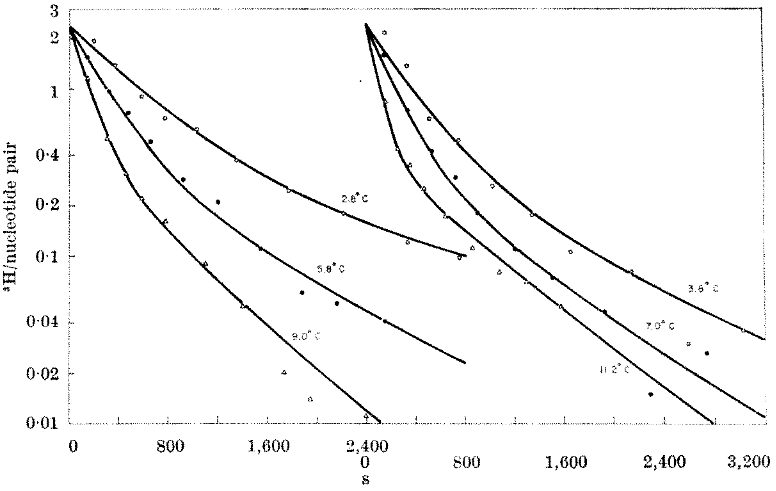


Fig. 2. A graphic representation of the data in Table 1. The slopes of the solid lines represent a Δ H$^+$ of 24 kilocalories/mole. The entropies of activation are 37·1 and 35·7 entropy units for components $\bar{A}$ and $\bar{B}$ respectively.

Table 1 lists the values of $K_1^*(T)$ and $K_2^*(T)$ obtained at the different temperatures. Fig. 2 presents the logarithm of these values as a function of $1/T$. With the exception of K_2^* (11·2°) all the values are consistent with a linear relationship between $\log_{10}K^*(T)$ and $1/T$ such that the slopes for $K_1^*(T)$ and $K_2^*(T)$ are the same. It is evident that the slope log $K_1^*(T)$ versus $1/T$ is much better defined than the slope of the same function of $K_2^*(T)$. This scatter is not unexpected if one considers that the counts at the later points of the curve are low and subject to greater error. In the following discussion we have assumed that the slopes of the two curves in Fig. 2 are the same.

From the curves in Fig. 2 we can calculate that the activation enthalpy for exchange is 24 ± 4 kilocalories/mole of activated complex and is almost the same for the fast and slow exchange components. There are about four times as many fast exchanging tritiums ($\bar{A}$) as slow ($\bar{B}$). The probability of these exchanging (entropy) is quite different: $\Delta S_1 = 37\cdot1$, $\Delta S_2 = 35\cdot7$. In the activated state, it is four times more probable for the fast component to exchange than for the slow component.

To explain these values, we suggest the following model. As proposed by Printz and von Hippel[2] the hydrogens that are exchanging are those involved in base pairing within the DNA helix. For exchange to take place, the rate limiting process would be the separation of base pairs and their subsequent orientation away from the helix centre toward the surrounding solvent. Subsequent exchange would be successful if the tritium could exchange with hydrogen bound to a solvent molecule before the bases re-orient toward the centre of the helix. This should be as probable for external tritiums as for those involved in the A·T base pair or two of those involved in the G·C base pair. The internal tritium involved in the bond between 1-nitrogens of the guanine and the cytosine groups should, however, encounter steric hindrance in exchanging with a solvent water. As a result, its exchange should occur less frequently, in spite of the similar activation energy dependence for the requirement to separate the base pair. For this difference to be observed, the probability of either a class $\bar{A}$ or class $\bar{B}$ exchange to occur during any activation event must be small—of the order of one or less exchange events per opening of the helix.

The activation enthalpy of 24 kilocalories/mole of activated complex is too high for the separation of a single base pair, and it seems more likely that the activation event involves the simultaneous separation of two or three base pairs, a thermodynamically stable situation that will preserve the stacking energy within the helix[4].

R. E. B. was supported by a training grant, and K. G. L. by a career development award, from the National Institute of General Medical Sciences. The research was supported by the National Institute of Allergy and Infectious Diseases and the US National Science Foundation.

ROBERT E. BIRD*
KARL G. LARK

Department of Biology,

BASIL CURNUTTE

Department of Physics,

JOHN E. MAXFIELD

Department of Mathematics,
Kansas State University,
Manhattan, Kansas 66502.

Received September 12, 1969.

* Currently at Oak Ridge National Laboratory.

[1] Englander, S. W., *Biochemistry*, **2**, 798 (1963).

[2] Printz, M. P., and von Hippel, P. H., *Proc. US Nat. Acad. Sci.*, **53**, 363 (1963).

[3] Lark, K. G., Repko, T., and Hoffman, E. J., *Biochim. Biophys. Acta*, **76**, 9 (1963).

[4] Eigen, M., in *Nobel Symposium 5: Fast Reactions and Primary Processes in Chemical Kinetics* (edit. by Claesson, S.) (Interscience Publishers, NY, 1967).

Interaction between Papova-like Virus and Paramyxovirus in Human Brain Cells: a Hypothesis

SUBACUTE sclerosing panencephalitis (SSPE), a progressive neurological disease of children, is characterized by intracytoplasmic and intranuclear inclusions in the neurones and glial cells. Although no infectious agent was isolated directly from the diseased brain tissue, a paramyxovirus, related serologically to the measles–rinderpest virus group, was recovered from SSPE brain cells maintained in tissue culture in serial passages[1]. These cultures were characterized by the formation of syncytia and by their capacity to undergo transformation[2]. Only by fusion with African green monkey kidney cells (AGMK) could an infectious syncytiogenic paramyxovirus be recovered from the brain cells.

Under the electron microscope, particles resembling paramyxovirus nucleocapsids were seen in nuclei and cell cytoplasm of all three human brain cultures[3], even though infectious paramyxovirus was recovered from only two cultures. Budding particles resembling paramyxovirus structurally were also seen in cells of the infected AGMK cultures. In addition, symmetrical particles of a different type, without membrane, were seen under electron microscope in the cell cytoplasm of one SSPE human brain culture and in AGMK cells infected with the syncytiogenic paramyxovirus isolated from the other two cultures (Table 1). In morphology and size range, particles of the second type resembled virions of the Papova virus group—for example, polyoma or SV40. These particles were observed in the cytoplasm of the human brain cell (Fig. 1*a*) and were found frequently in the same cells with paramyxovirus nucleocapsids. They were also seen in AGMK cells showing presence of paramyxovirus at the cell surface (Fig. 1*b*). In the brain of ferret injected with homogenates of infected AGMK cells (Table 1), they were seen in cells in which paramyxovirus was not observed.

Table 1. PAPOVA-LIKE AGENT IN HUMAN BRAIN TISSUE

Subacute sclerosing panencephalitis case	Syncytiogenic agent nucleocapsids in cells of brain cultures	Electron microscopic visualization of Papova-like virions in Human brain cell cultures	AGMK cells	Ferret brain tissue
ROB	+	+	NT	NT
JAC	+	−	+*	NT
LEC	+	−	+*	+ †

NT: not tested.

* African green monkey kidney cultures infected with material from heterokaryon cultures obtained by fusion of JAC brain cells with AGMK cells.

† Ferret killed two weeks after intracerebral inoculation of extracts from AGMK cells infected with material obtained from heterokaryon cultures produced by fusion of LEC brain cells with AGMK cells.

Preliminary results of gradient centrifugation of homogenates of cells showing presence of Papova-like particles revealed presence of 20S peak corresponding to the sedimentation velocity of circular DNA of the known Papova viruses[4].

Cells showing presence of Papova-like particles did not stain for either T or VP antigen in the presence of SV40 antibody conjugated with fluorescein[1].

Although particles resembling Papova virions were actually seen in cells of only one human brain culture (ROB; Table 1), intracellular particles of the same type must have been present in the two other cultures (JAC and LEC), because they were recovered, together with the paramyxovirus, in AGMK cells after fusion of the latter cells with JAC and LEC cells (Table 1).

If Papova-like virus particles are indeed present in human brain cells, then further consideration should be given to their role in the pathogenesis of chronic encephalopathies and other diseases, especially in the light of the following observations made during this study. (1) Neither tissue culture medium nor cell extracts of SSPE human brain cell cultures were infectious for normal human

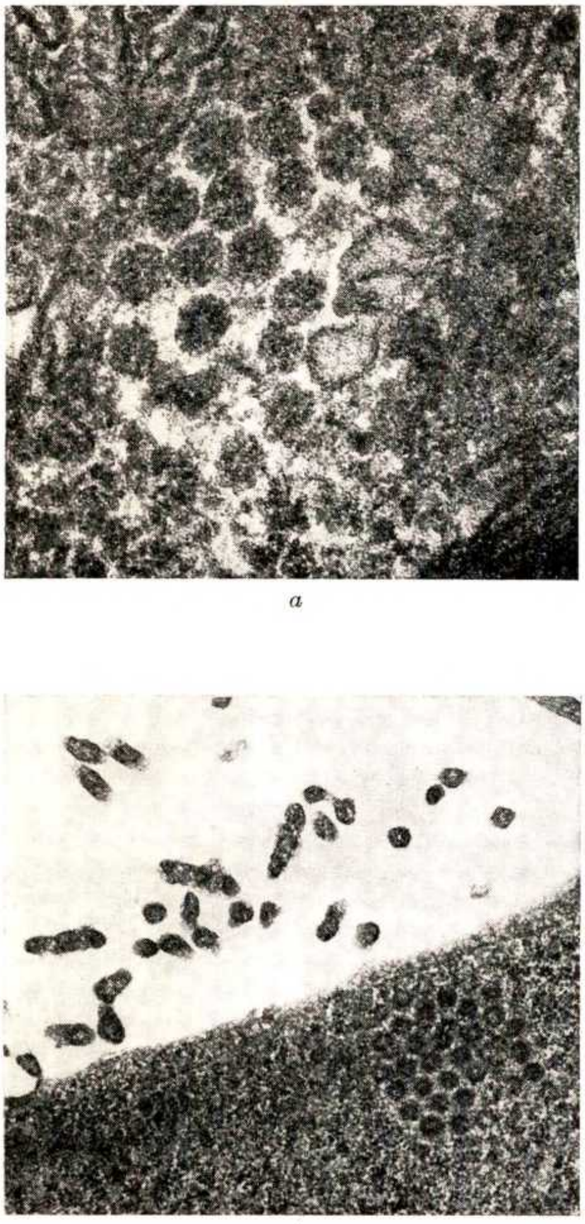


Fig. 1. *a*, Brain cell from subacute sclerosing panencephalitis patient ROB showing a cluster of Papova-like virions in the cytoplasm. Preparation kindly provided by Dr Shinsaku Oyanagi. *b*, AGMK cell infected with the viruses isolated from brain cells of patient JAC, showing a cluster of Papova-like virions in the cytoplasm and structures resembling paramyxovirus particles escaping from the cell.

cells or for cells of other animal species. Only after fusion of the human brain cells with cells of other types have particles of Papova-like virus been "rescued" together with the syncytiogenic agent[1]. (2) Papova-like virus particles in AGMK cultures were seen only in cells forming syncytia. (3) Cell-free extracts or tissue culture media from human brain cultures or from infected AGMK cultures did not produce encephalitis in ferrets, but inoculation of the same animals with viable cells from the same cultures caused encephalitis[5]. (4) Human brain cell cultures have undergone transformation[2].

We postulate that Papova-like virus particles are present as unobtrusive symbionts in human tissue, particularly brain tissue, incapable of causing signs of illness *per se*, and also unable to spread to other cells of the organism in normal physiological conditions. Cell fusion engendered by viruses belonging to the paramyxovirus group and found in cells containing the Papova-like agent enables the latter to spread from cell to cell. When a sufficient number of brain cells becomes involved in this process, the disease syndrome manifests itself.

Ferrets injected with viable AGMK cells containing both the syncytiogenic agent and the Papova-like agent developed encephalitis. Those injected with cell-free extracts from the same cultures did not, and their brain cells showed presence of Papova-like virus but not paramyxovirus particles. In addition, encephalitis was not observed in animals injected with cells infected with measles virus alone. Thus it is possible that neither a syncytiogenic paramyxovirus in the absence of the Papova-like agent nor the Papova-like agent in the absence of the syncytiogenic agent is capable of causing encephalitis.

If the Papova-like agent is "activated" by a syncytiogenic paramyxovirus, an analogy may be drawn between their interaction and that observed between syncytiogenic Sendai virus and another member of the Papova virus group, SV40. The SV40 genome can be activated[6] in transformed cells only after it spreads to other types of cells following fusion mediated by non-infectious Sendai virions adherent to the cell surfaces. The interaction between the two agents in SSPE cells may occur at the molecular level, where the DNA of the Papova virus "activates" the RNA of the paramyxovirus, or primarily at a "mechanical" level, whereby the presence of the paramyxovirus activates the fusion processes which result in the spread to other cells of the Papova virus DNA. Thus the syncytiogenic agent may represent not one virus but a group of viruses which may be encountered in man in various conditions. Acute childhood infections, such as measles, in which the brain of the patient becomes infected with the virus more frequently than has been supposed[7], may lead to SSPE if cells containing Papova-like agent become superinfected with the measles virus, thus enabling the Papova-like virus to spread from cell to cell through the mechanism of fusion. Moreover, apparent or non-apparent infections of the human organism with other paramyxoviruses which are just as capable as measles virus of fusing cells, may lead to the same disease syndrome by the same mechanism. In the first case, the syncytiogenic agent isolated from brain cells will be measles virus; in the second, it will be one of the other paramyxoviruses. The Papova-like virus, however, may be the same in both cases.

Within this context, the pathogenesis of progressive multifocal leucoencephalopathy (PML), a neurological disease in which Papova-like particles are found in abundance in brain tissue (Fig. 2), may not differ greatly from that of SSPE. PML develops almost invariably in patients suffering from other conditions such as Hodgkin's disease, leukaemia, neoplasms of various organs, sarcoidosis and others[8]. If any of these underlying conditions is accompanied by a non-apparent infection with a syncytiogenic agent such as paramyxovirus, which, after invasion of brain tissue, activates the Papova-like agent, massive concentrations of Papova-like particles[9] may result from their replication after "activation". It has not yet been possible to isolate a biologically active Papova-like agent from PML cases. This difficulty may be overcome, however, if fusion of viable cells, obtained either directly from PML brain tissue or after cultivation *in vitro*, with other cell types is accomplished in the presence of a syncytiogenic agent such as Sendai. Thus virus isolation in the case of SSPE may be duplicated in PML.

Finally, one should not overlook the fact that human brain cells containing Papova-like particles have become transformed in cultures and that the process of transformation is strikingly similar to that observed after infection of human cells with an oncogenic virus, SV40 (ref. 10). Also it should be noted that the striking hyperplasia of astrocytes in PML and SSPE resembles a neoplastic process and that interaction of two viruses similar

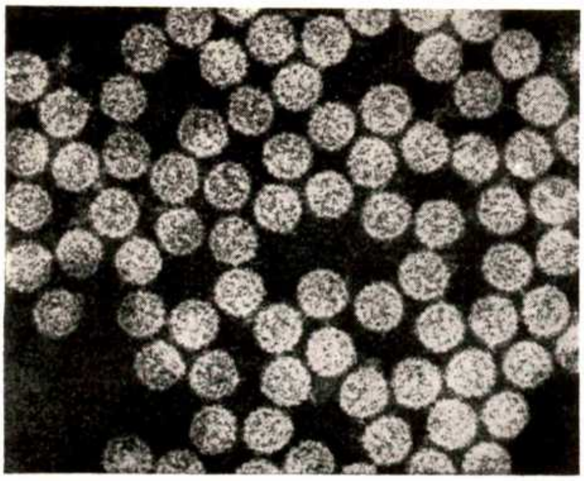


Fig. 2. Papova-like virions from brain tissue of a patient with PML. The biopsy was furnished by Dr Fusahiro Ikuta, Niigata University, Japan. Preparation kindly provided by Dr Klaus Hummeler, Children's Hospital, Philadelphia. (× 159,000.)

to that postulated for SSPE and PML may be involved in the mechanism of neoplasia.

This work was supported, in part, by the National Multiple Sclerosis Society and by US Public Health Service grants. Dr Barbanti-Brodano is a fellow at the Wistar Institute from the University of Bologna, Italy.

HILARY KOPROWSKI
GIUSEPPE BARBANTI-BRODANO
MICHAEL KATZ

Wistar Institute of Anatomy and Biology,
36th and Spruce Streets,
Philadelphia,
Pennsylvania 19104.

Received September 18; revised October 29, 1969.

[1] Barbanti-Brodano, G., *et al.*, *Proc. Soc. Exp. Med.* (in the press).
[2] Katz, M., Koprowski, H., and Moorhead, P., *Exp. Cell Res.*, **57**, 149 (1969).
[3] Katz, M., Oyanagi, S., and Koprowski, H., *Nature*, **222**, 888 (1969).
[4] Hirt, B., *J. Mol. Biol.*, **26**, 365 (1967).
[5] Katz, M., Rorke, L. B., Masland, W. S., Barbanti-Brodano, G., and Koprowski, H., *J. Infect. Dis.* (in the press).
[6] Koprowski, H. Jensen, F. C., and Steplewski, Z., *Proc. US Nat. Acad. Sci.*, **58**, 127 (1967).
[7] Gibbs, F. A., Gibbs, E. L., Carpenter, P. R., and Spies, H. W., *J. Amer. Med. Ass.*, **171**, 1050 (1959).
[8] Richardson, jun., E. P., *New Engl. J. Med.*, **265**, 815 (1961).
[9] Zu Rhein, G. M., in *Progress in Medical Virology* (edit. by Melnick, J. L.), **11**, 185 (Karger, Basel, 1969).
[10] Koprowski, H., Ponten, J. A., Jensen, F., Ravdin, R. G., Moorhead, P., and Saksela, E., *J. Cell. Comp. Physiol.*, **59**, 281 (1962).

Surface Leukaemic Antigen and Malignancy of Cells in Mice infected with Mazurenko Virus

IT is well known that virus induced mice leukaemic cells carry a specific surface antigen, which can be detected by a cytotoxicity reaction or by immunofluorescent labelling[1-3]. We have studied the time of appearance of the specific antigen in cells of various organs and tissues of mice after inoculation with a leukaemia virus, and compared this with the time when malignant transformation becomes apparent. This cellular change can be detected with the aid of histocytological examination and by the ability of such cells to grow after transplantation.

Concentrated suspensions of Mazurenko virus[4,5] were prepared by differential centrifugation[6]. Leukaemia was induced in 2 week old mice of the CC57Br strain by intraperitoneal injection of 0·2 ml. doses of these suspensions (1 ml. of virus suspension is equivalent to 1·5 mg of leukaemic tissue). Specific antileukaemia antiserum was prepared from mice which had been given a two-fold injection of a 20 per cent suspension of centrifuged virus particles and subsequently immunized five to seven times with increasing concentrations of leukaemic tissue homogenate. Before injection, leukaemic tissue was subjected to γ-irradiation at a dose equivalent to 9,600–12,000 rad.

Specific surface antigen in the cells of organs and tissues infected by the virus was demonstrated by Möller's modification of Coon's indirect method[7]. The intensity of the reaction was calculated from the index of fluorescence[8]. Values of 0·2 and higher were considered positive. Cells of thymus, lymph nodes, spleen and bone marrow were taken from three mice, and incubated with serum. The same cells incubated with serum from adult healthy animals of the same strain were used as controls. Corresponding cells from healthy mice incubated with anti-leukaemic serum were also used as controls. The specificity of the resulting fluorescence was confirmed in the fluorescence suppression experiments where leukaemic cells treated with anti-leukaemic serum were used. The same organs were examined histologically and cytologically.

Commencing on the fifth day after inoculation of the mice with virus, a suspension of spleen cells taken from three mice (prepared for the immunofluorescence reaction) was injected intraperitoneally into three 5–7 week old mice of the CC57Br strain, each animal being given four to six million cells. The recipients were kept under observation for 3·5 months. Leukaemia was diagnosed on the basis of the characteristic features of the disease.

Table 1. IMMUNOFLUORESCENCE AND MORPHOLOGICAL STUDY OF ORGAN AND TISSUES OF MICE INFECTED WITH MAZURENKO VIRUS

Days after injection	Index of immunofluorescence				Histological data	Cytological data	Transplantation result*
	Spleen	Thymus	Bone marrow	Lymph nodes			
2	0·16	0	0·1	0	Normal	Normal	—
4	0·13	0	0·08	0	Normal	Reticuloendothelial inflammation	—
5	0·05	0·1	—	0·08	Normal		0/2
7	0·24	0	0·39	0·09	Normal		0/3
8	0·06	0·04	0·11	0·07	Normal		0/2
10	0·21	0·02	0·1	0·06	Normal		0/3
12	0·30	0·03	0	0·16	Normal		0/3
13	0·22	0·18	0·17	0·01	Normal		0/3
14	0·41	0·16	0·21	0·17	Increase of poorly differentiated cells in spleen and lymph nodes		0/3
15	0·24	0·25	0·20	0·30			0/3
16	0·32	0·33	0·21	0·40			0/3
18	0·32	0·30	0·20	—			1/3
22	0·41	0·31	0·25	0·43	Leukaemia	Leukaemia	2/3
28	0·59	0·71	0·44	0·66	Leukaemia	Leukaemia	3/3

* Proportion of recipients which developed leukaemia.

A total of 109 10–14 day old mice of strain CC57Br were infected with concentrated virus. The kinetics of appearance of specific antigens in the organs of these animals and the morphological findings and results of transplantation are presented in Table 1. Microscopic studies showed that specific surface antigens were detectable in the cells of spleen and bone marrow on the seventh day after injection of virus. From the tenth to the thirteenth day the immunofluorescence reaction was positive only for the spleen cells. Commencing on the twelfth day, however, the index of fluorescence for the lymph nodes, thymus and bone marrow approached significant levels. Surface antigen was clearly detected in all organs studied from the fifteenth day onwards, the index of fluorescence gradually increasing subsequently.

Increased numbers of poorly differentiated cells were detected by histological examination of the spleen and lymph nodes commencing on the fourteenth day. They were usually present in the wide centres of multiplication, in the follicles, and also in the form of separate small foci in the splenic pulp. From the twenty-second day of the investigation, marked disorganization of normal spleen and lymph node structure took place, on account of diffuse or large focal hyperplasia of reticular or homocytoblastic cells. A similar disorganization was noted in the thymus. Bone marrow retained its content of polymorphonuclear cells at all times. It was only from the twenty-second day after infection with virus that small solitary centres of leukaemic cells were observed.

Focal growth of reticular cells was observed around lymphoid follicles and in the red pulp of spleen as well as in the bone marrow as early as the fourth day after infection. In the liver and kidneys, degenerative changes of parenchymal cells, micronecrosis and haemorrhages were found. Large numbers of histiocytes, fibrocytes and reticulocytes were usually observed in the spleen, the liver and the kidneys. The appearance of a large number of monocytes in the peripheral blood was also characteristic. Such evidence for an active reticuloendothelial system continued up to the twenty-second day after injection of virus, by which time leukaemic symptoms were fully developed. Transplantations of cells were positive from the eighteenth day after the injection of virus: leukaemia developed in recipient mice within 4–5 weeks.

Clearly, the appearance of specific leukaemic antigen in animals infected with virus precedes the malignant transformation of normal cells into ones that are leukaemic, according to morphological studies and their capacity for survival after transplantation. The nature of the specific surface antigen detected by immunofluorescence studies in leukaemia is still unclear. If an analogy is drawn with studies of DNA-containing viruses, the appearance of the specific surface antigen may be connected with changes in the cell surface in the process of leukaemogenesis. Our findings would then agree with those of other authors who, using the immunofluorescence test, detected the appearance of specific surface antigen before the morphological transformation of cells in tissue culture inoculated with SV40 virus and polyoma virus[9,10]. Specific fluorescence in these experiments is connected only with the formation of new cellular surface antigen and is not conditioned by the virus itself or by antigen present in the virus particles. In our experiments we have not attempted to differentiate cellular antigen from the antigen of virus particles, because in mouse leukaemia caused by RNA-containing virus the viruses are situated on the surface of cells in the tissues and organs of virus-inoculated animals. Apparently, the specific fluorescence on the surface of the cells we have studied is to be attributed to the appearance of new cellular antigen and to viral antigen.

V. E. GURTSEVICH
N. P. MAZURENKO
Laboratory of the Virology of Leukaemias,
E. I. JAROVA
Laboratory of Systemic Diseases of Blood,
N. A. PROBATOVA
G. N. STEPANOVA
Department of Pathological Anatomy of Human Tumours,
Institute of Experimental and Clinical Oncology,
Moscow.

Received October 6, 1969.

[1] Zueva, Yu. N., *Vestnik Acad. Med. Sci. USSR*, **11**, 51 (1964).
[2] Dirlugjan, R. P., and Stepina, V. N., *Vestnik Acad. Med. Sci. USSR*, **9**, 7 (1966).
[3] Klein, E., and Klein, G., *Nature*, **204**, 339 (1964).
[4] Mazurenko, N. P., *Rep. Conference on Experimental and Clinical Oncology, Kiev*, **8** (1957).
[5] Mazurenko, N. P., *Questions of Virology*, **1**, 11 (1962).
[6] Moloney, J. B., *J. Nat. Cancer Inst.*, **24**, 933 (1960).
[7] Möller, J., *J. Exp. Med.*, **114**, 415 (1961).
[8] Klein, E., and Klein, G., *J. Nat. Cancer Inst.*, **32**, 547 (1964).
[9] Kluchareva, T. E., Shachanina, K. L., Belova, S., Chibisova, V., and Deichman, G. I., *J. Nat. Cancer Inst.*, **39**, 825 (1967).
[10] Irlin, I. S., *Virology*, **32**, 725 (1967).

Leucine Naphthylamide: an Appropriate Substrate for the Histochemical Detection of Cathepsins B and B'

Sylvén and Snellman[1] have reported that highly purified beef spleen cathepsin B catalyses the hydrolysis of both benzoylarginine β-naphthylamide (*p*H 6·0 optimum) and leucine β-naphthylamide (*p*H 7·3 optimum). The latter effect was termed "leucine naphthylamidase" activity. Action on both substrates was attributed to a single enzyme, namely, cathepsin B. Maximal rates of hydrolysis were obtained only when cysteine and EDTA were included in the reaction mixtures, a finding that is consistent with the needs of cathepsin B (ref. 2).

Recently, we have obtained contrary evidence: we find that benzoylarginine β-naphthylamide and leucine β-naphthylamide are hydrolysed by different enzymes, and that neither of these is cathepsin B. We feel compelled to present this evidence because Sylvén and his associates[3], as well as others[4], are using leucine β-naphthylamide in a histochemical procedure based on the leucine naphthylamidase effect for observing enlarged lysosomes such as autophagic vacuoles. They recommend this technique for the early detection of cellular injury and the onset of regression and support the validity of the method by their substrate specificity studies[1]. Our data suggest, however, that those studies were conducted with a partially purified preparation of lysosomal cathepsin B′ that was contaminated with an aminopeptidase. There are indications that the latter may have been a sulphydryl-dependent, puromycin-sensitive aminopeptidase like that described by Ellis and Perry[5,6].

First of all, we have confirmed the findings of Otto[7], namely, that gel filtration can be used (Fig. 1) to isolate a second trypsin-like enzyme, called cathepsin B′, from cathepsin B preparations made according to Greenbaum and Fruton[2]. Although Otto demonstrated that cathepsins B and B′ are both sulphydryl-dependent enzymes that hydrolyse benzoylarginine amide at acid *p*H, these activities were readily distinguished by the fact that only cathepsin B′ hydrolysed benzoylarginine *p*-nitroanilide. We observe (Fig. 1) that cathepsin B′ also hydrolyses benzoylarginine β-naphthylamide, while the classical cathepsin B attacks only benzoylarginine amide. This, of course, has implications for the use of benzoylarginine β-naphthylamide as a histochemical substrate for the classical cathepsin B. Any confusion regarding the subcellular localization of cathepsins B and B′ may not prove to be serious because both were found by Otto and Schepers[8] in lysosome-rich fractions prepared from rat liver by the method of Sawant *et al.*[9].

We prepared cathepsins B and B′ from bovine spleen by a modified version of Otto's procedure, the basis of which was developed by Greenbaum and Fruton[2]. Acid

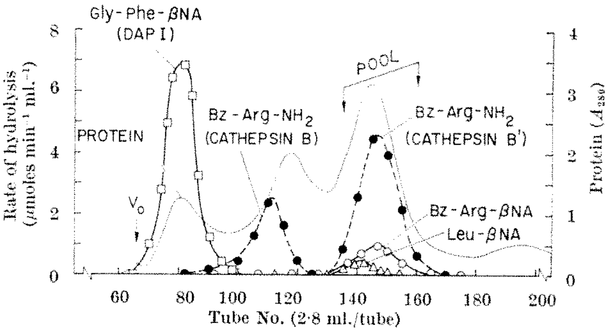


Fig. 1. Gel filtration of the ammonium sulphate fraction of beef spleen on 'Sephadex *G*-100' column (3·2 × 94 cm) equilibrated with 4 mM 2-mercaptoethanol–0·5 mM EDTA–0·1 M acetate buffer (*p*H 4·2) at 5° C. 580 mg of protein was applied in 5 ml. of column buffer; the flow rate was maintained at 19·5 ml. per h; 2·8 ml. fractions were collected. V_0 designates the void volume of the column. βNA = β-naphthylamide group; Bz = benzoyl group; DAP I = dipeptidyl aminopeptidase (cathepsin C).

extraction of 1 kg of fresh bovine spleen for 22 h at *p*H 3·5 and 37° C was followed by the recovery from the extract of the protein precipitated with ammonium sulphate between 40 and 70 per cent of saturation. The next step (passage through 'IRC-50' resin) was by-passed. The specific activity gain that was accomplished by performing the acid extraction at 37° C, rather than at 3° C, compensated to a large extent for the omission of the resin treatment. Gel filtration on 'Sephadex *G*-100', Otto's fourth and final step, was then performed instead of the final step used by Greenbaum and Fruton[2] (ethanol precipitation of the mercury derivative of cathepsin B). In this way Otto's cathepsin B′ remains, preferentially, in the supernatant.

Dipeptidyl aminopeptidase I (cathepsin C) was readily eliminated: it emerged from 'Sephadex *G*-100' only slightly retarded (Fig. 1). Confirming the results of Otto[7], we found that cathepsin B emerged before cathepsin B′. It seemed particularly significant, in view of the findings of Sylvén and Snellman[1], that the cathepsin B′ peak contained leucine naphthylamidase activity. It is obvious from Fig. 1 that leucine naphthylamidase tends to be closely associated with cathepsin B′, and this may help to explain why Sylvén and Snellman found "leucine naphthylamidase" activity in their preparation, which was also a 'Sephadex *G*-100' fraction. The results in Fig. 1 show that only cathepsin B′ hydrolyses benzoylarginine β-naphthylamide, so that the "cathepsin B" preparation of Sylvén and Snellman[1], purified as recently described by Snellman[10], must have been an enriched preparation of cathepsin B′. Considering the molecular weight of cathepsin B (about 50,000, ref. 7), one would expect cathepsin B to be present in Snellman's cathepsin D fraction.

Assays on benzoylarginine amide were conducted according to Greenbaum and Fruton[2] for cathepsin B. The methods of ammonia diffusion and quantitation are described elsewhere[11]. Rates of naphthylamide hydrolysis were determined at 37° C by a direct, recording fluorometric method[12]. Benzoylarginine β-naphthylamide (0·2 mM) was incorporated into reaction mixtures containing 4 mM cysteine–9 mM EDTA–17 mM phosphate buffer (*p*H 6·0). Dipeptidyl aminopeptidase I (cathepsin C) was assayed fluorometrically on glycylphenylalanine-β-naphthylamide[11]. Leucine β-naphthylamide was assayed by the method of Ellis and Perry[6]. The enzyme solution (0·1 ml.) was combined with 2·9 ml. of 5 mM 2-mercaptoethanol–0·15 M Tris–HCl buffer (*p*H 7·3), and preincubated 5 min at 37° C before adding 1·0 ml. of 0·8 mM leucine β-naphthylamide at 37° C; the final volume was 4 ml.

Subsequently (Fig. 2), the leucine naphthylamidase and cathepsin B′ activities were completely separated by subjecting the 'Sephadex' fraction to gradient elution chromatography on DEAE-cellulose. In designing our purification procedure it was necessary to consider only specific activity, and we were able to generate a preparation of cathepsin B′ having a specific activity comparable with that of Otto[7]. Otto did not use benzoylarginine β-naphthylamide, so we could only compare activities measured on the amide. The bovine spleen leucine naphthylamidase isolated in Fig. 2 had many properties in common with the puromycin-sensitive, sulphydryl-dependent aminopeptidase first demonstrated in the soluble fraction (cell sap) of bovine pituitary glands by Ellis and his associates[5,6]. It was strongly inhibited by puromycin, displayed a preference for the arylamide derivatives of basic amino-acids (see Fig. 2) and a sulphydryl requirement, and had a *p*H optimum of about 7·3. The latter three characteristics were reportedly exhibited by the beef spleen "leucine naphthylamidase" activity that Sylvén and Snellman[1] and Snellman[10] attributed to cathepsin B. Related studies by Barrett and Poole[13] have shown that the leucine naphthylamidase activity contained in human rheumatoid synovium can be inhibited by puromycin, both in crude extracts and at the histochemical level. They also reported that the cathepsin B activity contained in crude synovium extracts, assayed on benzoylarginine amide, was unaffected by puromycin.

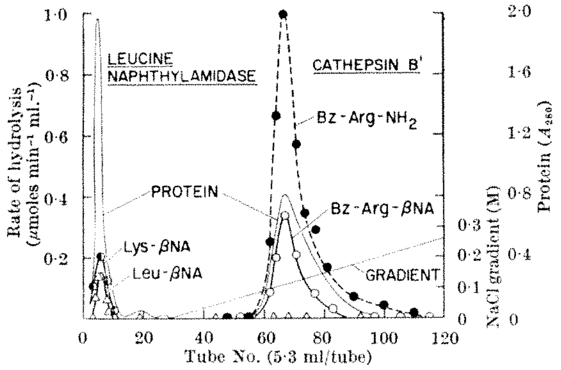


Fig. 2. Separation of beef spleen leucine naphthylamidase from cathepsin B′ by gradient elution chromatography (of the 'Sephadex *G*-100' fraction) on a microcrystalline DEAE-cellulose (Whatman 'DE-52') column (1·3 × 23 cm) equilibrated with 0·01 M sodium phosphate buffer–4 mM 2-mercaptoethanol (*p*H 7·1) at 5° C. 70 mg of protein was applied in 4·7 ml. of column buffer. A linear NaCl gradient was generated using a limiting buffer of 0·01 M phosphate–1·0 M NaCl–4 mM 2-mercaptoethanol (*p*H 7·1); flow rate was 24 ml. per h; 5·3 ml. fractions were collected.

Because leucine naphthylamidase activity is obviously distinct from cathepsin B′, and possibly arises from the cell sap, we have wondered how Niemi and Sylvén[3] have apparently been able to use a leucine naphthylamidase histochemical reaction to visualize autophagic vacuoles. It should be noted that their sections have to be incubated at *p*H 5·5 in order to obtain localization of reaction product by simultaneous coupling with a diazonium salt. Because the *p*H optimum of the leucine napthhylamidase reaction is 7·3 (ref. 1), we suspect—as one possibility—that the reaction product formed at *p*H 5·5 appears slowly enough to allow time for the lysosomes to scavenge and concentrate the dye, a property of lysosomes that seems to be extremely significant[14,15]. Niemi and Sylvén[3] did report that between *p*H 6·0 and 7·0 a generalized cytoplasmic staining occurred that obscured lysosomal structures, but they attributed this reaction to separate cytoplasmic aminopeptidases[12] and related the *p*H 5·5 reaction to cathepsin B.

Regardless of whether any leucine naphthylamidase activity resides within lysosomal structures, our results demonstrate: (*a*) that this activity is distinct from cathepsins B and B′, and (*b*) that only cathepsin B′ acts on benzoylarginine β-naphthylamide.

J. KEN McDONALD
BENJAMIN B. ZEITMAN
STANLEY ELLIS

Environmental Biology Division,
Ames Research Center,
National Aeronautics and Space Administration,
Moffett Field, California 94035.

Received November 25, 1969.

[1] Sylvén, B., and Snellman, O., *Histochemie*, **12**, 240 (1968).

[2] Greenbaum, L. M., and Fruton, J. S., *J. Biol. Chem.*, **226**, 173 (1957).

[3] Niemi, M., and Sylvén, B., *Histochemie*, **18**, 40 (1969).

[4] Chayen, J., Bitensky, L., Butcher, R. G., and Poulter, L. W., *Nature*, **222**, 281 (1969).

[5] Ellis, S., and Perry, M., *Biochem. Biophys. Res. Commun.*, **15**, 502 (1964).

[6] Ellis, S., and Perry, M., *J. Biol. Chem.*, **241**, 3679 (1966).

[7] Otto, K., *Z. Physiol. Chem.*, **348**, 1449 (1967).

[8] Otto, K., and Schepers, P., *Z. Physiol. Chem.*, **348**, 482 (1967)

[9] Sawant, P. L., Shibko, S., Kumta, V. S., and Tappel, A. L., *Biochim. Biophys. Acta*, **85**, 82 (1964).

[10] Snellman, O., *Biochem. J.*, **114**, 673 (1969).

[11] McDonald, J. K., Zeitman, B. B., Reilly, T. J., and Ellis, S., *J. Biol. Chem.*, **244**, 2693 (1969).

[12] McDonald, J. K., Ellis, S., and Reilly, T. J., *J. Biol. Chem.*, **241**, 1494 (1966).

[13] Barrett, A. J., and Poole, A. R., *Nature*, **244**, 279 (1969).

[14] Dingle, J. T., and Barrett, A. J., *Biochem. J.*, **105**, 19p (1967).

[15] Barrett, A. J., and Dingle, J. T., *Biochem. J.*, **105**, 20p (1967).

Histochemistry of some Lysosomal "Proteolytic" Enzymes of Human Rheumatoid Synovia

ONE subcellular manifestation of rheumatoid arthritis is that the membranes of the lysosomes in the synovial lining cells are virtually functionless; by contrast, in non-rheumatoid synovia—even after recent mechanical trauma—the lysosomal membranes in the lining cells are functional in that they retard entry of the substrates used for the demonstration of the intra-lysosomal enzymes[1]. Barrett and Poole[2], while not disputing this conclusion, questioned the precise nature of the lysosomal enzyme used in the study, which was visualized and measured microdensitometrically on the basis of the hydrolysis of leucine-2-naphthylamide, the liberated naphthylamine being trapped by 'Fast Blue B' (tetra-azotized dianisidine) to form an insoluble dye. By precise biochemical analysis, they demonstrated that human synovial tissue, taken from rheumatoid joints, contained cathepsins B and D as well as aminopeptidase activity. They showed that cathepsin D cannot hydrolyse leucine-2-naphthylamide and quoted unpublished data from other workers which suggested that cathepsin B could not hydrolyse this substrate. From this evidence they concluded that the particulate enzyme activity which was demonstrated by this substrate was a lysosomal aminopeptidase.

The recent detailed work of Snellman[3] showed that highly purified and electrophoretically discrete cathepsin B does, in fact, hydrolyse this substrate. But Barrett and Poole also claimed that the specific nature of this lysosomal enzyme could be elucidated because, although puromycin is a powerful inhibitor of aminopeptidases, it has much less effect on cathepsins B and D. Their results with 'Hyamine 1622' were less valuable because we find that this compound interferes with the naphthylamine–'Fast Blue' reaction and produces spurious histochemical effects.

Synovial tissue, especially that taken from rheumatoid joints, contains a large number of different cell types. Attention was previously focused[1] only on the synovial lining cells which, it was argued, might be significantly involved in the erosion of the articular cartilage. These cells probably correspond to the electron microscopists' type A and B cells[4–6] or to the "synovial membrane" in the looser terminology. Even when the surgeon is specifically attempting to sample these linings, they are frequently absent in the biopsy. Barrett and Poole's photographs of their histochemical "leucine naphthylamidase" reaction, which was largely inhibited by puromycin, depict cells which resemble fibroblasts or macrophages of the type which occur at a depth not less than ten cells below the synovial surface.

Rheumatoid synovia were chilled in hexane at –70° C, sectioned with a cooled knife in a cryostat at –30° C, and tested with leucine-2-naphthylamine and tetra-azotized dianisidine (Serva) by methods[7] very similar to those used by Barrett and Poole. Serial sections were incubated in the same medium to which 1×10^{-3} M puromycin (Nutritional Biochemicals) had been added. The amount of enzyme activity was measured by means of a scanning and integrating microdensitometer; the reaction time was kept to 15 min, at which time the response of the tissue, and of the instrument, was linear with activity[8]. With this instrument the cell type can be determined by visual microscopy and the activity of the selected cell, or group of two cells, can be measured. All measurements, whether of synovial cells or those of the deeper layers, were made with the same apertures, to ensure the same projected area of the tissue on to the photo-multiplier. Consequently, the measurements are of activity per unit area of section and are directly comparable. Tests were also made for non-specific absorption, caused by the scattering of light; this was found to be negligible in these sections which were mounted in Farrant's medium. Measurements were made of as many fields (of two cells each) as possible in the synovial lining, and of a sufficient number of deeper cells to yield a meaningful mean value. The reactions with and without puromycin were performed at the same time and the measurements were made within a day of the reactions, to avoid errors as a result of the fading of the dye produced, especially if the sections are not kept in the dark. The results obtained with rheumatoid synovia from the knees of two patients were plotted as population histograms (Figs. 1*A* and *B*) and the mean activities were calculated (Table 1). In agreement with Barrett and Poole, we find that puromycin strongly inhibited the reaction in the deeper cells. Inspection of the sections indicated that this inhibition varied

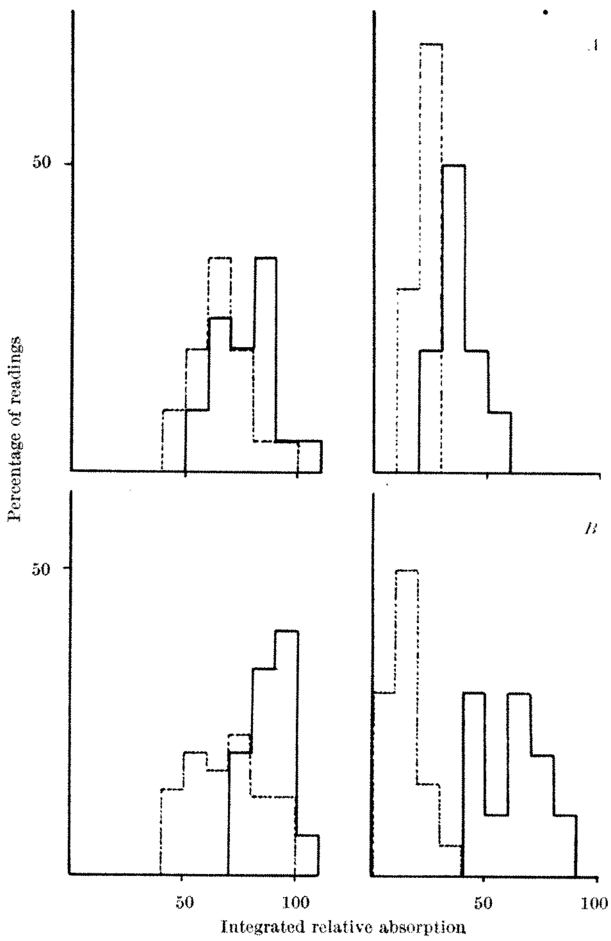


Fig. 1. Population histograms of the measurement of "leucine naphthylamidase" activity ("integrated relative absorption" measured by scanning and integrating microdensitometry). ———, Activity; - - - -, activity of the same region of the tissue when tested in the presence of puromycin. *A*, Activity measured in the synovial surface cells and in cells about ten cells below the surface; *B*, activity measured in the surface cells and in cells about twenty cells below the surface.

Table 1. MEAN ACTIVITY* OF "LEUCINE NAPHTHYLAMIDASE" AND THE EFFECT OF PUROMYCIN

	Without puromycin		With puromycin		
	Activity	No. of readings	Activity	No. of readings	Inhibition (per cent)
Case 1					
Synovial lining cells	76·2	(20)	66·1	(20)	13·3
Deeper cells	37·5	(10)	19·8	(10)	47·2
Case 2					
Synovial lining cells	88·7	(15)	69·0	(30)	22·2
Deeper cells	60·0	(10)	14·0	(20)	76·7

* Integrated relative absorption, in arbitrary units. A relative absorption of 50 units corresponds to a completely homogeneous field of absolute extinction value of 0·34. The relation between relative absorption and extinction is linear.

slightly at different levels below the synovial surface. Quantitatively it was found that puromycin caused between 47 and 77 per cent inhibition in these cells (Table 1), the inhibition being greater in the deeper cells. The scatter of the readings is shown in Fig. 1. But in marked contrast, puromycin had much less effect on this enzymatic reaction in the synovial lining cells (13 and 22 per cent inhibition).

The enzyme activity in the deeper cells might be attributable solely to leucine naphthylamidase because 1 mM puromycin can inhibit 89 per cent of the activity of purified leucine naphthylamidase[2], although apparently it is less effective in tissue sections[2]. The relatively slight inhibition produced by puromycin in the synovial lining cells might indicate that all this activity represents cathepsin B; or that about 20 per cent of the total "leucine naphthylamidase" activity in these cells represents true aminopeptidase activity and up to 80 per cent cathepsin B. Either way, the use of puromycin indicates that synovial lining cells contain a high proportion of apparently catheptic activity, whereas the cells of the deeper layers contain predominantly aminopeptidase activity.

We thank the Medical Research Council for a grant for this work, the Arthritis and Rheumatism Council for their support, and Mr J. C. R. Hindenach of the West London Hospital for his help in obtaining surgical specimens.

J. CHAYEN
LUCILLE BITENSKY
L. W. POULTER

Division of Cellular Biology,
Kennedy Institute of Rheumatology,
Bute Gardens,
London W6.

Received December 29, 1969.

[1] Chayen, J., Bitensky, L., Butcher, R. G., and Poulter, L. W., *Nature*, **222**, 281 (1969).
[2] Barrett, A. J., and Poole, A. R., *Nature*, **224**, 279 (1969).
[3] Snellman, O., *Biochem. J.*, **114**, 673 (1969).
[4] Barland, P., Novikoff, A. B., and Hamerman, D., *Amer. J. Pathol.*, **44**, 853 (1964).
[5] Ghadially, F. N., and Roy, S., *Ann. Rheum. Dis.*, **26**, 426 (1967).
[6] Wyllie, J. C., Haust, M. D., and More, R. H., *Lab. Invest.*, **15**, 519 (1966).
[7] Chayen, J., Bitensky, L., Butcher, R. G., and Poulter, L. W., *A Guide to Practical Histochemistry* (Oliver and Boyd, Edinburgh, 1969).
[8] Butcher, R. G., Poulter, L. W., and Chayen, J., *Biochem. J.*, **107**, 27*P* (1968).

Morphogenetic Substratum for Differentiation of Cartilage in Tissue Culture

WE report here a new system for studying the development of cartilage from post foetal mesenchymal cells *in vitro*. The chondrocytes develop under the influence of the matrix pattern of a substratum prepared from decalcified bone. In this system, a chemically defined medium is introduced and embryo extracts are avoided in order to exclude all the soluble or particulate material that may be present in cultures of skeletal tissue[1–5].

Fifty samples of minced muscle, approximately 2 mm^3, were excised from the thigh muscles of newborn Sprague–Dawley rats and immediately transferred to plastic flasks 'T-30' (Falcon Plastics) or organ culture dishes (Falcon) containing 5·0 ml. and 1·0 ml. of 'medium 199' (Gibco). Care was taken to exclude muscle origins and insertions. Foetal calf serum (Hyland Laboratories) was added in sufficient quantities to produce a final protein concentration of 15–20 per cent. The samples were washed in Ca^{2+} and Mg^{2+} free Tris buffer three times to remove old blood. Flasks and dishes were placed in a water jacketed incubator at 37° C with a gaseous phase of 5 per cent CO_2 in air, and changed every other day, for periods of 10 to 36 days. The tissue was exposed to the nutrient media and fragments were cultured in five different ways: directly on plastic; directly on plastic with media covered with a thin layer of mineral oil; on wire grid; packed loosely into the medulla of a segment of lyophilized cortical bone matrix decalcified with 0·6 M HCl[6–8]; packed into similar segments of cortical bone matrix after denaturation with acid–ethanol, decalcification and lyophilization[8]. At 5 day intervals all cultures were fixed in formalin for histological sections and stained in haematoxylin–eosin and azure II.

Free fragments of explanted muscle led to a profuse outgrowth of amoeboid and spindle shaped mesenchymal cells. The new cells grew out within a few days, and the population density increased until a monolayer covered the bottom of a plastic flask measuring 25 cm^2. The monolayer pattern of cell growth was modified in seven of the twenty cultures of muscle that were placed in the interior of a cylinder of HCl decalcified diaphyseal adult rat bone matrix: in these, the mesenchymal cells migrated from the muscle tissue interstices but also reaggregated and differentiated into hyaline cartilage. Cells, after proliferating along the surface of the bone matrix, produced small nodules of cartilage in the space between the old matrix and muscle—or on the surface of the old matrix—but never inside a fragment of muscle. A greater percentage of cultures producing cartilage might have been obtained with natural media, but this meant introduction of too many undefined substances. A mass of cartilage developed (Fig. 1) from young mesenchymal cells proliferating on the surface of the decalcified bone matrix when the culture was confined to a chemically defined medium. The intimate relationship between new growth and old matrix is illustrated (Fig. 2). Cartilage was formed only from that part of the cell population in close contact with the old matrix. The old bone matrix collagen was not resorbed by cells cultured in 'medium 199'. In the same medium, control cultures, in which the explants of muscle were packed into the medullary cavities of segments of bone matrix denatured with acid–ethanol, produced an outgrowth. There was no clonal aggregation or differentiation of cartilage cells, however.

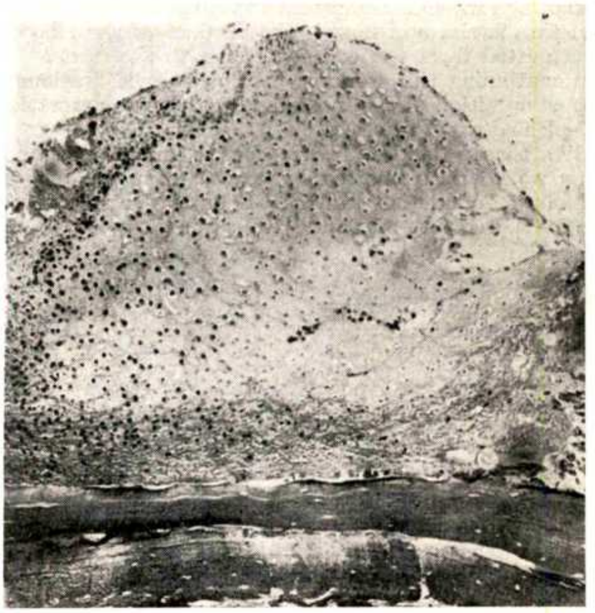


Fig. 1. Photomicrograph showing new proliferation and differentiation of cartilage (above) on a substratum (below) of rat bone matrix prepared by decalcification in 0·6 M HCl at 2° C for 96 h.

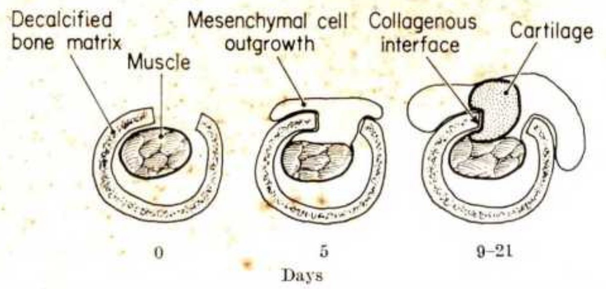


Fig. 2. Diagrammatic representation of the sequence of events leading to differentiation of cartilage from mesenchymal cells under the control of a substratum of decalcified native bone matrix. The substratum dissuades a segment of the population from monolayer formation, enhances cell aggregation and promotes cartilage cell differentiation.

These observations demonstrate that post foetal mesenchymal cells differentiate into cartilage in response to a complex set of conditions. One possible set of conditions would be a particular mixture of CO_2 and O_2, which exists in the interior of a segment of diaphyseal cortical bone. Another would be a morphogenetic substratum provided by undenatured decalcified bone matrix. Evidence for the first set comes from experiments on explants of differentiated skeletal tissues including cartilage[2]. Evidence for the second set comes from recent experiments on implants of morphogenetic matrices prepared from bone and other calcified tissues[6-8]. The highly stable acid insoluble matrix of calcified tissues is a morphogenetic, firmly cross-linked pattern which is imposed on a young mesenchymal cell population by some presently unknown mechanism.

Physico-chemical conditions in tissue culture activate a mesenchymal cell biosynthetic mechanism characteristic of a species. They do not stimulate a new biosynthetic pathway[1,5,9]. In an enriched medium under mineral oil, somite cells differentiate into cartilage at an earlier stage of development than in an unenriched medium[10]. In our system, no enrichment of the medium and only contact between cells and surface of undenatured bone matrix is sufficient to switch the pathway of development of mesenchyma from fibrous tissue to cartilage. Various concepts for cell differentiation[11-15] have been proposed and constitute valid interpretations. The questions are, what is the nature of the receptor in a mesenchymal cell population which is competent to respond to the morphogenetic matrix, and how is the product of the response transmitted from one cell generation to the next? We are continuing this work with stepwise modifications in the composition of the media and with new preparations of calcified tissue matrices.

We thank Sandra Harris for technical assistance. This work was supported by grants from the John A. Hartford Foundation and the US Public Health Service, National Institute of Dental Research.

MARSHALL R. URIST
HIROSHI NOGAMI

Bone Research Laboratory,
Department of Surgery,
UCLA Medical School,
Los Angeles, California 90024.

Received October 3; revised December 1, 1969.

[1] Coon, H. G., *Proc. US Nat. Acad. Sci.*, **55**, 66 (1966).
[2] Basset, C. A. L., *J. Bone and Joint Surg.*, **44A**, 1217 (1962).
[3] Grobstein, C., and Parker, G., *Proc. Soc. Exp. Biol.*, **85**, 477 (1954).
[4] Lash, J. W., *The Stability of the Differentiated State* (edit. by Ursprung, H.) (Springer-Verlag, New York, 1968).
[5] Holtzer, H., and Abbott, J., *The Stability of the Differentiated State* (edit. by Ursprung, H.) (Springer-Verlag, New York, 1968).
[6] Urist, M. R., Hay, P. H., Dubric, F., and Büring, K., *Clin. Orthopaed. Rel. Res.*, **64**, 194 (1969).
[7] Urist, M. R., *Science*, **150**, 893 (1965).
[8] Urist, M. R., Dowell, T. A., Hay, P. H., and Strates, B. S., *Clin. Orthopaed. Rel. Res.*, **59**, 59 (1968).
[9] Young, R. W., *Bone Biodynamics* (edit. by Frost, H. M.) (Little, Brown and Co., Boston, 1964).
[10] Ellison, M. L., Ambrose, E. J., and Easty, G. C., *J. Embryol. Exp. Morphol.*, **21**, 331 (1969).
[11] Ambrose, E. J., *Cell Differentiation* (edit. by De Reuck, A. V. S., and Knight, J.) (Little, Brown and Co., Boston, 1967).
[12] Grobstein, C., *Amer. Zool.*, **6**, 89 (1966).
[13] Hauschka, S. D., and Konigsberg, I. R., *Proc. US Nat. Acad. Sci.*, **55**, 119 (1966).
[14] Cahn, R. D., *Stability of the Differentiated State* (edit. by Ursprung, H.) (Springer-Verlag, New York, 1968).
[15] Fell, H. B., *Ann. Rheum. Dis.*, **28**, 213 (1969).

Uniquely Oriented Epidermal Lipid

AN important function of the stratum corneum of skin is to limit the penetration of substances[1]. This is dependent on a lipid-protein complex which can be disrupted by a variety of polar organic solvents[2], and diffusion studies suggest that filamentous protein and certain polar lipids are involved[3]. We describe here a lipid which has a characteristic orientation with respect to the long axis of the keratin filaments and which may be an important component of the lipid-protein complex.

X-ray diffraction studies of mammalian stratum corneum have shown the keratin filaments to be randomly arranged in planes parallel to the skin surface[4], but after stretching the tissue an oriented α-protein pattern can be seen with meridional arcs at 5·14 Å and equatorial arcs at 9·8 Å. In addition to the usual keratin reflexion, meridional arcs were seen at 4·15 Å in the α-type epidermis of mammals, amphibians, birds and reptiles (Fig. 1*a*). The 4·15 Å spacing was absent from hair, nails and feather-type tissue of birds and reptiles.

The subcellular localization of the material giving rise to the 4·15 Å spacing was studied by differential centrifugation of isotonic sucrose homogenates of trypsin or heat separated epidermis. The 4·15 Å spacing was observed in the 1,000*g* precipitate and not in the 1,000*g* supernatant. The 1,000*g* precipitate was then extracted with 6 M urea in the presence of a 0·1 M mercaptoethanol at *p*H 10·5[5]. The solution was dialysed, lyophilized, and fibres were drawn from the isolated protein. These isolated keratin fibres showed, in addition to the 9·8 Å and 5·14 Å keratin reflexions, a 4·15 Å reflexion on the meridian. The results strongly suggest that the material producing the 4·15 Å spacing is closely related to the keratin filaments and does not arise from some other part of the cell. Extraction of pulverized stratum corneum with hexane or hot water failed to remove the 4·15 Å arcs, but treatment with chloroform–methanol (3 : 1) abolished them. The X-ray diffraction pattern of material extracted with chloroform–methanol showed a complete circle at 4·15 Å (Fig. 1*b*) instead of arcs, suggesting that the orientation observed in intact epidermis arises from the spatial relationship between the keratin filaments and the lipid producing the 4·15 Å spacing. A 4·15 Å spacing was not observed in X-ray diffraction patterns of material isolated with chloroform–methanol from subcutaneous fat, liver or cerumen. Chloroform–methanol extracts from stratum corneum were purified further by chromatography on silica-gel in a solvent of 85 per cent

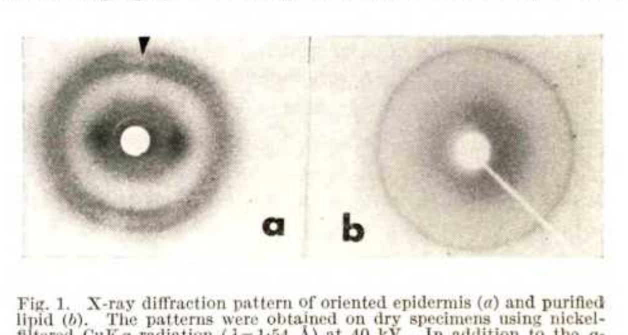


Fig. 1. X-ray diffraction pattern of oriented epidermis (*a*) and purified lipid (*b*). The patterns were obtained on dry specimens using nickel-filtered CuKα radiation ($\lambda = 1{\cdot}54$ Å) at 40 kV. In addition to the α-pattern, the epidermis shows a reflexion at 4·15 Å accentuated on the meridian.

benzene and 15 per cent methanol. The component which was used for the subsequent analyses had an approximate R_f of 0·55 in the benzene–methanol system. The isolated material had characteristic wide and low angle X-ray diffraction patterns with reflexions at 4·15 Å, 42·5 Å, 49·6 Å and 134·0 Å. Spacings of approximately 4·15 Å are sometimes associated with lecithin–cholesterol–water complexes[6], but melting and drying failed to alter the X-ray pattern, negating such a hypothetical complex as the cause of the 4·15 Å spacing. Treatment of the material with acid and alkali failed to change its X-ray diffraction pattern, suggesting that it is not a fatty acid or fatty acid salt.

Mass spectrometric analysis revealed a compound with $m/e^+ = 284$ and a disintegration suggestive of a long chain aliphatic compound. Such a relatively polar non-saponifiable lipid could have a wide angle X-ray diffraction pattern with a 4·15 Å spacing, representing the distance between adjacent hydrocarbon chains. To obtain X-ray diffraction patterns with 4·15 Å arcs parallel to the 5·14 Å reflexions of keratin, the long axis of the lipid molecule would have to be perpendicular to the axis of the keratin filament. Fraser *et al.* found a similar orientation of a lipid in an α-keratin[7].

Although the lipid has not been specifically identified, its physical properties do not correspond with any of the well described lipids known to be present in the epidermis. Further characterization may make it possible to relate the structure of the protein–lipid complex to the properties of epidermis.

We thank Dr James Orr for his work on mass spectrometric analysis. The work was supported by grants from the US National Institutes of Health.

LOWELL A. GOLDSMITH
HOWARD P. BADEN

Department of Dermatology,
Harvard Medical School and
Massachusetts General Hospital,
Boston, Massachusetts 02114.

Received July 14, 1969.

[1] Malkinson, F. D., in *The Epidermis* (edit. by Montagna, W., and Lobitz, W. C.), 435 (Academic Press, New York, 1964).
[2] Mali, J. W. H., *J. Invest. Derm.*, **27**, 451 (1956).
[3] Crounse, R. G., *Arch. Environ. Health*, **11**, 522 (1965).
[4] Swanbeck, G., *Acta Dermato-venereol.*, **39**, Suppl. 43 (1959).
[5] Baden, H. P., and Bonar, L., *J. Invest. Derm.*, **51**, 478 (1968).
[6] Luzzati, V., and Husson, F., *J. Cell Biol.*, **12**, 207 (1962).
[7] Fraser, R. D. B., MacRae, T. P., Rogers, G. E., and Filshie, B. K., *J. Mol. Biol.*, **1**, 90 (1963).

Myosin Filaments in Vertebrate Smooth Muscle

THE sliding filament mechanism for muscular contraction was first established in the case of vertebrate striated muscle[1,2]. Shortly afterwards good evidence for the existence of a similar mechanism was obtained in a molluscan smooth muscle[3]. Attempts to explain contraction in vertebrate smooth muscle, however, met with considerable difficulties, mainly because of uncertainty about the state of myosin in the living relaxed muscle. This still cannot be decided from the biochemical[4–6], electron optical[7–16] and X-ray diffraction[17–19] evidence available so far. Here we present evidence from X-ray diffraction studies suggesting that in living relaxed vertebrate smooth muscle myosin is present in the form of filaments.

We have used low angle X-ray diffraction techniques to study the taenia coli muscle of the guinea-pig in the living relaxed state as well as during spontaneous rhythmic contractile activity. The muscle was dissected and maintained in a circulated Ringer solution of a composition given by Bülbring and Golenhofer[20]. A mixture of 97 per cent O_2 and 3 per cent CO_2 was bubbled through this Ringer solution. For examination in the X-ray beam the muscle was mounted in a 'Perspex' cell between windows of polyester film. To monitor the state of the muscle, connexion was made to either isometric or isotonic recording devices. After mounting in the cell, the muscle was allowed to equilibrate to a constant length or tension over a period of hours before the X-ray exposure, the aim being to obtain well-oriented specimens.

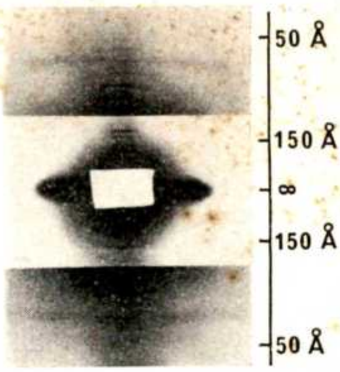


Fig. 1. Low angle X-ray diffraction pattern from a living relaxed taenia coli muscle holding a 20 g weight at 15° C. Specimen-to-film distance 36 cm. × 2. Fibre axis vertical. Centre of print underexposed to show: (*a*) broad reflexion on equator near 120 Å (actin lattice); (*b*) strong meridional 130 Å (collagen, fifth order of 650 Å); (*c*) weaker meridional 144 Å (myosin). Outer part of print shows higher order meridionals from collagen, and diffuse layer lines near 59 Å and 51 Å from actin.

The X-ray cameras were of the mirror–monochromator type described by Huxley and Brown[21], with a specimen-to-film distance of about 36 cm. A quartz crystal was used to focus the CuKα_1 radiation ($\lambda = 1·5405$ Å) from an Elliott-Automation rotating anode generator, operated at 750 W with a nominal focal size of 1 × 0·1 mm. With this combination of focal size and specimen-to-film distance, the resolution between orders was about 3000 Å. Exposure times varied between 2 and 24 h depending on the region of the pattern examined.

Our experiments with the living relaxed muscle, maintained at 12°–15° C, have confirmed all the previously described features of the low angle diffraction pattern, including meridional reflexions from collagen at orders of an approximately 650 Å repeat, layer lines from actin in the region from 59 Å out to higher angles, and a equatorial reflexion from actin filament lateral organization at a spacing of 110–120 Å (refs. 17–19, 22). We also observed a meridional reflexion at 144 ± 1 Å, and two diffuse layer lines at axial spacings of about 200 Å and 400 Å. Here we propose to deal only with the 144 Å reflexion.

In patterns from living relaxed muscles (Fig. 1) we find that the ratio of peak intensities of the 144 Å reflexion and of the adjacent fifth order collagen reflexion at about 130 Å usually lies between 1 : 2 and 1 : 1. Patterns from muscles in a Ringer solution containing 0·1 per cent phosphotungstic acid show enhancement of the diffracted intensity from collagen; the above-mentioned ratio comes to at least 1 : 10.

Spontaneous rhythmic contractions can be induced in the muscle by raising the temperature of the Ringer solution to about 37° C. Patterns obtained during such activity show a very marked decrease in the intensity of the 144 Å reflexion. By contrast, the actin layer lines from 59 Å outwards and the "400" Å layer line become clearer. The 120 Å is less sampled. No appreciable changes are seen in the collagen pattern. On lowering the temperature to about 12°–15° C, the whole diffraction pattern very nearly returns to that characteristic of resting muscle.

We consider that the 144 Å meridional reflexion arises from the structure of myosin filaments, and (as in vertebrate[21] and insect[23,24] striated muscles) at least in part represents the axial periodicity of heavy meromyosin projections. The size of the myosin filaments in vertebrate smooth muscle is difficult to estimate, because from the width of the 144 Å reflexion (in a direction perpendicular to the fibre axis), it seems that the coherently diffracting region is larger in cross-section than can reasonably be supposed to be the diameter of a single myosin filament. We are investigating the nature of this filament organization, details of the actin structure, the origin of the "400" Å layer line, and the high angle diffraction pattern.

Note added in proof. The 5·1 Å reflexion characteristic of α-protein structure has now been seen in diffraction patterns from the taenia coli muscle (J. L., F. R. P., P. J. V. and J. M. Squire), as has the 72 Å meridional reflexion due to myosin. Observations made on sectioned material with the electron microscope (J. L. and J. V. Small) show that, in this muscle, myosin is present in very long ribbon-like elements the individual dimensions of which account for the shape of the meridional reflexions. We also see small scale arrays of actin filaments the orderly packing of which is responsible for the observed equatorial diffraction pattern.

J. LOWY
F. R. POULSEN
P. J. VIBERT

Biophysics Institute,
University of Aarhus,
Denmark.

Received December 15, 1969.

[1] Hanson, J., and Huxley, H. E., *Nature*, **172**, 530 (1953).
[2] Huxley, A. F., and Niedergerke, R., *Nature*, **173**, 971 (1954).
[3] Hanson, J., and Lowy, J., *Nature*, **184**, 286 (1959).
[4] Needham, D. M., and Shoenberg, C. F., *Proc. Roy. Soc.*, B, **160**, 517 (1964).
[5] Needham, D. M., and Williams, J. M., *Biochem. J.*, **89**, 552 (1963).
[6] Shoenberg, C. F., Ruegg, J. C., Needham, D. M., Schirmer, R. H., and Nemetchek-Gansler, H., *Biochem. Z.*, **345**, 255 (1966).
[7] Choi, J. K., *Proc. Fifth Int. Conf. Electron Microsc., Philadelphia*, M9 (1962).
[8] Fawcett, D. W., *An Atlas of Fine Structure, The Cell: Its Organelles and Inclusions*, 241 (Saunders, Philadelphia, 1966).
[9] Hanson, J., and Lowy, J., *Proc. Roy. Soc.*, B, **148**, 523 (1964).
[10] Kaminer, B., *J. Mol. Biol.*, **39**, 257 (1969).
[11] Kelly, R. E., and Rice, R. V., *J. Cell Biol.*, **37**, 105 (1968).
[12] Kelly, R. E., and Rice, R. V., *J. Cell Biol.*, **42**, 683 (1969).
[13] Needham, D. M., and Shoenberg, C. F., *Proc. Roy. Soc.*, B, **160**, 517 (1964).
[14] Nonomura, Y., *J. Cell Biol.*, **39**, 741 (1968).
[15] Panner, B. J., and Honig, C. F., *J. Cell Biol.*, **35**, 303 (1967).
[16] Pease, D. C., *J. Ultras. Res.*, **23**, 280 (1968).
[17] Elliott, G. F., *Proc. Roy. Soc.*, B, **160**, 467 (1964).
[18] Elliott, G. F., *J. Gen. Physiol.*, **50**, 171 (1967).
[19] Huxley, H. E., quoted in Shoenberg, C. F., *Tissue and Cell*, **1**, 83 (1969).
[20] Bülbring, B., and Golenhofer, K., *J. Physiol.*, **193**, 213 (1967).
[21] Huxley, H. E., and Brown, W., *J. Mol. Biol.*, **30**, 383 (1967).
[22] Elliott, G. F., and Lowy, J., *Nature*, **219**, 156 (1968).
[23] Reedy, M. K., Holmes, K. C., and Tregear, R. T., *Nature*, **207**, 1276 (1965).
[24] Tregear, R. T., and Miller, A., *Nature*, **222**, 1184 (1969).

Platelet Adhesion in Rabbit Arteries observed by Scanning and Transmission Electron Microscopy

THE adhesion of blood platelets to the wall of an artery at a site where the endothelium has been destroyed is usually the first event in the formation of an experimental thrombus. When the endothelium is removed from the mid-abdominal aorta of a rabbit, by passing a roughened probe into the lumen, only single platelets or small platelet clumps adhere to the denuded surface when the blood flow is restored[1]. Because in these circumstances the platelets attached to the wall are not obscured by an overlying mass of thrombus material, the preparation provides a suitable model for studying the morphology of the adherent platelets both by scanning electron microscopy *en face* and by conventional transverse sections.

The segment of aorta is fixed (glutaraldehyde, followed by osmium tetroxide) and dehydrated through graded ethanol at arterial pressure so that its normal dimensions are preserved[2]. It is then opened longitudinally so that the injured area can be identified, and is divided into two. One part is mounted flat with the inner surface uppermost on the standard chuck for the Stereoscan scanning microscope. The second part is further divided into thin transverse or longitudinal sections and embedded in 'Araldite'. This communication describes the preliminary results obtained from such a procedure.

The appearances of adherent platelets shown by scanning electron microscopy (Fig. 1) are very similar to those described for platelets adherent to a foreign surface such as glass[3]. Many have a central hump, suggesting that the organelles are concentrated in this region; others are more flattened and may correspond to those which in transverse section appear to have lost their granules. Long cytoplasmic projections, or pseudopods, some of them branched, extend from the peripheral hyaloplasm. This spreading seems to occur fairly evenly over the surface of the exposed internal elastic lamina with no clear relationship to the ramifying sub-endothelial fibres on its surface. Transverse sections confirm the presence of elongated pseudopodia and reveal a narrow space between the platelet surface membrane occupied by occasional fibres and diffuse material. The latter stains intensely when the dye ruthenium red is added to the fixative (ref. 4 and personal communication from J. H. Luft) and probably represents the ground substance of the sub-endothelial space which is rich in polysaccharide complexes (Fig. 2).

Studies on the properties of platelets *in vitro* have

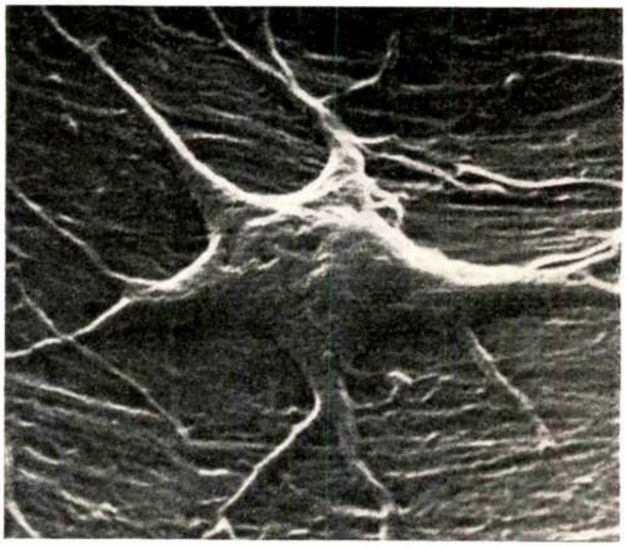


Fig. 1. Adherent platelet as seen by scanning electron microscopy. (×21,000.)

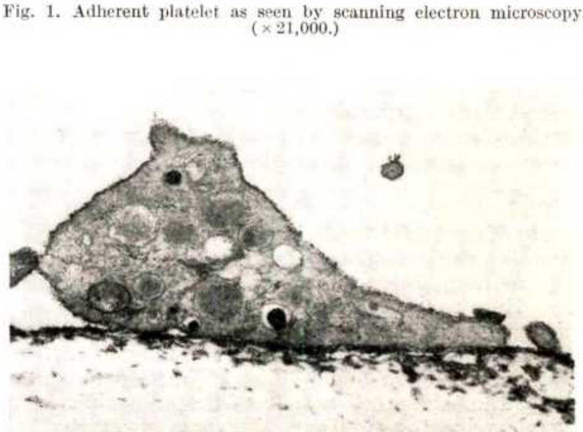


Fig. 2. Adherent platelet containing characteristic granules, in transverse section. Material on inner surface of internal elastic lamina is stained with ruthenium red. (×20,500.)

indicated that platelets will adhere readily to collagen, but not to elastin, and that mucopolysaccharides obtained from the ground substance of the aorta will not by themselves cause platelet aggregation[5]. Adhesion *in vivo* of platelets to collagen in the haemostatic plug[6] and to basement membrane in injured veins has been observed by electron microscopy[7]. The combination of scanning and transmission electron microscopy, which permits a three dimensional view of the platelets, indicates, however, that typical collagen fibres or basement membrane are not always present at the sites where platelets adhere; this keeps open the possibility that components of the injured intima other than collagen act differently in their native state and have a significant effect in platelet adhesion.

The micrograph in Fig. 1, taken on a Cambridge Mark IIa Stereoscan scanning microscope, is reproduced by permission of the Cambridge Instrument Company.

B. L. SHEPPARD
J. E. FRENCH

Sir William Dunn School of Pathology,
University of Oxford.

Received November 3, 1969

[1] Poole, J. C. F., Sanders, A. G., and Florey, H. W., *J. Pathol. Bact.*, **75**, 133 (1958).
[2] Wolinsky, H., and Glagov, S., *Circ. Res.*, **14**, 400 (1964).
[3] Scarborough, D. E., Mason, R. G., Dalldorf, F. G., and Brinkhous, K. M., *Lab. Invest.*, **20**, 164 (1969).
[4] Luft, J. H., *Fed. Proc.*, **25**, 1773 (1966).
[5] Packham, M. A., Nishizawa, E. E., and Mustard, J. F., *Suppl. Biochem. Pharm.*, 171 (1968).
[6] Kjaerheim, A., and Hovig, T., *Thromb. Diath. Haemorrh.*, **7**, 1 (1962).
[7] Baumgartner, H-R., Tranzer, J. P., and Studer, A., *Thromb. Diath. Haemorrh.*, **18**, 592 (1968).

Inhibition by Bile Salts of the Jejunal Transport of 3-O-Methyl Glucose

THE action of bile salts on the intestinal absorption of fat is well known[1] although their role on the transport of water soluble substrates remains poorly defined. Inhibition of monosaccharide transport by both unconjugated and conjugated bile salts has been reported[2,3]. Pope and co-workers, however, using highly purified preparations of bile salts in jejunal loops from normal rats, concluded that conjugated bile salts have no physiological action on the absorption of glucose in normal animals[5]. We have reassessed this problem in bile depleted rats using extracorporeally perfused segments of rat jejunum removed with their intact circulation.

A bile fistula was established by inserting a cannula in the common duct 5 mm distal to the bifurcation of the hepatic ducts in ten male Sprague–Dawley rats weighing 200 to 280 g. The position of the cannula did not interfere with a normal flow of pancreatic secretions as shown by trypsin assays[6]. The bile collected at 4° C and pooled had a bile salt concentration of 11 μmoles/ml. determined by a spectrofluorometric method[7]. A comparable number of animals were sham operated, the cannula lying in their peritoneal cavity. The two groups of animals were placed in restraining cages and offered *ad libitum* a 5 per cent sucrose solution containing 40 mequiv/l. of NaCl and 20 mequiv/l. of KCl. Forty-eight hours after operation, the absorption of 3-O-methyl glucose (3-O-MG), a non-metabolizable monosaccharide which shares the transport characteristics of glucose[8], was studied in segments of upper jejunum.

Under pentobarbital anaesthesia, 20 cm segments of upper jejunum immediately distal to the ligament of Treitz were completely removed from the animals and perfused extracorporeally through the superior mesenteric artery[9,10]. The blood perfusate consisted of 20 per cent washed bovine red cells suspended in a physiological electrolyte solution, 2·5 per cent dextran (mol. wt. 78,000), 2·5 per cent bovine serum albumin, 100 mg per cent of D-glucose and 100 mg per cent of 3-O-MG. It was oxygenated by means of a rotating mesh screen using a humidified 95 per cent O_2 and 5 per cent CO_2 gas mixture. The circulation was maintained at a rate of 2·5 ml./min by a servo-controlled peristaltic roller type pump with an asymmetrical head ('Ambec' extracorporeal perfusion unit, Beck, Colorado). Both ends of the jejunal segments were cannulated. The intestinal lumen was perfused at a rate of 1 ml./min with 100 mg per cent of 3-O-MG (Calbiochem, Los Angeles) and 2 μCi of ^{14}C-labelled 3-O-MG (New England Nuclear Corp., 5·3 mCi/mmole) in a physiological electrolyte solution or in pooled bile. Once the luminal perfusate was started, the total portal venous effluent was collected at one minute intervals and plasma ^{14}C was assayed in a Packard '300 Tri-Carb' liquid scintillation spectrometer. The percentage minute absorption (per cent abs./min) was calculated by dividing the product of the substrate concentration and the volume per min of the portal venous effluent by the mean luminal substrate concentration. The latter was considered equal to the average substrate concentration in the proximal and distal intestinal catheters. The per cent abs./min was then corrected per g of freeze-dried mesentery free bowel. Fig. 1 illustrates the perfusion sequence and the absorption characteristics observed in bile fistula and in sham animals. Results show a significantly increased absorption of 3-O-MG in the bile depleted animals (Table 1). On the other hand, the substitution of bile for the electrolyte solution as a vehicle for the substrate under study had no effect in either group of animals (Fig. 1).

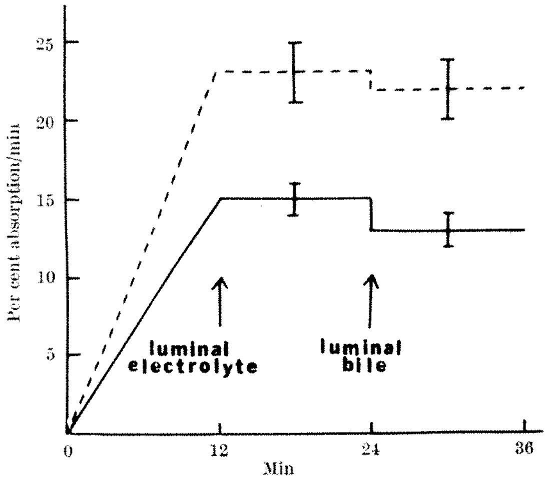


Fig. 1. Once placed in the tissue chamber at 37° C, the jejunal preparation was perfused for about 5 to 10 min before the constant luminal perfusion of 3-O-MG was started. As illustrated, the concentration of substrate in the venous effluent rose promptly. This accumulative tissue and vascular phase lasted 8 to 10 min and was followed by a relatively steady rate of substrate influx into the venous effluent. The portal venous ^{14}C activity was measured in duplicate from one minute samples taken throughout the two consecutive 12 min periods of luminal 3-O-MG electrolyte solution and the final 12 min interval during which pooled rat bile was the vehicle for the substrate. - - -, Bile fistula; ———, sham animals.

To validate this initial observation, three groups of ten rats with bile fistulae were studied. After creating the fistula, an intraduodenal cannula was positioned through a gastrostomy. The three groups of animals with bile fistulae were restrained, fasted and intraduodenally perfused for 48 h at a rate of 1 ml./h with either pooled rat bile, an electrolyte solution, or with a solution containing 35 μmoles/ml. of sodium taurocholate, the reported concentration of bile salts in rat bile[11]. The *p*H of these duodenal perfusates was 6·8 and their osmolality 285–310 mosM/kg. The commercial preparation of sodium taurocholate (Maybridge Research Chemicals, UK) was repurified by extracting an aqueous solution (*p*H 4·0) with diethyl ether five times. The acid *p*H was maintained between extractions. The final ether phase was decanted, flash evaporated at 24° C and the water phase lyophilized. Thin layer chromatography[12] showed trace amounts of other taurine conjugates, but no detectable unconjugated fractions. After 48 h of bile drainage and constant duodenal perfusion of either bile, electrolyte or sodium taurocholate, the weight loss of the animals averaged 11·4, 11·1 and 12·2 per cent for the three groups. The capacity of their isolated segments of upper jejunum to transport actively monosaccharides *in vitro* was assessed during a 30 min period of perfusion using an electrolyte solution of 100 mg per cent 3-O-MG and 2 μCi of ^{14}C-labelled 3-O-MG.

Table 1. PER CENT ABSORPTION PER MINUTE OF 3-O-MG/g DRY WEIGHT OF UPPER JEJUNUM PERFUSED *in vitro* AFTER 48 H OF BILE DEPLETION THROUGH A COMMON DUCT CANNULA

	No.	$\bar{X} \pm S.E.$	*P*
Sham	10	15·1 ± 1·13	< 0·01
Bile fistula	10	23·2 ± 1·95	

Table 2. PER CENT ABSORPTION OF 3-O-MG/g DRY WEIGHT OF UPPER JEJUNUM PERFUSED *in vitro*

	No.	$X \pm S.E.$	*P*
Pooled bile	10	20·6 ± 1·04	< 0·001
Electrolyte solution	10	33·6 ± 1·86	< 0·001
Sodium taurocholate	10	21·8 ± 1·07	

These intestinal preparations were studied in bile fistula animals after 48 h of continuous intraduodenal perfusion of either pooled rat bile, electrolyte solution or sodium taurocholate.

Table 3. DETERMINATIONS ON MUCOSAL SCRAPINGS

Determination	Perfusion liquid Electrolyte (5 rats)	Pooled bile (5 rats)	Taurocholate (5 rats)
Protein (mg/g wet weight)	113·3 ± 9·1	122 ± 9·4	100·3 ± 8·6
Hexokinase (nmoles of glucose metabolized per min/mg protein)	22·2 ± 2·9	24·1 ± 2·7	27·7 ± 2·5
Na^+, K^+ stimulated ATPase (μg P_i/mg protein/h)	86·2 ± 17·5	94·9 ± 19·1	77·7 ± 13·6
Glucose (mg/100 g wet tissue)	67·2 ± 8·0	68·1 ± 8·4	63·3 ± 7·7

Homogenates of mucosal scrapings frozen at −70° C were used for the determination of protein[13], hexokinase[14] and Na^+, K^+ stimulated ATPase[15]. Glucose determinations were carried out on whole bowel wall homogenates[16]. The results are expressed as means ± *S.E.* and show no significant difference between the three groups of animals.

Table 2 shows a marked difference in the *in vitro* per cent abs./min of 3-O-MG between the bile fistula rats perfused *in vivo* with the electrolyte solution and those perfused with either bile or sodium taurocholate for 48 h. There was no correlation between absorptive capacity and metabolic function as assessed by protein, hexokinase, Na^+, K^+ stimulated ATPase and glucose determinations carried out on tissue taken immediately adjacent to the jejunal segment removed from the animal (Table 3).

Thus when a segment of jejunum was removed from a bile depleted animal and perfused, it absorbed 3-O-MG more efficiently than a similar segment from a sham operated rat. In those bile fistula animals where bile depletion was countered by a constant *in vivo* bile infusion, however, the increase expected in bile fistula animals was not seen. More important, the experiments with a preparation of sodium taurocholate suggest that the biliary constituent responsible for modifying monosaccharide transport is a bile salt. On the other hand, when bile was acutely perfused through the *in vitro* jejunal preparation from both bile depleted and sham animals, it failed to affect the absorption of 3-O-methyl glucose (Fig. 1). This indicates that bile probably does not have a detergent effect modifying permeability characteristics of intestinal epithelial cells to monosaccharides. The observation that chronically administered bile salts in bile fistula animals exert an inhibitory effect on 3-O-MG transport is compatible with the idea that bile salts interfere with and modulate the absorption of monosaccharides. Because there was no apparent relationship between the absorption of 3-O-MG and metabolic function in the presence and absence of bile salts (Table 1), it is tempting to postulate that bile salts may have a specific effect on the monosaccharide active transport system localized in the plasma membrane of epithelial cell microvilli[17].

We thank Drs F. Kern and S. Gordon for their help and Miss C. Trombley and Mrs A. Montgomery for assistance. This investigation was supported by the US National Institutes of Health and Mead Johnson.

CLAUDE C. ROY
REUBEN S. DUBOIS
FERNAND PHILIPPON

Department of Pediatrics and Medicine,
University of Colorado Medical Centre,
Denver, Colorado.

Received September 15, 1969.

[1] Simmonds, W. J., *Amer. J. Clin. Nutrit.*, **22**, 266 (1969).
[2] Parkinson, T. M., and Olson, J. A., *Life Sci.*, **6**, 393 (1963).
[3] Nunn, A. S., Baker, D., and Searle, G. W., *Life Sci.*, **9**, 646 (1963).
[4] Smyth, D. H., and Whaler, B. C., *J. Physiol.*, **121**, 2P (1953).
[5] Pope, J. L., Parkinson, T. M., and Olson, J. A., *Biochim. Biophys. Acta*, **130**, 218 (1966).
[6] O'Brien, D., Ibbott, F. A., and Rodgerson, D. O., in *Laboratory Manual of Pediatric Micro-Biochemical Techniques*, 319 (Hoeber Medical Publishers, New York, 1968).
[7] Levin, S. J., Irvin, J. L., and Johnston, C. G., *Anal. Chem.*, **33**, 856 (1961),
[8] Wilson, T. H., and Landau, B. R., *Amer. J. Physiol.*, **198**, 99 (1960).
[9] Dubois, R. S., Vaughan, G. D., and Roy, C. C., in *Perfusion and Preservation of Organs* (edit. by Norman, J. C.), 863 (Appleton Century Crofts, New York, 1968).
[10] Dubois, R. S., and Roy, C. C., *Proc. Soc. Exp. Biol. and Med.*, **130**, 931 (1969).
[11] Dietschy, J. M., *J. Clin. Invest.*, **47**, 286 (1968).
[12] Hofmann, A. F., *J. Lipid Res.*, **3**, 127 (1962).
[13] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).
[14] Stifel, F. B., Rosensweig, N. S., Zakim, D., and Herman, R. H., *Biochim. Biophys. Acta*, **170**, 221 (1968).
[15] Hirschhorn, N., and Rosenberg, I. H., *J. Lab. Clin. Med.*, **72**, 28 (1968).
[16] Neale, R. J., and Wiseman, G., *J. Physiol.*, **198**, 601 (1968).
[17] Crane, R. K., in *Alimentary Canal* (edit. by Code, C. F.), **3**, 1323 (American Physiological Society, The Williams and Wilkins Co., Baltimore, 1968).

Parathyroid Hormone Secretion *in vitro*: Regulation by Calcium and Magnesium Ions

CONSIDERABLE evidence[1–5] has indicated that the secretion rate of parathyroid hormone (PTH) is controlled by the serum calcium; the release of hormone is proportional to calcium ion concentration, variations of which are followed within minutes by changes in circulating hormone activity[6]. Furthermore, studies[7–9] in animals suggest that the effects of magnesium ion on parathyroid function may resemble those of calcium.

We have recently developed an organ culture system utilizing normal bovine parathyroid tissue, which has provided direct evidence that the release of PTH is inversely proportional to both calcium and magnesium ion concentrations. Fresh bovine parathyroid glands were obtained at the time of slaughter and placed in sterile Earle's solution containing penicillin and streptomycin. The glands were plated out as 1 mm³ explants on stainless steel grids in organ culture dishes within 1 to 3 h. Incubations were performed in BGJ chemically defined medium modified by the addition of glycine and proline[10,11] plus 10 per cent foetal calf serum. Radioactive leucine (New England Nuclear) was added to low leucine medium in some experiments (0·5 μCi/ml. of ^{14}C-leucine). Tissue culture fluid was changed daily according to the experimental design, and the culture was continuously incubated in 5 per cent carbon dioxide in air. Calcium and magnesium ion concentrations were varied over the ranges 1–2·6 mM and 0·6–1·5 mM respectively, keeping chloride, phosphate and bicarbonate concentrations constant. Bovine PTH released into the tissue culture medium was measured by a highly specific and sensitive radio immunoassay[12], utilizing dextran coated charcoal to separate bound and free hormone[13]. In the perfusion studies, three or four explants were placed in a microchamber and perfused with varying calcium concentrations at a rate of 1·0 ml./h with a Sage pump. The calcium concentration of the perfusate was lowered in stepwise fashion, and three 30 min perfusates were collected at each calcium concentration.

Explants of normal glands remained functionally and histologically intact for periods as long as 3–4 weeks. Although central portions of the tissue eventually became necrotic, new cell growth at the borders was noted, and a functional layer of peripheral epithelial cells remained. Synthesis of new hormone in the tissue was documented by the incorporation of ^{14}C-leucine into a labelled protein (in tissue extracts and culture medium) which could be eluted on a column of 'Sephadex *G*-75' in a position corresponding to that of ^{131}I-labelled bovine PTH. Furthermore, ^{14}C-labelled hormone could be bound to antibodies against bovine PTH (moving its elution position on 'Sephadex *G*-75' to the void volume of the column) and then displaced by unlabelled bovine hormone, confirming its immunological identity with bovine PTH. Unlabelled PTH released into the tissue culture fluid behaved like purified bovine PTH in the radio immunoassay because it caused parallel displacement of ^{131}I-labelled hormone bound to antibody. When the medium was changed daily, and calcium concentrations maintained at either high (2·6 mM) or low levels (1·0 mM) for 2 day periods, hormone release varied in reciprocal fashion (Fig. 1*A*). The amount of hormone released into the medium varied between 0·1 and 1·6 μg/day. Tissue exposed to constant calcium concentrations of 2·0 mM, on the other hand, showed no significant variations in hormone release (dotted line, Figs. 1*A* and *B*). Similar variations in medium magnesium concentration (with calcium constant at 2·0 mM) in the range of 0·6 to 1·5 mM resulted in reciprocal variations in PTH secretion (Fig. 1*B*). In leucine incorporation studies performed under identical conditions, the amount of newly synthesized hormone secreted into the medium paralleled the release of unlabelled hormone.

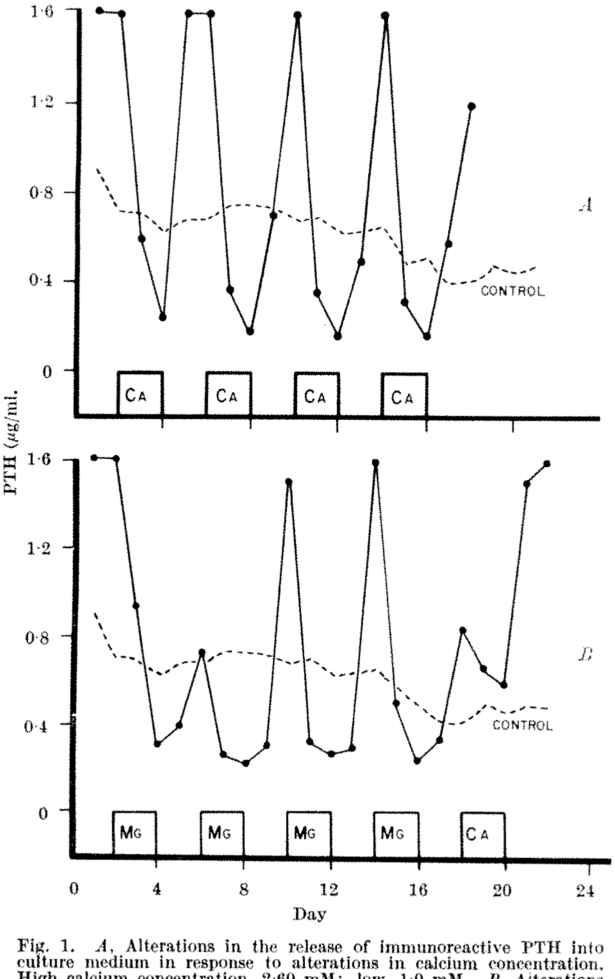


Fig. 1. *A*, Alterations in the release of immunoreactive PTH into culture medium in response to alterations in calcium concentration. High calcium concentration, 2·60 mM; low, 1·0 mM. *B*, Alterations in the release of immunoreactive PTH into medium in response to alterations in magnesium concentration. High magnesium concentration, 1·5 mM; low, 0·6 mM. Dotted line indicates results of constant calcium (2·0 mM) control culture.

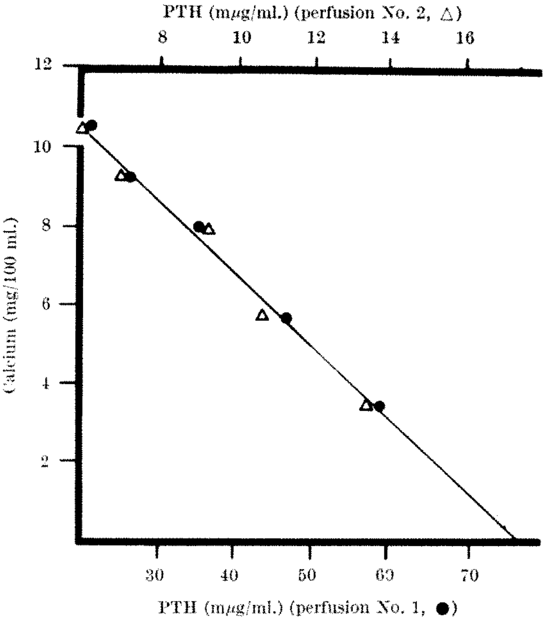


Fig. 2. Variations in PTH released from perfused explants at five calcium concentrations in two separate experiments. Each point represents mean of three 30 min perfusates.

In separate perfusion experiments, calcium concentration which was 2·6 mM (10·4 mg per cent) initially was sequentially decreased to 1·0 mM (4 mg per cent) in four steps. Parathyroid hormone released into the fluid was inversely related to the perfusing calcium concentration and was a proportional function when plotted against the calcium concentration of the medium (Fig. 2).

The current report describes an *in vitro* system which synthesizes PTH and which responds rapidly to changes in calcium concentration by varying the secretion of hormone. These studies fully confirm earlier observations[3] *in vivo* of a linear relationship between serum calcium concentration and the release of hormone and, furthermore, provide definite evidence that magnesium ion has a calcium like effect on parathyroid function. In a recent detailed and quantitative comparison of the effects of calcium and magnesium ions on parathyroid gland metabolism (J. H. Targovnik and L. M. S., in preparation), it is apparent that the two cations are equipotent in blocking hormone release. Further studies of the mechanism of this effect as well as the effects of the two ions on hormone synthesis are in progress.

This work was supported by a US Public Health Service grant and a grant from the Boston Medical Foundation. We thank D. Cluxton and S. Catousse for valuable technical assistance.

LOUIS M. SHERWOOD

Department of Medicine,
Harvard Medical School and Beth Israel Hospital,
Boston, Massachusetts 02215.

INGEBORG HERRMAN
C. ANDREW BASSETT

Department of Orthopedic Surgery,
Columbia College of Physicians and Surgeons,
New York, NY.

Received October 27; revised December 19, 1969.

[1] Patt, H. M., and Luckhardt, A. B., *Endocrinology*, **31**, 384 (1942).

[2] Carnes, W. H., Pappenheimer, A. M., and Stoerk, H. C., *Proc. Soc. Exp. Biol. and Med.*, **51**, 314 (1942).

[3] Copp, D. H., and Davidson, A. G. F., *Proc. Soc. Exp. Biol. and Med.*, **107**, 342 (1961).

[4] Raisz, L. G., *Nature*, **197**, 1115 (1963).

[5] Sherwood, L. M., Potts, jun., J. T., Care, A. D., Mayer, G. P., and Aurbach, G. D., *Nature*, **209**, 52 (1966).

[6] Sherwood, L. M., Mayer, G. P., Ramberg, jun., C. F., Kronfeld, D S., Aurbach, G. D., and Potts, jun., J. T., *Endocrinology*, **83**, 1043 (1968).

[7] Care, A. D., Sherwood, L. M., Potts, jun., J. T., and Aurbach, G. D., *Nature*, **209**, 55 (1966).

[8] Gitelman, H. J., Kukolj, S., and Welt, L. G., *Amer. J. Physiol.*, **215**, 483 (1968).

[9] Buckle, R. M., Care, A. D., Cooper, C. W., and Gitelman, H. J., *J. Endocrinol.*, **42**, 529 (1968).

[10] Biggers, J. D., Gwatkin, R. B., and Heyner, S., *Exp. Cell Res.*, **25**, 41 (1961).

[11] Jackson, S. F., *Biochem. J.*, **104**, 13P (1967).

[12] Berson, S. A., Yalow, R. S., Aurbach, G. D., and Potts, jun., J. T., *Proc. US Nat. Acad. Sci.*, **49**, 613 (1963).

[13] Herbert, V., Lau, K. S., Gottlier, C. W., and Bleicher, S. J., *J. Clin. Endocrinology-Metab.*, **25**, 1375 (1965).

Complexes between Acetylcholine and Catecholamines and their Tolerance to Mental Illness

THE biochemical theories of schizophrenia based on the presence of endogenous psychotogenic agents[1] relate to the hypothesis first advanced by Osmond and Smythies[2] of an aberrant catecholamine metabolism and take into account the studies on experimental animals and humans[3] which demonstrated the psychotomimetic properties of aminochromes.

Recently the importance of a direct chemical interaction between central chemical transmitters and neurotropic drugs has been pointed out[4] and postulated as the chief event responsible for the pharmacodynamic effect of some neurotropic drugs[5]. Moreover, the possibility of an interference between a well known[6] oxidation product of a central neurotransmitter such as noradrenaline and another neurotransmitter such as acetylcholine has been established[7]. In fact, acetylcholine is able to interact with oxidized noradrenaline, yielding a molecular complex that is stable toward further transformation to adrenolutin brought about by ascorbic acid.

In this work a new medium for the interaction was chosen with conductivity and oxidative properties quite close to the ones found for whole brain homogenates. A mixture of Tris-HCl 0·1 M *p*H 7·4 and dioxane (1 : 10) was used with a conductivity of about 3 microSiemens and an E_h value of 185 mV. Whole rabbit brain homogenates showed an average conductivity of about 5 microSiemens and E_h value of about 120 mV (with a radiometer *TTT 1C* equipped with platine-calomel electrode). The values found for the homogenate thus agree quite well with those found for the Tris-dioxane medium. This medium, in fact, brings about the spontaneous transformation of catecholamines first to aminochromes and later to dihydroxyindoles as shown by spectral and chromatographic evidence.

Table 1 shows the characteristics of the interaction between acetylcholine and oxidation products of noradrenaline both in the excited state and in the ground state of the molecules as shown by the good parallelism of spectral, chromatographic and conductometric data. The non-additive conductivity of acetylcholine and noradrenaline is mostly evident in the Tris-dioxane medium and the same is true for the chromatographic mobility that is slower for the acetylcholine–noradrenaline mixture in the Tris-dioxane medium. Further, the typical visible and ultraviolet absorption of aminochromes and a significant interaction documented by difference spectra is found only with the Tris-dioxane medium. If dopamine is used instead of noradrenaline a similar interaction can be noted.

Infrared spectra were taken after evaporating the solutions listed in Table 1 and redissolving the residue in chloroform. The chief variations were observed with Tris-dioxane medium; a new 1,040 cm^{-1} band not shown in either compound alone appeared in the acetylcholine–noradrenaline mixture. This band might be due to deformation and skeletal vibration of the CO–O–C portion of acetylcholine. A new stretching vibration appeared also in the 3,600–3,000 cm^{-1} region which can be assigned to the free OH of noradrenochrome. These results support the previous hypothesis[7] concerning a possible interaction between the O–C–C–N system common to the two molecules. The main finding reported here and in the preceding study[7] is related to the stabilization of toxic oxidation products of central catecholamines such as noradrenaline and dopamine by the central chemical transmitter acetylcholine. A detailed chemical study of such an interaction is now in progress.

The effects of ascorbic acid and nicotinamide, a compound used for the therapy of mental illness[8], are of great interest if studied on our system. In fact, the velocity of autoxidation of noradrenaline is increased by both acetylcholine and nicotinamide as shown in Table 2. It seems that either compound is able to form molecular complexes with aminochrome, but the effect of ascorbic acid strongly differs for each complex, as documented polarographically and by fluorimetric measurements (Table 3).

By measuring the velocity of noradrenochrome formation in the presence of different concentrations of acetylcholine and nicotinamide two apparent dissociation

Table 1*. ACETYLCHOLINE–NORADRENALINE INTERACTION

Compound†	Solvent‡	Absorption spectrum (λ max)§		Difference spectrum (ΔEλ)‖		Chromatography¶		Conductivity** microSiemens
		Ultraviolet	Visible	Ultraviolet	Visible	R_f	Fluorescence	
Acetylcholine	Water	—	—	—	—	—	—	2,800
Noradrenaline	Water	278	—	—	—	0·78	B	300
Acetylcholine–noradrenaline	Water	278	—	0	0	0·77	B	3,000
Acetylcholine	Tris-buffer	—	—	—	—	—	—	7,200
Noradrenaline	Tris-buffer	278	—	—	—	0·78	B	4,900
Acetylcholine–noradrenaline	Tris-buffer	278	—	0	0	0·70	B	7,000
Acetylcholine	Tris-dioxane	—	—	—	—	—	—	140
Noradrenaline	Tris-dioxane	280	485	—	—	0·80	Y	8
Acetylcholine–noradrenaline	Tris-dioxane	280	485	$0{\cdot}180_{277}$ $0{\cdot}320_{210}$	$0{\cdot}060_{485}$ $0{\cdot}320_{305}$	0·69	B	25

* The data reported here were collected 1 h after the solutions were prepared.

† The products were obtained from Calbiochem. The initial solutions were 0·1 M in water, Tris-HCl 0·1 M, *p*H 7·4 or Tris-dioxane (1 : 10). Dioxane (Merck, puriss.) was used without redistillation.

‡ The solvents indicated were used for the dilutions 1 : 100 or 1 : 1,000 made before taking the spectra.

§ The spectra were recorded with a Cary 15 recording spectrophotometer.

‖ Double-compartment cells were used. The acetylcholine–noradrenaline mixture was put into the upper chamber together with solvent and the two separate solutions of acetylcholine and noradrenaline were put into the lower chamber.

¶ Thin layer chromatography on silica gel (Eastman sheets 20 × 20 type K 301 R2) with acetic acid–water 2 per cent as solvent was used. The spots were detected after spraying with 10 per cent methanolic KOH. The fluorescence was viewed in an Original Hahau (No. 5201) apparatus with excitation wavelength of 3600 Å. B = blue, Y = yellow, G = green, BG = blue-green.

** A conductivity-meter CDM 2 radiometer type was used.

Table 2. MOLECULAR COMPLEXES OF NORADRENOCHROME WITH ACETYLCHOLINE AND NICOTINAMIDE

Compound added to noradrenaline*	Spectrophotometric velocity of autoxidation †	Polarographic relative velocity of autoxidation ‡	Chromatographic mobility§	
			Rf_1	Rf_2
None	0·050	100	0·86	—
Acetylcholine	0·200	137	0·70	—
Nicotinamide	0·425	220	0·73	0·58

* To 1·5 ml. of noradrenaline 1×10^{-3} M in Tris-HCl *p*H 7·4 0·1 M and dioxane (1 : 10) 1·5 ml. of acetylcholine or nicotinamide 1×10^{-2} M are added.

† Noradrenochrome formation is followed with a Beckman DB recording spectrophotometer as $\Delta E_{497\ \mathrm{nm}}$ in 10 min.

‡ A Gilson ME apparatus equipped with a Clark oxygen electrode is used.

§ Thin layer chromatography on silica gel with acetic acid 2 per cent in water as solvent is used. The spots are detected with an ultraviolet lamp after spraying the chromatoplates with 10 per cent methanolic KOH.

Table 3. EFFECT OF ASCORBIC ACID ON ACETYLCHOLINE–NORADRENOCHROME AND NICOTINAMIDE–NORADRENOCHROME COMPLEXES

Compound added to noradrenaline*	Relative velocity of formation of fluorescent compound †	Relative velocity of polarographic reduction ‡
None	100	100
Acetylcholine	19	36
Nicotinamide	590	430

* The solutions are the same as those described in the caption to Table 1. They are used 1 h after acetylcholine or nicotinamide addition. 0·03 ml. of 0·5 per cent ascorbic acid in water is added and the relative velocities of formation of reduction derivatives are measured.

† An Eppendorf recording spectrophotometer with the fluorescence attachment is used with 400 nm excitation and 500 nm emission filters.

‡ A Gilson ME oxygraph as described in Table 2 is used.

constants for acetylcholine–noradrenochrome ($K_1 = 1{\cdot}75 \times 10^{-4}$ M) and nicotinamide–noradrenochrome ($K_2 = 4{\cdot}16 \times 10^{-3}$ M) complexes were calculated.

From these data it appears that nicotinamide is able to compete with acetylcholine for noradrenochrome. In fact, a similar effect was observed by studying the formation of molecular complexes between thiamine and central chemical transmitters[9]. The acetylcholine–noradrenochrome complex is relatively stable toward reduction to dihydroxyindole by ascorbic acid as already shown[7], whereas the reduction of nicotinamide–noradrenochrome complex is much faster than the reduction of noradrenochrome alone. The data obtained after chromatography agree with the fluorescence measurements. In fact, the spot (Table 2) corresponding to adrenolutin[6] is present only when nicotinamide is added and the rate of formation of fluorescence products is maximal in the presence of nicotinamide.

A general scheme can summarize these results as follows

$$\begin{tikzcd}
 & \text{nicotinamide} \arrow[r] & \text{complex 2} \arrow[d] \\
\text{catecholamine} \arrow[r] & \text{aminochrome} \arrow[u] \arrow[r] \arrow[d] & \text{dihydroxyindole} \\
 & \text{acetylcholine} \arrow[r] & \text{complex 1}
\end{tikzcd}$$

If we consider adrenochrome reduction derivatives as non-toxic excretion products of the psychotogen aminochromes, a rationale for the clinical use of nicotinamide in megadoses[8] as a therapy for mental illness is now provided. In fact, our data suggest a chemical explanation for mental illness which may offer an alternative to the theory of orthomolecular psychiatry proposed by Pauling[10].

We can postulate that if a central catecholamine is leaking at any synapse devoid of the enzymatic system that destroys it, the slightly oxidative conditions together with the hydrophobicity of the medium are sufficient to bring about a physiological aminochrome formation, as occurs in our *in vitro* system. If this takes place at the level of cholinergic synapses the aminochromes so formed can accumulate and be stabilized by acetylcholine.

The leak of catecholamine and the stabilization of its oxidation products could produce an aberrant communication which, on the one hand, might be the chemical event leading to a short circuit between adrenergic and cholinergic systems and, on the other hand, might be the origin of irritative foci of stabilized psychotogenic agents at cortical level.

Both effects could possibly explain the onset of mental illness.

I thank Professor Linus Pauling and Dr Emile Zuckerkandl for scientific, moral and financial support.

LAURO GALZIGNA

Groupe de Recherches U–67 (Inserm),
B.P. 1018, 34–Montpellier,
and Department of Chemistry,
University of California, San Diego.

Received September 23; revised December 22, 1969.

[1] Shulgin, A. T., Sargent, T., and Naranjo, C., *Nature*, **221**, 537 (1969).
[2] Osmond, H., and Smythies, J. R., *J. Ment. Sci.*, **98**, 306 (1952).
[3] Hoffer, A., and Osmond, H., *The Hallucinogens* (Academic Press, New York, 1967).
[4] Galzigna, L., *FEBS Lett.*, **3**, 97 (1969).
[5] Galzigna, L., Manani, G., Manzin, E., Gritti, G., and Rizzoli, A. A., *Acta Anesth.*, **19**, 1 (1968).
[6] Heacock, R. A., *Chem. Rev.*, **52**, 181 (1959).
[7] Galzigna, L., *CR Acad. Sci.*, **268**, 2498 (1969).
[8] Hoffer, A., *Psychosomatics*, **8**, 95 (1967).
[9] Galzigna, L., *Biochem. Pharmacol.*, **18**, 2485 (1969).
[10] Pauling, L., *Science*, **160**, 265 (1968).

Changes in Enzymes involved in the Biosynthesis and Metabolism of Noradrenaline and Adrenaline after Psychosocial Stimulation

THE important role of catecholamines in behaviour has been recognized[1]. Increased adrenaline and noradrenaline excretion has been observed in anxiety-provoking situations and in states of anger and aggression[2,3]. Drugs used in affective disorders cause marked changes in uptake, storage, release and metabolism of the neurotransmitter noradrenaline in the peripheral and central nervous systems[4,5]. Because of the growing understanding of the implication of catecholamines in behavioural changes, we examined the effect of psychosocial stimulation and deprivation on the activity of the enzymes involved in the biosynthesis and metabolism of catecholamines. Groups of mice were raised in social isolation or were subjected to prolonged and increased social interaction, and the catecholamine synthesizing (tyrosine hydroxylase and phenylethanolamine N-methyltransferase) and metabolizing (monoamine oxidase and catecholamine-O-methyl transferase) enzymes were examined in the adrenal gland. We wish to report marked changes in enzymes involved in catecholamine synthesis and metabolism in the adrenal glands of these mice.

Control groups consisted of mice which had normal nursing and sibling contact until three to four weeks old and were then weaned. The sexes were separated and maintained six to eight siblings per standard cage 27 × 14 × 13 cm until eight months of age. Stimulus-deprived mice were removed from mother and sibling contact 24 h after birth for 2 h a day for 14 days. They were then isolated in pint size glass containers one-third full of wood shavings. A paper towel was taped around each jar to prevent visual contact, but they retained access to olfactory and auditory stimuli. The mice remained isolated until eight months of age. Groups which had increased social stimulation were deprived of mother and sibling contact 2 h per day for 14 days, as described for the stimulus deprived group. The sexes were separated and six to eight siblings were placed in a standard cage for 90 days. Ten males and ten females were then placed in a communicating box system until they were eight months old, as previously described[6]. This consisted of six cages connected with narrow tubes. Another tube from each cage led into a central box containing food and water. Floor space per mouse was about twice as much as that of the standard cage, but males and females could now intermingle. Such a box system forced the mice into frequent dominance-submission choices and increased social stimulation. At eight months, all males were killed with ether, the adrenals rapidly removed, cleaned, weighed and then frozen. Each adrenal gland was homogenized in 1 ml. of ice-cold isotonic sucrose with glass Duall homogenizer and an aliquot taken for the determination of noradrenaline[7]. The remaining homogenate was centrifuged at 27,000*g*, tyrosine hydroxylase[8], phenylethanolamine N-methyltransferase (PNMT)[9], and catechol-O-methyl transferase (COMT)[10] were assayed in the supernatant fraction and monoamine oxidase (MAO)[11] was determined in the particulate fraction.

Tyrosine hydroxylase, the initial and rate-limiting enzyme in catecholamine formation, is localized in sympathetic nerves and in the adrenal gland. PNMT is restricted to the adrenal medulla and certain areas of the brain and converts noradrenaline to adrenaline. In the socially deprived mice there was a significant decrease in adrenal tyrosine hydroxylase and PNMT activity (Table 1). The noradrenaline and adrenaline content was slightly but not significantly lower in these animals. No changes in MAO or COMT activity were observed. There was a marked elevation in tyrosine hydroxylase, PNMT, and MAO activity and in noradrenaline and adrenaline content in the adrenals of psychosocially stimulated mice, but no change in COMT activity. Adrenal gland weight was elevated in both deprived and stimulated mice.

Table 1. EFFECT OF PSYCHOSOCIAL STIMULATION ON THE ACTIVITY OF CATECHOLAMINE FORMING AND METABOLIZING ENZYMES IN MALE MOUSE ADRENALS

Treatment	Adrenal (weight/mg)	PNMT (units)	Tyrosine hydroxylase (units)	MAO (units)
Normal	1·05 ± 0·12	0·29 ± 0·013	1·8 ± 0·29	19 ± 3·0
Stimulus deprived	1·55 ± 0·05†	0·19 ± 0·026†	1·1 ± 0·21†	21 ± 1·5
Stimulus increased	2·5 ± 0·15*	0·48 ± 0·41*	4·3 ± 0·41*	36 ± 3·5†

Treatment	COMT (units)	Noradrenaline (μg)	Adrenaline (μg)
Normal	0·053 ± 0·006	0·42 ± 0·1	1·42 ± 0·20
Stimulus deprived	0·042 ± 0·005	0·36 ± 0·07	1·01 ± 0·17
Stimulus increased	0·057 ± 0·006	1·01 ± 0·15*	3·23 ± 0·25*

* $P < 0·001$. † $P < 0·01$. ‡ $P < 0·05$.

Results are expressed as mean ± *S.E.* per adrenal. One unit of enzyme activity is the pmole product formed per h.

These results demonstrate changes in enzymes concerned with the biosynthesis of noradrenaline and adrenaline in the adrenal glands of mice subjected to different social interactions. In socially deprived mice there was a fall in tyrosine hydroxylase and PNMT activity although adrenal weight was increased, while in socially stimulated mice there was a rise in both biosynthetic enzymes and one metabolizing enzyme (MAO). The elevation of these enzymes might be the result of increased discharge of corticoids from the adrenal cortex, or an increased stimulation of the adrenal gland by sympathetic nerves. We have previously shown that tyrosine hydroxylase and PNMT can be elevated by a reflex increase in sympathetic nerve activity, but not by pituitary or corticoid hormones[12]. Furthermore, it was found that the increase in enzyme activity was trans-synaptically induced, dependent on new protein synthesis, and required one to three days before an elevation was apparent[13]. Prolonged, forced immobilization of rats also produces an elevation of tyrosine hydroxylase and PNMT activity and this rise could be abolished by cutting the splanchnic nerve to the adrenal gland (personal communication of R. Kvetnausky, I. J. Kopin and V. Weise). These findings suggest that the increase in the catecholamine-forming enzymes in psychosocial stimulation may also be neuronally mediated and is not an immediate response, as in the case of a sudden discharge of noradrenaline and adrenaline in states of anger, fear or aggression[2,3].

A reduction of adrenal PNMT and tyrosine hydroxylase activity occurs after hypophysectomy and this decrease can be prevented with ACTH. Adrenal tyrosine hydroxylase activity decreases after cutting the splanchnic nerve to the adrenal[14]. Whether the reduced PNMT and tyrosine hydroxylase activity in psychosocially deprived animals is due to a depletion of ACTH, reduced sympathetic nerve stimulation or other factors remains to be established.

JULIUS AXELROD
ROBERT A. MUELLER

Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Maryland.

JAMES P. HENRY
PATRICIA M. STEPHENS

Department of Physiology,
University of Southern California,
Los Angeles, California.

Received December 17, 1969.

[1] Schildkraut, J. J., and Kety, S. S., *Science*, **156**, 21 (1967).
[2] Elmadjian, F., Hope, J. M., and Lamson, E. T., *J. Clin. Endocrinol.*, **17**, 608 (1957).
[3] Levi, L., *Psychosomat. Med.*, **27**, 80 (1965).
[4] Axelrod, J., Whitby, L. G., and Hertting, G., *Science*, **133**, 383 (1961).
[5] Glowinski, J., and Axelrod, J., *Pharmacol. Rev.*, **18**, 775 (1966).
[6] Henry, J. P., Meehan, J. P., and Stephens, P. M., *Psychosomat. Med.*, **29**, 408 (1967).
[7] von Euler, U. S., and Lishajko, F., *Acta Physiol. Scand.*, **61**, 348 (1961).
[8] Mueller, R. A., Thoenen, H., and Axelrod, J., *J. Pharmacol. Exp. Therap.*, **169**, 74 (1969).
[9] Axelrod, J., *J. Biol. Chem.*, **237**, 1657 (1962).
[10] Wurtman, R. J., and Axelrod, J., *J. Biol. Chem.*, **241**, 2301 (1966).
[11] Wurtman, R. J., and Axelrod, J., *Biochem. Pharmacol.*, **12**, 1439 (1963).
[12] Thoenen, H., Mueller, R. A., and Axelrod, J., *J. Pharmacol. Exp. Therap.*, **169**, 249 (1969).
[13] Mueller, R. A., Thoenen, H., and Axelrod, J., *Mol. Pharmacol.*, **5**, 463 (1969).
[14] Thoenen, H., Mueller, R. A., and Axelrod, J., *Endocrinology* (in the press).

Direct Postsynaptic Responses to Stimulation of Serotonin-containing Neurones

THERE is one giant serotonin-containing neurone in each metacerebral ganglion of the slug *Limax maximus* and of the snail *Helix pomatia*. These cells can be identified histochemically and their content of serotonin can be measured by bioassay of individually dissected neurones. The amine is localized in small electron dense granules in the cytoplasm of these cells and also, in certain conditions, in particles with the appearance of lysosomes (ref. 1 and unpublished data of G. A. C. and N. N. Osborne). Dye injection experiments with 'Procion Yellow', using the method of Stretton and Kravitz[2], show that each giant neurone in *Helix pomatia* gives off three main axonal branches. One branch passes across the cerebral commissure to the other group of cerebral ganglia, another can be traced to the ipsilateral cerebral-buccal connective, and the third passes into the ipsilateral exterior lip nerve.

Kandel and Tauc[3,4] have previously studied the membrane properties of these neurones in *Helix aspersa* and *Helix pomatia* and they have discovered that they show a high degree of anomalous rectification. They also showed, using electrophysiological methods, that each cell sends an axon to the ipsilateral exterior lip nerve and one to each cerebral-buccal connective.

In this study a search was made for cells directly innervated by these giant serotonin-containing neurones in *Helix pomatia*. The circum-oesophageal ganglionic complex, together with the buccal ganglia and connecting nerves, were dissected from active *Helix pomatia* and pinned to a nylon sheet fixed at the bottom of a small bath (volume 0·7 ml.). The preparation was perfused with snail saline[5]. Double-barrelled glass micro-electrodes were used to impale the giant cell and to explore possible postsynaptic cells. One barrel was filled with 3 M KCl and used for recording and the other, used for applying current, was filled with 0·6 M Na_2SO_4. Input leads from the electrodes were fed through cathode followers to a dual beam oscilloscope.

Giant serotonin cells usually fired a burst of spikes when impaled with a micro-electrode and then remained inactive. They were made to fire action potentials by passing a depolarizing pulse of 1 s or shorter duration through the second electrode. In each buccal ganglion a neurone was found which was stimulated by a burst of spikes in a giant serotonin cell. The cell was located close to the entry of the cerebral-buccal connective in each ganglion. The response recorded in some preparations was a slight depolarization at normal membrane potential (Fig. 1*a*) which was usually within the range of 50 to 60 mV. In other preparations, usually when the buccal cell showed some prior spike activity, a burst of spikes in a giant serotonin cell resulted in depolarization and a spike firing in the buccal cell. In a few preparations a small individual response was recorded with each spike fired in a giant serotonin cell. There was facilitation of such responses with repetitive stimulation and the potentials summed (Fig. 1*b*). There was a constant delay of about 100 ms in appearance of these potentials, suggesting a direct link between the giant serotonin cell and the buccal cell described.

Further information on the connexions between the cells was obtained in other preparations by hyperpolarizing one of the buccal cells described and repeatedly stimulating a giant serotonin cell. The size of the responses varied slightly from one preparation to another, but they were increased in amplitude by hyperpolarization. The potentials summed with repeated stimulation of a giant cell and in most cases facilitation of the responses could be observed. Increasing the level of hyperpolarization did not result in a loss of the potentials. Depolarizing pulses of different durations which did not result in spike firing in a giant cell did not cause any potential changes in the buccal cells.

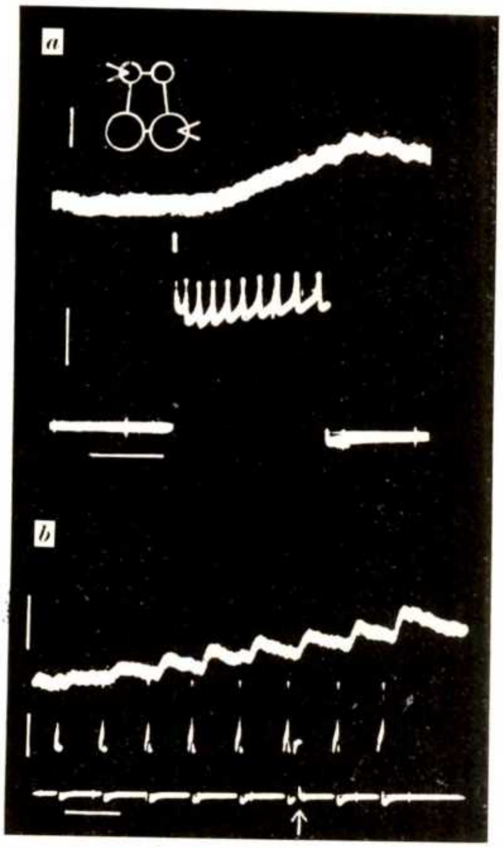


Fig. 1. Responses of a cell of the left buccal ganglion to spike firing in the right giant serotonin cell. The records are from two preparations. The buccal cell response is recorded on the upper beam in each case. (Sometimes, as in *a*, a giant serotonin cell would not fire spikes without relatively intense depolarizing pulses. Frequently axon spikes alone were recorded in these conditions.) In *b*, 50 ms depolarizing pulses, each resulting in only one spike, were applied to the giant serotonin cell. Each spike was followed by a small depolarizing potential in the buccal cell. The responses summed and were facilitated with repeated stimulation. A square wave alone did not affect the buccal cell (arrow). Calibrations: upper beam 2 mV; lower beam 20 mV; 0·5 s.

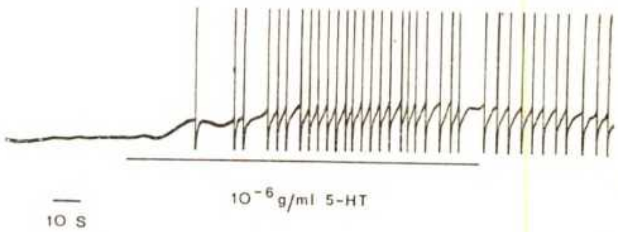


Fig. 2. Response of a cell, of the left buccal ganglion, which was previously shown to respond to spikes in the left giant serotonin cell, to perfusion with serotonin (5-HT). (The pen recorder did not record the full amplitude of the spikes.)

The data suggest that the small potentials observed are chemically mediated excitatory postsynaptic potentials (EPSPs). The view that the linkage was monosynaptic was supported by the observations that the EPSPs could not be lost by repeated stimulation, and the delay in appearance of EPSPs was always constant in a given experiment at about 100 ms.

The delay in response of the buccal cell was about what would be expected from information on conduction velocity of spikes in gastropod nerves. Conduction velocity along the intestinal nerve of *Helix pomatia* is at 5 cm/s (ref. 7). The distance between the giant serotonin cells and the buccal ganglia is about 0·7 cm.

Each of the two giant serotonin cells had an input on to a cell in each buccal ganglion and the conduction time for ipsilateral and contralateral connexions was observed to be the same. In some preparations both giant serotonin cells were stimulated while recording from the same buccal cell. No consistent difference in responses was observed depending on whether one or the other of the giant serotonin cells was stimulated.

It is thought that serotonin may be a transmitter substance in molluscs[7]. If this is the case with the giant serotonin cells, it would be expected that buccal cells would be activated by the amine. This was, indeed, shown to be so. The cells were depolarized and started firing spikes when serotonin, at a concentration of 10^{-7} g/ml. or greater, was added to the perfusion liquid (Fig. 2), whereas the giant serotonin cells were not depolarized and generally did not spike when the amine was added. Reduction in the potential, produced by applying a constant hyperpolarizing current, when serotonin was added to the bath showed that the amine increases membrane conductance of the specified buccal cells. Acetylcholine also stimulated the postsynaptic buccal neurone (as well as the giant serotonin cells). With respect to this observation, it may be important that each of the buccal cells receives at least one excitation input other than those from the giant serotonin cells. Dopamine, in concentrations of about 10^{-7} g/ml., hyperpolarized the postsynaptic cells. In the case of each of these drugs the responses of the specified buccal cells resemble those of the "CILDA cells" of gastropods which have been shown to have specific serotonin receptors[8,9].

The response of the cells in the buccal ganglia to activation of the giant serotonin cells was blocked with relatively high concentrations of the serotonin antagonists lysergic acid diethylamide (5×10^{-5} g/ml.) and "Methysergide" (10^{-4} g/ml.) but not by perfusing lower concentrations of these drugs for periods up to about 15 min. Further studies, however, need to be completed in order to evaluate the significance of the experiments with high concentrations of the blocking drugs in view of the observations[10] that the closely related compound 2-bromolysergic acid diethylamide can interfere with axonal conduction in another gastropod mollusc, *Aplysia*.

These results show that two symmetrically arranged serotonin-containing neurones form excitatory synaptic links with other neurones in the snail brain. They also provide evidence that serotonin is a transmitter in the central nervous system of the snail. The system that I have described may provide a favourable preparation for further pharmacological studies with drugs which modify the effects, or levels, of serotonin in nervous tissue.

I thank the MRC for financial support and Mr C. J. Roemmélé and Mr J. Brown for technical assistance.

G. A. COTTRELL

Wellcome Laboratories of Pharmacology,
Gatty Marine Laboratory,
University of St Andrews,
Fife, Scotland.

Received November 7; revised December 29, 1969.

[1] Cottrell, G. A., and Osborne, N. N., *Nature*, **225**, 471 (1970).
[2] Stretton, A. O. W., and Kravitz, E. A., *Science*, **162**, 132 (1968).
[3] Kandel, E. R., and Tauc, L., *J. Physiol.*, **183**, 269 (1966).
[4] Kandel, E. R., and Tauc, L., *J. Physiol.*, **183**, 287 (1966).
[5] Meng, K., *Zool. Jber.*, **68**, 539 (1960).
[6] Krijgsman, B. J., *Z. vergleich. Physiol.*, **28**, 286 (1941).
[7] Cottrell, G. A., and Laverack, M. S., *Ann. Rev. Pharmacol.*, **8**, 273 (1968).
[8] Stefani, E., and Gerschenfeld, H. M., *J. Neurophysiol.*, **32**, 64 (1969).
[9] Ascher, P., *J. Physiol.*, **198**, 48 P (1968).
[10] Gerschenfeld, H. M., and Stefani, E., *J. Physiol.*, **185**, 684 (1966).

Acute Toxicity of Aflatoxin B_1 in Wethers

THE original objective of this study was the preparation of milligram quantities of aflatoxin M_1. Holzapfel *et al.*[1] and Masri *et al.*[2] had collected sufficient quantities of the toxin from the urine of sheep which had eaten food containing aflatoxin B_1 for structural studies. We administered an exploratory dose of 4 mg of aflatoxin B_1/kg, a conservative value compared with a reported single dose LD_{50} of approximately 500 mg/kg (ref. 3). This was lethal to our wether. In subsequent correspondence Purchase advised that an (intraperitoneal) dose of 2 mg/kg had killed a Merino sheep, and Masri noted that a repeated oral dose of 0·38 mg/kg per day for seven days was lethal to both a Hampshire ewe and a crossbred western white-face wether. If the toxin is to be given over a period of time, a sublethal dose must be used. We undertook a toxicological study to satisfy this requirement and now report the results of our LD_{50} study.

The aflatoxin B_1 was obtained from extracts of cultures of *Aspergillus flavus* (FDA, DM Isolate No. M141) grown on sterile moist rice and purified by the procedure of Rodricks[4]. Recrystallization from chloroform yielded aflatoxin B_1 of 98 per cent purity based on thin layer chromatography and ultraviolet analysis (λ_{max} (361 nm) in methanol, ε 21,500). The required quantities of aflatoxin B_1 were weighed into gelatine capsules which were completely filled with powdered sucrose and sealed.

The animals used were two year old wethers, crossbred Hampshire–Shropshire–Merino–Southdown and an experimental Morlam breed derived from crossbred Dorset–Rambouillet–Merino sheep. They were kept in individual metabolism stalls adapted from a design by Briggs and Gallup[5] which allowed separate collection of faeces and urine. A mixture of alfalfa, clover and timothy hay was freely offered, and a grain feed, Beltsville 20C, was restricted to 0·9 kg per animal each day. While the animals were in the stalls, rectal temperatures were taken, and the droppings and general clinical appearance were observed. Sheep showing irregular or questionable results and abnormal behaviour without treatment were rejected.

After dosing, the clinical signs and body temperatures were recorded at 2–5 h intervals. Laparotomy was performed on surviving sheep, usually at day 7 and again after several weeks, to follow the course of liver restitution. Dead animals were necropsied. If the sheep survived, faeces were collected for 5 days and urine for 3 days after dosing and analysed for the content of aflatoxins B_1 and M_1.

Another single exploratory dose was given at 1·28 mg/kg and multiple doses were given on successive days (shown in parentheses): 0·9 (2), 0·59 (2), 0·23 (2) and 0·14 (6) mg/kg to separate wethers. None of these died.

Table 1. ACUTE EFFECT OF AFLATOXIN B_1 ON 2 YEAR OLD WETHERS

Daily dose mg/kg body weight	Days	No. of animals	No. dead
4·00	1	3	3
2·84	1	3	3
2·00	1	3	1
1·42	1	3	0
1·28	1	1	0
0·90	2	1	0
0·59	2	1	0
0·23	2	1	0
0·14	6	1	0

The single dose LD_{50} for crossbred wethers, calculated by the Weil[6] procedure, was 2·0 with a 95 per cent confidence interval of 1·6 to 2·4 mg/kg, where, according to the notations used by Weil, $K=3$, $n=2$, $r=0$, 1, 2, 2, $d=0{\cdot}1505$ and $Da=1{\cdot}42$ mg/kg. When one Morlam wether was added to each group, the LD_{50} was 2·0 (1·7–2·6) mg/kg where $n=3$, $r=0$, 1, 3, 3, and the remaining constants are as before. The dosing and mortality data are summarized in Table 1.

Loss of appetite and diarrhoea occurred at 0·23 mg/kg and higher, and persisted several days after a sublethal dose. Excessive salivation, frothing at the mouth and increased rectal temperatures of 0·9° ($P<0{\cdot}025$) within 12 h were seen at 1·28 but not 0·59 mg/kg. Higher doses caused an increased rate of respiration and increased the temperature 1·5° C or more. Much mucus and some bloody rectal discharge accompanied the scours after days 1–2 among sheep dosed at 1·28–2·00 mg/kg.

All the sheep that died in 15–18 h had massive centrolobular necrosis of the liver. One animal, dosed at 4 mg/kg, which died on day 6, had marked centrolobular necrosis of the liver with necrotic bridges extending to contiguous central veins. This produced an apparent reversal of the normal lobular architecture in that the portal triads, and surrounding normal parenchyma, were now central to a new pseudolobular pattern.

In liver biopsies of surviving sheep on day 7 there was periportal congestion with widely dilated sinusoids and loss of liver cells in these areas. On day 53 there was still focal congestion, along with microfocal liver cell degeneration.

An oral dose of aflatoxin B_1 0·5 mg/kg followed by 1–2 weeks' rest and subsequent redosing at the same level was tolerated by several sheep. This schedule, however, may have contributed to the cause of later death in two wethers. Other schedules therefore need to be examined for converting aflatoxin B_1 to M_1 without endangering the animals.

The susceptibility of sheep to an acute aflatoxin poisoning is thus similar to that observed with other animal species[7]. A limited number of biopsy observations indicate that the animals require several months to recover from sublethal doses of aflatoxin B_1.

We thank Dr George M. Sidwell, Sheep Branch, ARC, USDA, Beltsville, for supplying several sheep and technical advice; and Mr James E. Jackson and Mr Harry E. Zimmerman, jun., for technical assistance.

BERNARD H. ARMBRECHT
WILLIAM T. SHALKOP
LARRY D. ROLLINS

Division of Veterinary Research,
Food and Drug Administration,
Washington DC 20204.

ALBERT E. POHLAND
LEONARD STOLOFF

Division of Food Chemistry
and Technology,
Food and Drug Administration,
Washington DC 20204.

Received August 5; revised December 9, 1969.

[1] Holzapfel, C. W., Steyn, P. S., and Purchase, I. F. H., *Tetrahedron Lett.*, **25**, 2799 (1966).

[2] Masri, M. S., Lundin, R. E., Page, J. R., and Garcia, V. C., *Nature*, **215**, 753 (1967).
[3] Wogan, G. N., *Fed. Proc.*, **27**, 932 (1968).
[4] Rodricks, J. V., *J. Amer. Oil Chem. Soc.*, **46**, 149 (1969).
[5] Briggs, H. M., and Gallup, W. D., *J. Animal Sci.*, **8**, 479 (1949).
[6] Weil, C. S., *Biometrics*, **8**, 249 (1952).
[7] Newberne, P. M., and Butler, W. H., *Cancer Res.*, **29**, 236 (1969).

Vertebrate Fauna from the Lower Trias of Australia

AUSTRALIA'S lack of abundant Permian and Triassic tetrapod vertebrate fauna has long been an enigma to palaeontologists. Both labyrinthodont and reptilian remains are common in South America, Southern Africa and Madagascar, and fairly common in India, while part of a labyrinthodont mandible has recently been reported from Antarctica[1]. Apart from a recent find in Western Australia, and a small collection from Tasmania, the few described Australian labyrinthodonts have been isolated specimens, while a single individual from Tasmania is the only Australian pre-Jurassic reptile.

In 1965 Alan Bartholomai, following up a report of bone in the previously barren Upper Rewan (Lower Trias) red beds south of Rolleston in Central Queensland, found scattered labyrinthodont and thecodont reptilian remains. He added to the collection in 1966 and 1968, and in September 1969 was joined in the field by Alex Ritchie and Kingsley Gregg from the Australian Museum, and by Anne Howie. Most of the remains were found as isolated bone fragments which had weathered out from the Rewan's banded red and lime green mudstone, a small proportion occurred in rare sandstone layers, while the smaller specimens tended to be preserved in purple ferruginous nodules.

Although the greater part of the collection consists of labyrinthodont remains (Fig. 1), it also contains dipnoan tooth plates, a small quantity of thecodont material and some twelve small skulls of the earliest known Australian reptiles. Dissociated remains of at least thirty labyrinthodonts were recovered and four partial skulls were found in association with postcranial material. The most common species is a small parotosaur while a second species has a broad skull with large, laterally placed orbits in the anterior half of the skull roof and prominent tabular horns. Apparently associated with this second skull type are poorly ossified neorhachitomous vertebrae. These specimens are being prepared in Sydney by Anne Howie, who will publish a full description later.

Alan Bartholomai is describing the small reptiles, all of which are preserved in acid resistant nodules and thus present considerable problems of preparation. They appear on initial investigation to be two or three species of lepidosaurian reptile and are provisionally allied with the Paliguanidae (Fig. 2), or short skulled eosuchians, other members of which have been described from South Africa[2,3] and Central Asia[4]. It seems that, apart from the glaring absence (so far) of any trace of the Synapsida, a vertebrate fauna similar to that found in other southern continents did exist in the Lower Trias of Australia, and it is to be expected that a further search will produce more material.

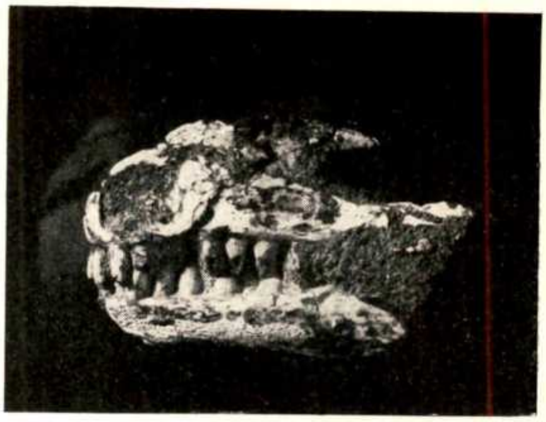


Fig. 2. Left lateral view of the front end of the skull of one of the small ?Paliguanid reptiles (× 2).

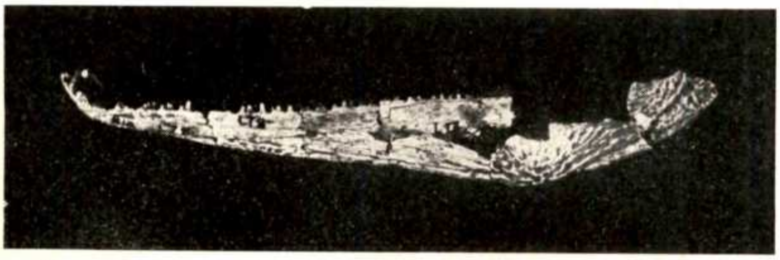


Fig. 1. Left ramus of the lower jaw of a labyrinthodont from the Rewan seen in lateral view (× 4/5).

ALAN BARTHOLOMAI

The Queensland Museum, Brisbane.

ANNE HOWIE

School of Biological Sciences,
University of Sydney.

Received November 28, 1969.

[1] Barrett, P. J., Baillie, R. J., and Colbert, E. H., *Science*, **161**, 460 (1968).
[2] Broom, R., *Rec. Albany Mus.*, **1**, 1 (1903).
[3] Broom, R., *Proc. Zool. Soc.*, 487 (1926).
[4] Koh, T. P., *Bull. Geol. Soc. China*, **20**, 73 (1940).

Sperm Transfer by Spermatophores in *Glossina austeni* Newstead

SPERMATOPHORES have apparently never been reported from higher flies. Davies[1] named three nematocerous families that have spermatophores, and more recently Downes[2] added a probable fourth to the list. Here I describe the spermatophore of *Glossina austeni* (Muscidae, Cyclorrhapha), and briefly discuss the implications of the discovery for current experimental techniques of studying *Glossina* reproduction.

Female flies were 2–4 days old when used for the mating studies, and males were at least 10 days old; observations were made at 25° C. To obtain mated flies, virgin pairs were left undisturbed in glass tubes for 24 h.

The spermatophore is a prominent colourless object in the uterus of recently mated females (Fig. 1). A typical example measured 700 × 500 × 400 μm, but the size varies. By microscopical examination of fresh and fixed specimens, a single closely packed sperm mass is easily seen inside the transparent body of the spermatophore; the mass can be dissected out whole from the surrounding material even when fresh. When viewed through the microscope, sperm are active in spermatophores placed in saline solution. The part of the spermatophore in immediate contact with the rounded sperm mass appears more transparent than remoter parts and is firmer in consistency. It keeps its shape even after the sperm mass is dissected out, but breaks easily on compression. As shown in Fig. 1, the spermatophore and the sperm mass are visible through the uterus wall.

Spermatophores are found adhering to the antero-dorsal wall of the uterus, and project backwards into the uterine cavity to an extent depending on spermatophore size. At this point on the wall is the opening of the fused spermathecal ducts.

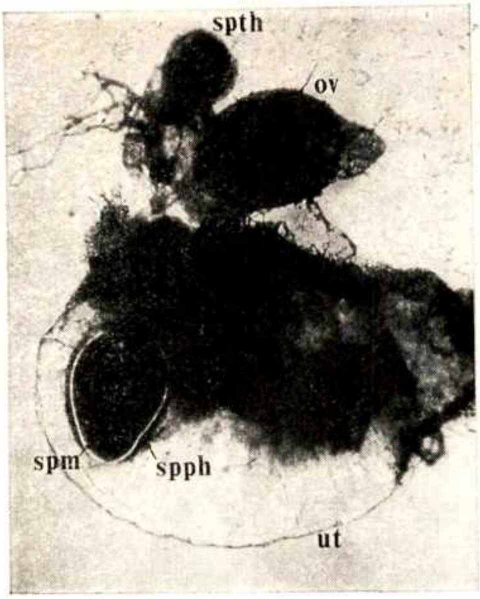


Fig. 1. Photograph of the reproductive system dissected from a recently mated female *Glossina austeni*, showing a spermatophore within the uterus. Lateral view (×25). *Ov*, ovary; *spm*, sperm mass; *spph*, spermatophore; *spth*, spermathecae; *ut*, uterus.

Some dissections of fixed, recently mated female flies have yielded a sperm mass bearing a bifid process that has precisely the shape of the sphermathecal ducts, showing that the sperm mass, though otherwise completely enclosed within the spermatophore, is placed exactly at the entrance to the common spermathecal duct. Whether the bifid process is made of the cuticular lining of the ducts or of fixed material taken intact from within them is not yet known.

Because the accessory glands of the male are partially exhausted after mating, it is reasonable to assume that at least the bulk of the spermatophore is of male accessory gland secretion, though the possibility that the female makes some contribution cannot be ruled out.

Most inseminated females dissected within a few hours of mating were found to have a spermatophore in the uterus, but some had none. To find out what happens to a spent spermatophore, fifty pairs of flies were set up in glass tubes and examined the next day, when twenty-nine tubes were found to contain expelled spermatophores. The spermatophores were somewhat shrunken but rapidly swelled to nearer normal size when placed in water. They contained considerable quantities of sperm; I noted also that spermatophores dissected from the uterus often contained more sperm than could evidently be accommodated within the spermathecae of the recipient female.

It seemed reasonable to suppose that the presence of an expelled spermatophore showed that the female concerned had been inseminated. This idea was tested by dissection, when it was found that all twenty-nine female flies had sperm in the spermathecae. It is worth recording that in two of these females spermatophores were also found in the uterus, showing that sperm transfer may occur twice between the same pair of flies within 24 h. The other twenty-one females were also dissected; eleven had spermatophores in the uterus and, of these, ten had sperm in the spermathecae, with one doubtful case. The remaining ten females had neither a spermatophore in the uterus nor sperm in the spermathecae. There was therefore an excellent correlation between the presence of a spermatophore in either the tube or in the uterus, and the presence of sperm in the spermathecae. I regard this as good preliminary evidence that the expelled spermatophore can be used to show that the female has been properly inseminated. On the other hand, the non-appearance of an expelled spermatophore after a day or two does not show failure of sperm transfer. Females tubed with males for 24 h and then isolated for another 24 h sometimes retained the spermatophore throughout the second period. Probably even these spermatophores would be expelled ultimately, but this point has not been verified.

The discovery of a spermatophore in *Glossina* has certain practical implications. First, the only current method for detecting whether a female has been inseminated (other than by breeding) is to dissect and examine the spermathecae for sperm content[3]. Empty spermathecae are taken to show that the fly is virgin. As already noted, the presence of an expelled spermatophore in the mating tube shows that sperm transfer has taken place, so that dissection is not necessary, and the female can be used for further studies. Second, I noticed that females dissected immediately after mating showed very few sperm in the spermathecae, though a full spermatophore was present in the uterus. There can be little doubt that in such cases sperm had not had time to migrate from the uterus to the spermathecae. Evidently, dissection of the females to determine their mated status would yield more information if the uterus were inspected as well as the spermathecae, especially when the latter seem to be empty.

Glossina morsitans was also found to use a spermatophore. A closer study of sperm transfer in other genera of advanced flies would perhaps reveal similar instances, for it is unlikely that *Glossina* is an isolated case. *Lucilia sericata* does not use one, and Khalifa[4] has listed species of Diptera in which he likewise failed to find spermatophores.

I thank Dr C. T. Lewis for his advice and encouragement, and Dr T. A. M. Nash, Tsetse Research Laboratory, Bristol, for the supply of tsetse flies. I also thank Professor T. R. E. Southwood for providing laboratory facilities and Mr R. H. Williams for taking the photographs. My study of mating in Diptera is supported by the Agricultural Research Council.

J. N. POLLOCK

Field Station, Imperial College,
Ashurst Lodge, Sunninghill,
Ascot, Berkshire.

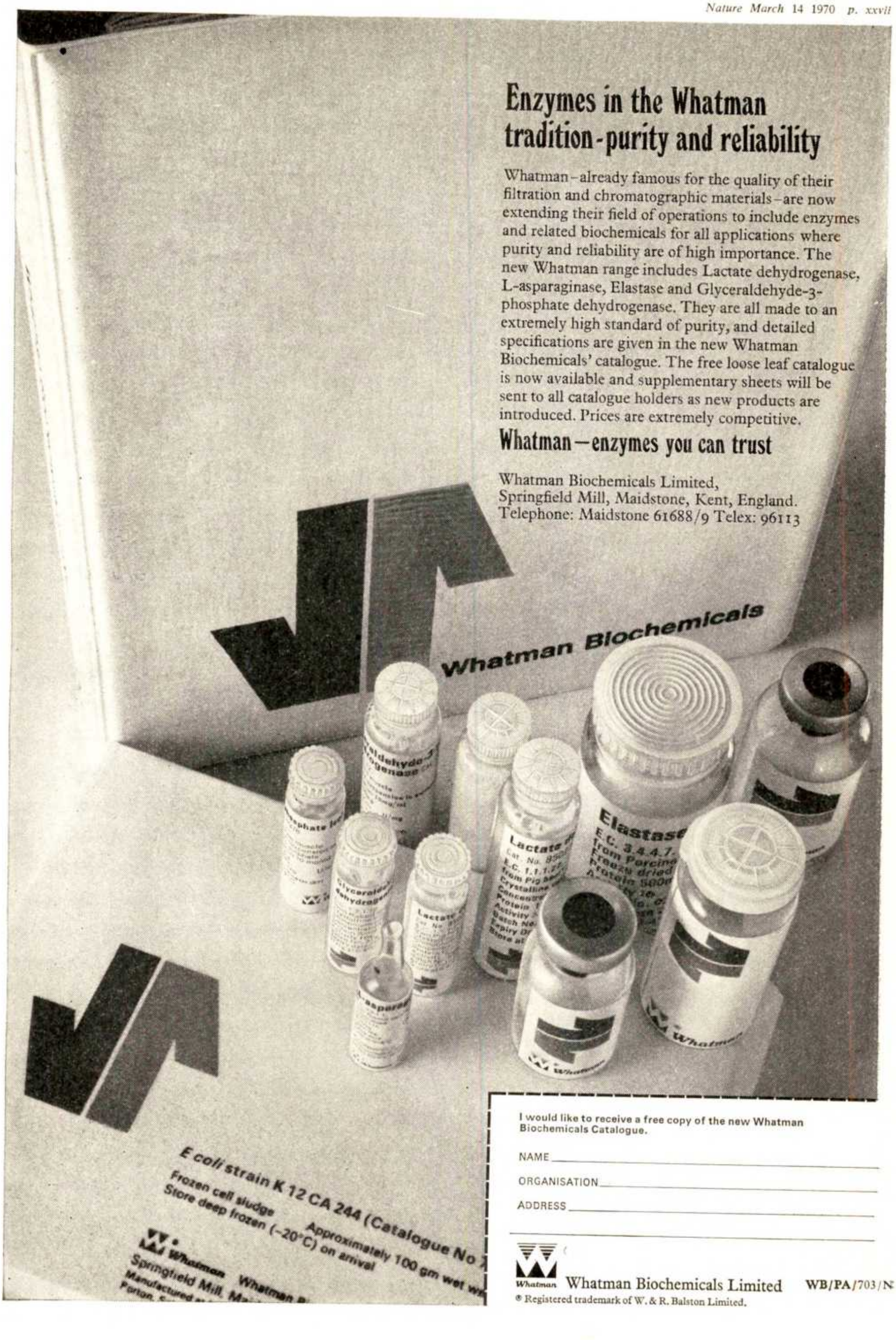
Received December 1, 1969.

[1] Davies, L., *Proc. Roy. Ent. Soc. Lond.* (*A*), **40**, 30 (1965).
[2] Downes, J. A., *Canad. Ent.*, **100**, 608 (1968).
[3] Mellanby, K., *Bull. Ent. Res.*, **27**, 611 (1936).
[4] Khalifa, A., *Trans. Roy. Ent. Soc. Lond.*, **100**, 449 (1949).

Courtship Behaviour of some *Drosophila melanogaster* Mutants

Hotta and Benzer (ref. 1), using loss of phototactic response as an indicator of defective vision in ebony^{11} and tan^{1} mutants of *D. melanogaster*, have shown that these mutants also possess abnormal electroretinograms (ERGs). Certain other body colour mutants, however, such as black, show normal ERGs, suggesting that body colour abnormality and defective ERG are unrelated. Pearce (now Crossley)[2,3] reported that the ebony gene influenced behaviour in a number of ways, not all of which could be related to defective vision. A more detailed analysis of the mutants studied by Hotta and Benzer is therefore of interest.

We have examined the behaviour of ebony^{11}, tan^{1} and black mutants kindly supplied by S. Benzer. The basis for comparison was ebony outcrossed flies (ebony^{0}) analysed previously[2,3] and the wild-type stock A which was used for outcrossing. Male behaviour was recorded on alternate beats of a metronome set at 80 beats/min. The numbers of single pair matings studied were 101 wild-type A; 91 ebony^{0}; 6 ebony^{11}; 17 tan^{1} and ^{10}black.



Enzymes in the Whatman tradition-purity and reliability

Whatman – already famous for the quality of their filtration and chromatographic materials – are now extending their field of operations to include enzymes and related biochemicals for all applications where purity and reliability are of high importance. The new Whatman range includes Lactate dehydrogenase, L-asparaginase, Elastase and Glyceraldehyde-3-phosphate dehydrogenase. They are all made to an extremely high standard of purity, and detailed specifications are given in the new Whatman Biochemicals' catalogue. The free loose leaf catalogue is now available and supplementary sheets will be sent to all catalogue holders as new products are introduced. Prices are extremely competitive.

Whatman – enzymes you can trust

Whatman Biochemicals Limited,
Springfield Mill, Maidstone, Kent, England.
Telephone: Maidstone 61688/9 Telex: 96113

I would like to receive a free copy of the new Whatman Biochemicals Catalogue.

NAME __________

ORGANISATION __________

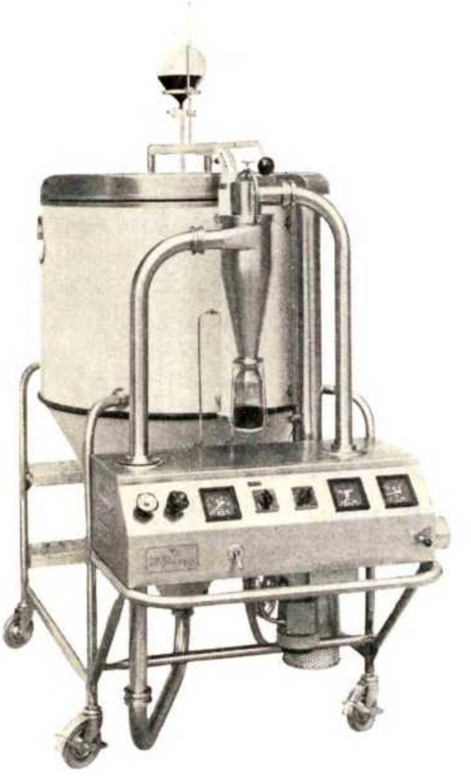
ADDRESS __________

Whatman Whatman Biochemicals Limited WB/PA/703/N

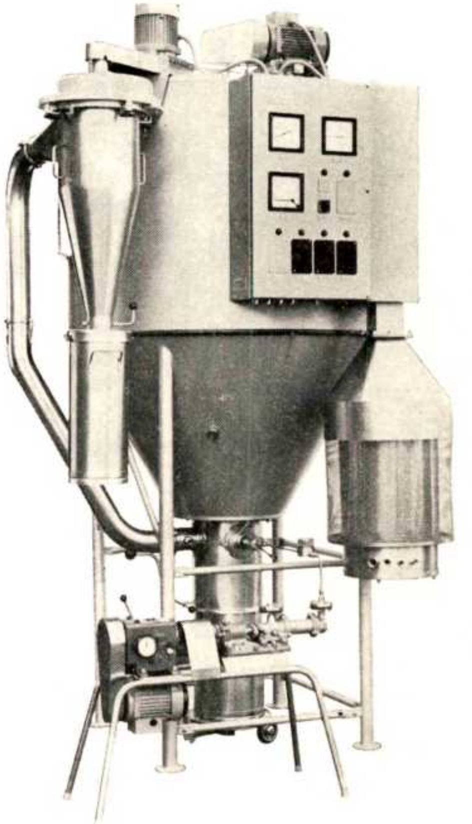
® Registered trademark of W. & R. Balston Limited.

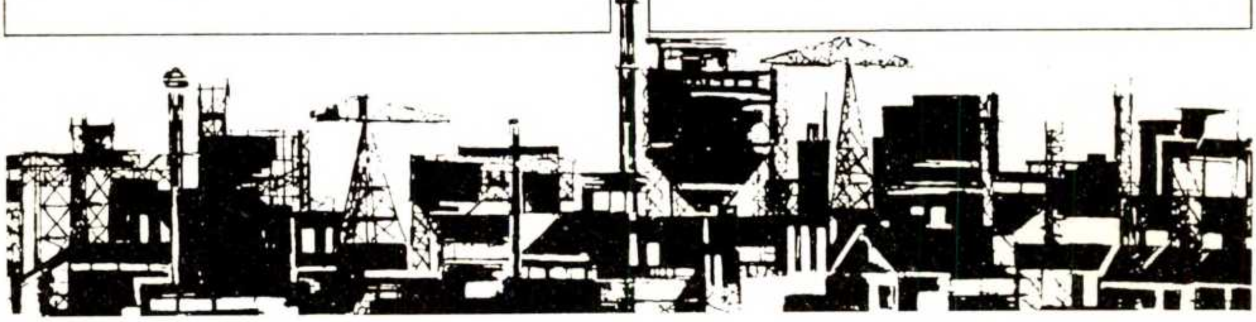
Spray Drying

Mobile Minor plant for research and development



Production Minor for research and semi-industrial drying





NIRO ATOMIZER

Niro Atomizer Ltd., Copenhagen. 305 Gladsaxevej. DK-2860 Soeborg. Denmark. Phone (01) 691011. Cable: Atomniro. Telex: 5603

U. K. Representative: **CORNWELL PRODUCTS Ltd.**
56-60, Hallam Street . London W1N 6 HP . Phone 01-580 5025-6-7 . Cable: Atomniro . Telex: 26 35 33

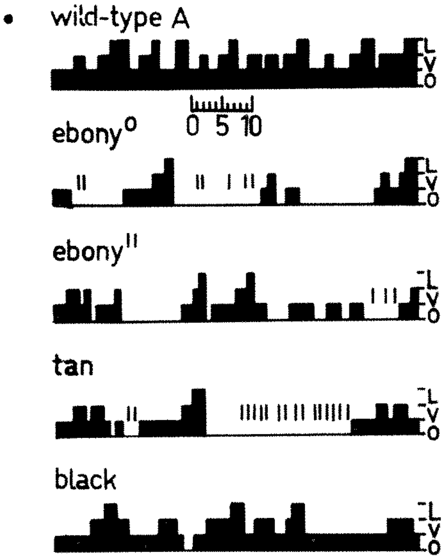


Fig. 1. Diagrammatic representation of typical courtships by different kinds of males courting their own females. Each sequence reads from left to right and the scale shows time units of 1·5 s. The height of shading indicates the courtship element being performed; the three levels are marked on the right (*L*, licking plus attempted copulation; *V*, vibration; *O*, orientation). Gaps in the records represent breaks in courtship. Wing flicks appear on the vibration row as vertical dashes.

Fig. 1 describes the courtship patterns recorded. Normal courtship is shown by wild-type A. The male first taps the female with his fore tarsi; he then faces her (orientation), and next extends one wing and vibrates it (vibration). During or after vibration he licks the female's genitalia (licking) and then attempts copulation. If this attempt is unsuccessful the sequence from orientation is repeated until copulation occurs. Black courtship pattern is essentially similar to that of wild-type A. The courtships of the two unrelated ebony stocks and tan^{1} are similar, but they differ strikingly from wild-type and from black. The main difference concerns breaks in courtship. These occur when a male ceases to follow a female or turns away from her. Wild-type males seldom break off courtship. Black males break off courtship more than wild-type but very much less than the other three forms studied. Another difference is that during a break ebony^{0}, ebony^{11} and tan^{1} males run in a zigzag path opening and closing their wings as they run (wing flicking). Tan^{1} males wing flick more than ebony^{0} and ebony^{11} males. Neither wild-type nor black males wing flick during breaks. Further analysis is incomplete, but the records so far suggest that, as in ebony^{0}, the courtship of these mutants contains fewer of the highly stimulating elements of vibration and licking than the courtship of wild-type A males. All the mutant males take longer to mate than wild-type males because of their inferior courtship. There are no obvious differences in female behaviour.

To what extent is the abnormal behaviour of ebony^{11} and tan^{1} related to defective ERG ? Behavioural observations suggest that courtship breaks occur because ebony^{0}, ebony^{11} and tan^{1} males do not use visual cues when courting. For example, all these males, unlike wild-type and black, make mistakes during courtship by licking and attempting to copulate with a female's head. The female then decamps and initiates a courtship break. Similarly, Crossley[3] showed that courtship of ebony^{0} males is uninfluenced by darkness but in contrast wild-type A males in red light (which to flies is darkness) behave as ebony^{0} males, making licking errors, breaking off courtship and zigzag running during breaks. The behavioural abnormalities shared by tan^{1} and ebony mutants probably stem, therefore, from impaired vision caused by changes in that part of the eye concerned with the ERG. Reduction in vibration and licking, however, is probably not the result of impaired vision, for ebony and tan mutants vibrate and lick in the dark and black, which has a normal ERG, also shows reduced vibration and licking. It is therefore unlikely that all the abnormal behaviour of tan and ebony mutants will be found attributable to their defective ERGs.

It is important to note that Rendel's[4] and Jacobs's[5] findings that ebony flies mate better in the dark than in the light are not supported here. Ebony^{0} did not mate better in the dark than in the light, so presumably selection of genes favouring improved mating in the dark had occurred in the stocks used by the earlier workers.

It may be concluded that certain courtship abnormalities are good indicators of impaired vision. Mutation may influence phototactic score or courtship behaviour or both without changing the visual system, for example, by changing activity. It is therefore suggested that in identifying behavioural mutants with defects in their visual system a more complete analysis than simple measurement of phototactic score is worthwhile.

S. CROSSLEY

Department of Psychology,
Monash University,
Clayton, Victoria, Australia.

E. ZUILL

Department of Zoology,
University of Oxford.

Received September 22; revised December 23, 1969.

---

[1] Hotta, Y., and Benzer, S., *Nature*, **222**, 351 (1969).
[2] Pearce, S., *Anim. Behav.*, **8**, 232 (1960).
[3] Crossley, S., thesis, Oxford Univ. (1963).
[4] Rendel, J. M., *Evolution*, **5**, 226 (1951).
[5] Jacobs, M. E., *Ecology*, **41**, 182 (1960).

Brassins—a New Family of Plant Hormones from Rape Pollen

WE report the discovery of new hormones, termed brassins, in rape pollen (*Brassica napus* L.). Following the general practice of designating gibberellins as plant hormones, we speak of brassins as hormones because they are specific translocatable organic compounds isolated from a plant and have induced measurable growth control when applied in minute amounts to another plant.

The chromatographic methods we used in purifying hormones from rape pollen were as follows: 1·5 g of finely ground air-dried rape pollen was extracted with 18 ml. of anhydrous ether for 30 min at room temperature. Decantate from this extraction was reduced in volume (40° C) and the concentrate was streaked across a 20 × 20 cm silica gel chromatographic plate 2 mm thick (Brinkmann). The plate was developed in benzene–methanol–acetic acid (45 : 8 : 4). The zone containing activity was located with the second internode assay[1] and visualized by spraying the plate with a 10 per cent alcoholic solution of phosphomolybdic acid followed by 15 min at 100° C. A zone of silica gel containing the activity (bounded by R_f 0·35 and 0·45) was removed, ground and put in a centrifuge tube with 10 ml. of absolute ethyl alcohol. We used the alcohol to avoid formation of condensation products[2]. The silica gel was stirred with the alcohol for 30 s at room temperature and separated by centrifugation, and the decantate was recovered. The extraction process was repeated and the second decantate added to the original one. The combined decantate was evaporated to dryness (40° C) in an evaporating dish. The residue was dissolved by swirling repeatedly five small portions of anhydrous ethyl ether (0·2–0·4 ml.) one at a time. The volume of the five combined extractions was reduced and the sample spotted across half the width of a silica gel plate of the type used before. The plate was developed with isopropyl

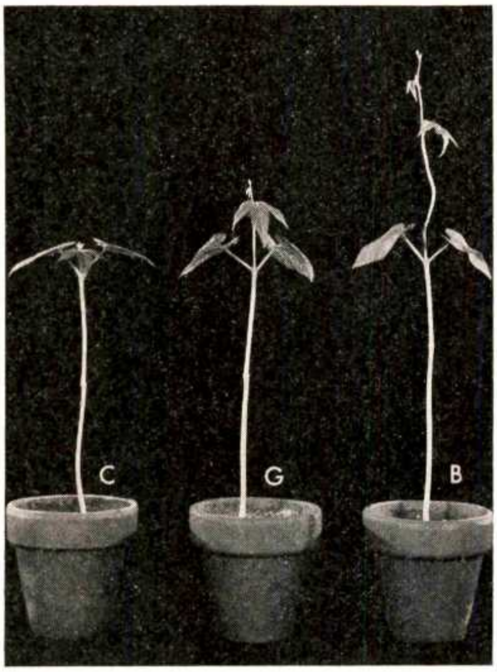


Fig. 1. Elongation of plants treated in the second internode assay with 10 μg of brassins (*B*); 0·001 μg of gibberellic acid (*G*); and the fractionated lanolin carrier alone (*C*). Duration of treatment 4 days.

ether and acetic acid (190 : 10). The activity remained at R_f 0·0. Silica gel in a zone that extended approximately 2 mm on each side of the zero R_f line was removed and extracted with absolute alcohol as described. The purified extract was evaporated to dryness, subjected to vacuum to remove traces of solvents and stored at 4° C for further studies.

The oily product obtained, termed "brassins", was assayed by the bean second internode test. Collectively the brassins induced very marked elongation of both second and third internodes of the intact plants when applied at the rate of 10 μg per plant. For example, second internodes grew an average of 155 mm during four days immediately after treatment, while controls treated with the fractionated lanolin alone grew only 12 mm. During nine days immediately after treatment, the third and fourth internodes grew an average of 8·9 and 11·7 times respectively more than the controls (Fig. 1). Further, the response to brassins was histologically different from the response induced by gibberellic acid.

The nuclear magnetic resonance spectrum of the brassins in deuterochloroform exhibited a pattern characteristic of fatty acid esters. The signals from internal tetramethyl silane (delta scale) appeared at 0·9 for a terminal methyl group split into triplet; a strong CH_2 (straight chain) absorption at 1·26, as a singlet; and other CH_3, CH_2 groups attached to the double bonds at 1·55 and 2·0 p.p.m., respectively. The resonance signal at 3·8 denoted the CH_2 attached to oxygen and the olefinic protons showed a multiplicity character at 5·3 p.p.m. There seemed to be no exchangeable protons, suggesting the absence of carboxyl and hydroxyl groups. The appearance of absorption at about 3·8 p.p.m. suggested that the brassins have a glyceride structure.

Analysis of the brassins by combined gas chromatography and mass spectrometry showed that the product contained several components with molecular weights ranging from 250 to 580. As well as the fragmentation of long chain hydrocarbon derivatives, we observed two characteristic and intense peaks at m/e 259 and 577 for two components. Furthermore, separations by 'Sephadex *LH*-20' gel chromatography with methylene dichloride resulted in five products that could be distinguished by ultraviolet absorption at 254 nm[3,4]. Each fraction proved to be active.

Strong ultraviolet absorption at 207 and 226 nm suggests unsaturation in all the components. Our data show that brassins are a family of new plant hormones that appear to have a glyceride structure.

We are investigating the fractionation of the five-component family of brassins and the structural determination of each compound. We did not determine a dosage response curve using the brassins collectively, for data based on response to the entire family of compounds would have relatively little meaning compared with response curves obtained from individual components of the brassin family.

Fats and similar compounds have been reported to control plant growth in some conditions (refs. 5, 6 and bibliography in ref. 6).

Pollen from plants other than rape may also contain hormones that are similar to brassins. For example, pollen from alder, *Alnus glutinosa* (L). Gaertn., was extracted and chromatographed as described above along with a parallel sample of pollen from rape. After simultaneous development in the same tank both plates showed similar fluorescence. The activity of these bands was typical of that obtained from all previous tests with brassins. The hormones obtained from alder and rape pollen thus seemed similar when compared biologically and chromatographically. Because the alder pollen was hand collected and not taken from bees, the brassins must be of plant origin.

We thank Marjorie E. Drowne and Bruce A. Sidwell for help with chromatographic separations; Norman E. Beckett for growing the necessary test plants; and L. N. Standifer, S. E. McGregor, and C. B. Reed for providing pollen.

J. W. MITCHELL
N. MANDAVA
J. F. WORLEY
J. R. PLIMMER

Crops Research Division,
Agricultural Research Service,
US Department of Agriculture,
Beltsville, Maryland 20705, USA.

M. V. SMITH

Department of Agriculture,
Ontario Agricultural College,
University of Guelph,
Guelph, Ontario, Canada.

Received October 6; revised December 23, 1969.

[1] Mitchell, J. W., and Livingston, G. A., *Methods of Studying Plant Hormones and Growth-Regulating Substances*, Agriculture Handbook No. 336 (US Government Printing Office, Washington, 1968).

[2] Mitchell, J. W., Mandava, N., Worley, J. F., and Drowne, M. E., *Nature*, **223**, 1386 (1969).

[3] Joustra, M. K., *Protides Biol. Fluids*, **14**, 533 (1967).

[4] Determan, H., *Gel Chromatography* (Springer-Verlag, New York, 1968).

[5] Hayashi, F., and Rappaport, L., *Plant Physiol.*, **41**, 53 (1966).

[6] Stowe, B. B., and Hudson, V. W., *Plant Physiol.*, **44**, 1051 (1969).

Localization of Antigens associated with the Pollen Grain Wall by Immunofluorescence

MUCH of the protein detectable in short-term leachates from intact pollen grains[1] is probably released from the inner layer of the wall, the cellulosic intine, where protein and enzyme activity have been detected by cytochemical methods[2,3]. Extracts from intact grains are usually used in tests for pollen allergenicity, and we have considered the possibility that the proteins principally responsible come from the wall sites and not from within the cells. We report here the results of a study of the antigens present in leachates in which we have shown by immunofluorescence that they are indeed contributed largely from the intine and other wall locations.

We tested pollen from *Gladiolus gandavensis* Van Houtte cv. and *Ambrosia trifida* L. Earlier work showed that the relative thinness of the exine of gladiolus pollen facilitated the detection and localization of intine held proteins; *Ambrosia* (ragweed) pollen was selected for test because of its importance as a human allergen in the United States. The gladiolus pollen was taken from fresh flowers and partly dried at 2°–5° C, and that of ragweed was obtained from Greer Drug and Chemical Corp. One gram samples of intact pollen were extracted with 4–8 ml. of 0·85 per cent NaCl, adjusted to *p*H 7·5, in roller tubes for 24 h at 2° C. Rabbits were immunized with freshly prepared extracts in complete Freund adjuvant. Serum was collected on day 28 after three weekly injections. Double diffusion in agar showed that the extracts contained several antigens, and at least seven bands could be distinguished on immunoelectrophoresis. Faint cross-reactions occurred between the two pollens, but most of the antigenicity seems to be species specific.

30 min at room temperature, rinsed in buffer and mounted for observation with the fluorescence microscope.

RITC-labelled goat anti-rabbit globulin (7S fraction) was used in the indirect method. Samples of the unlabelled unfractionated or partly purified pollen leachate antisera were layered on to cryostat-cut sections, and the preparation incubated at room temperature for 30 min. The excess serum was then removed by rinsing with saline for 5–10 min, and the RITC-labelled goat anti-rabbit serum (globulin concentration, 1·5–2·0 mg/ml.) spread as before and left for 30–60 min at room temperature. The slides were then rinsed thoroughly with buffer and mounted. Control sections received RITC-labelled rabbit anti-guinea-pig serum direct, or rabbit anti-guinea-pig serum followed by RITC-labelled goat anti-rabbit serum.

Both methods gave good localization of antigens in the sectioned grains with appropriate antisera, but specificity was higher with the indirect technique. The clarity of the intine localization in gladiolus can be seen in Fig. 1. In the more complicated wall of ragweed pollen the intine was also labelled, and there was some indication of concentration of antigens at the apertures.

Immunofluorescence gave little evidence of antigens outside of the exine of gladiolus pollen, but the surface materials of ragweed pollen did give a reaction. These are derived from the tapetum, and are therefore products of the diploid parent and not of the haploid spores. The sporophytic origin of some of the antigens borne by the mature pollen grains was indicated also by a reaction between the tapetal plasmodium and labelled ragweed pollen–leachate antiserum in young anthers sectioned before maturation of the wall.

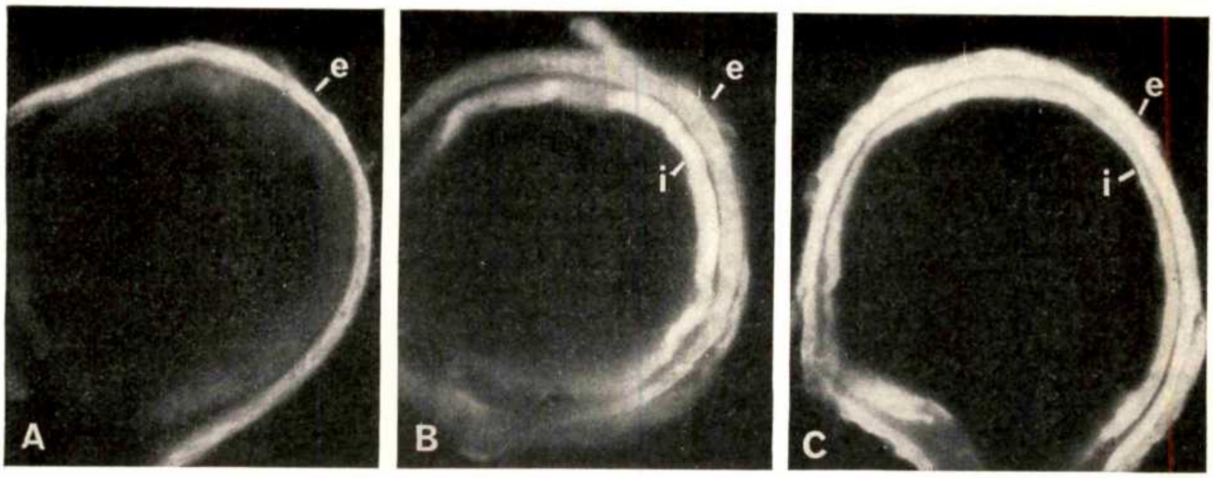


Fig. 1. Fluorescence photomicrographs (Bausch and Lomb 7–37 excitation filter, T2 barrier filter) of sections of unfixed pollen grains of *Gladiolus gandavensis* cut at −15° C. *A*, Control, treated with RITC-labelled rabbit anti-guinea-pig serum. The exine shows native fluorescence (*e*); the intine is barely visible. *B*, Direct method, RITC-labelled rabbit anti-gladiolus pollen–leachate serum. The exine shows native fluorescence, and the intine (*i*) is now heavily labelled. This section is not equatorial so that the thickness of the wall layers relative to the lumen is exaggerated. *C*, Indirect method, unlabelled rabbit anti-gladiolus pollen–leachate serum followed by RITC-labelled goat anti-rabbit globulin serum. Again the intine is heavily labelled. The micrographs were made from original colour transparencies in which a distinction could be made between the greenish fluorescence of the exine and the orange–red fluorescence of the RITC-labelled precipitins. Protoplasm is present in all three grains illustrated, but its fluorescence was too slight to be apparent in these prints. × *c*. 950.

The antibody globulin was partly purified with ammonium sulphate[4], and the concentration was estimated by absorption at 285 nm against a bovine γ-globulin standard. The concentration was adjusted to 10–20 mg/ml. for use. The most commonly used label, fluorescein isothiocyanate, proved unsatisfactory because its fluorescence matched the natural fluorescence of the exine too closely. Rhodamine B isothiocyanate (RITC) (Nutritional Biochemicals Corp.), which gives an orange–red fluorescence, provided better contrast.

Both direct and indirect methods of antigen localization were used. For the former, the labelling method of Cherry *et al.*[5] as modified by Goldman[4] was adopted. For use in controls, rabbit anti-guinea-pig serum was labelled in the same manner as the pollen diffusate antisera.

Pollen grains were sectioned in a cryostat to minimize loss of diffusible material[2,3], and the sections were brought quickly up to room temperature. The labelled antiserum, full strength or diluted with 0·02 M phosphate buffer at *p*H 6·5, was pipetted on to the sections and spread rapidly. The slides were then incubated in a moist atmosphere for

Various enzymes—for example, acid phosphatase and ribonuclease—are common to the intines of the pollens of gladiolus and ragweed[2,3]. The mixture of antigens present in leachates presumably includes these enzymes, but it remains possible that some of the wall-associated antigenic material is concerned with incompatibility reactions.

In emphasizing the wall sites as the source of the rapidly leached antigens, we do not suggest that saline extraction does not release proteins and other materials from within the vegetative cell of the pollen grain, but merely that these may not contribute very greatly to the total antigenicity of extracts.

This work was supported by the Graduate School of the University of Wisconsin and the US National Science Foundation.

R. B. Knox*
J. Heslop-Harrison

Institute of Plant Development,

C. Reed

Department of Medicine,
University of Wisconsin,
Madison.

Received October 6; revised December 4, 1969.

---

* Permanent address: Department of Botany, Australian National University, Canberra.

[1] Stanley, R. G., and Linskens, H. F., *Physiol. Plant.*, **18**, 47 (1965).
[2] Knox, R. B., and Heslop-Harrison, J., *Nature*, **223**, 92 (1969).
[3] Knox, R. B., and Heslop-Harrison, J., *J. Cell Sci.*, **16**, 1 (1970).
[4] Goldman, M., *Fluorescence Antibody Methods* (Academic Press, New York and London, 1968).
[5] Cherry, W. B., Goldman, M., and Carski, T. R., *US Public Health Service Publ. 729* (1960).

Hydramoeba hydroxena (Entz) discovered on *Hydra graysoni* sp. nov.

While a population of hydras from the outflow of Hodson's Tarn in north Lancashire, England, was being examined, several specimens were observed to be parasitized by *Hydramoeba hydroxena* (Entz). A full taxonomic description has been compiled for the hydra concerned. It is to be called *Hydra graysoni* and is closely related to the group of hydras which possess a plano-convex embryotheca[1]. Although the hydras were examined periodically since September 1968, it was only in November 1969, when they went into a depression after 3 weeks in the laboratory, that the amoebae were discovered.

The amoebae were examined both live and after staining with iron haematoxylin. They generally resemble *Hydramoeba hydroxena*, as described already[2-5], and most closely resembles Ito's description, particularly in size and nuclear detail (Fig. 1). When at rest on the tentacles or column of the hydra, the so-called verrucosa-type[5] varies from 36 to 65 µm long. When detatched from the host the proteus-type[5] varies from 52 to 144 µm long. After being detached for a few minutes the proteus-type often turns into the limax-type[5], which varies from 90 to 220 µm long. Up to two nuclei have been observed, although only one is usually present. This is spherical and ranges from 8 to 17 µm in diameter. Polygonal crystals present in the cytoplasm vary from 1 to 3 µm across and granules can be seen which are up to 1 µm in size. Up to two contractile vacuoles have been seen, although there is usually only one, which is up to 23 µm in diameter.

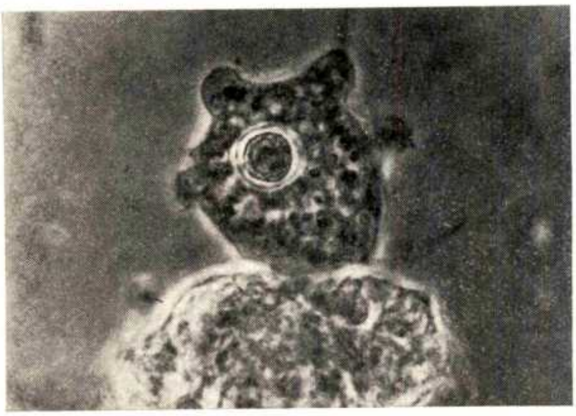


Fig. 1. A stained specimen of *Hydramoeba hydroxena* on the tip of a tentacle of *Hydra graysoni*. (× 500.)

This amoeba has been found to be a serious pathogen on several different species of *Hydra*. Infection experiments were carried out in culture water, *p*H 7·5 – 7·8, at 20° C. The hydras were not fed during the course of the infection. *Hydra graysoni* and *Hydra oligactis* (Pallas) died within 3 days. *Chlorohydra viridissima*, *Hydra pirardi* and *Hydra littoralis* died within 4 days. *Hydra pseudoligactis* died within 5 days and *Hydra vulgaris* (Pallas) was the most resistant to the parasite, surviving for 12 days. Many hydra populations have been examined from all over Britain by myself and my colleagues, and Hodson's Tarn is the only place, except for Esthwaite Lake[6], where *Hydramoeba hydroxena* has been found. The amoebae from both these localities are morphologically identical.

Thomas R. A. Maxwell

Department of Zoology,
King's College,
London.

Received December 15, 1969.

[1] Hadley, C., and Forrest, H., *Amer. Mus. Nov.*, No. 1423, 1 (1949).
[2] Entz, G., *Arch. Protistenk.*, **27**, 19 (1912).
[3] Reynolds, B. D., and Looper, J. B., *J. Parasitol.*, **15**, 23 (1928).
[4] Rice, N. E., *J. Protozool.*, **7**, 151 (1960).
[5] Ito, T., *Sci. Rep. Tohoku Univ.*, Fourth Ser. Biol., **18**, 205 (1949).
[6] Grayson, R. F., and Hayes, D. A., *J. Brit. Naturalists Assoc.*, Spring, 1 (1968).

Umbellulidae Distribution extended in the Atlantic

On a recent Atlantic cruise of the USNS Kane, 1,540 exposures of the sea bed were obtained at 77 stations (Fig. 1). The Alpine multi-exposure camera system that was used consisted of a 35 mm camera fitted with a 33 mm *f*/11 Hopkins water corrected lens actuated by a bottom contact switch. At a typical station, twenty contacts would be made in the course of an hour, at locations varying with the drift of the ship.

The most remarkable forms of life photographed were organisms of the Phylum Cnidaria (Coelenterata) Family Umbellulidae (Fig. 2). Umbellulidae have previously been reported from the Atlantic by the Challenger Expedition (1873–1876)[1]. Dredged samples from this expedition form the basis for knowledge of the species of this family.

The basic appearance of the Umbellulidae is shown clearly in Fig. 2. The tall slender stalk (estimated height 1 m) is topped by a cluster of large flower-like polyps. The polyps are deep red, shading towards orange-red or purplish-red, each with eight long pennate tentacles[2,3]. The tentacles are used for feeding and defence. The base of the Umbellula is a long, hollow, muscular bulb which embeds itself into the soft-mud bottom and anchors the animal colony to the ocean floor[2,3].

Specimens of Umbellulidae have been found[2,3] at depths of 3,400–5,000 m. Fig. 2 comes from the lower continental rise of Africa at 05° 08·7′ N, 18° 06·5′ W at a depth of 5,300 m. Other stations that gave Umbellulidae sightings are shown in Fig. 1.

The sea floor at the camera station of Fig. 2 is fairly smooth. There are many scattered holes and small oblate mounds with a poorly developed bottom lineation. Occasional holes are distinguished by irregular groove patterns radiating from the centres. A sediment core taken at the camera station reveals the top 20 cm to be a greyish-brown fossiliferous lutite. The bottom water temperature is 1·78° C (unpublished results of B. C. Heezen and W. Fedukowicz). Other Kane camera stations that gave Umbellulidae sightings reveal a similar ocean bottom.

In Fig. 2 the specimen seems to be standing vertically, because of the camera's angle. A subsequent exposure

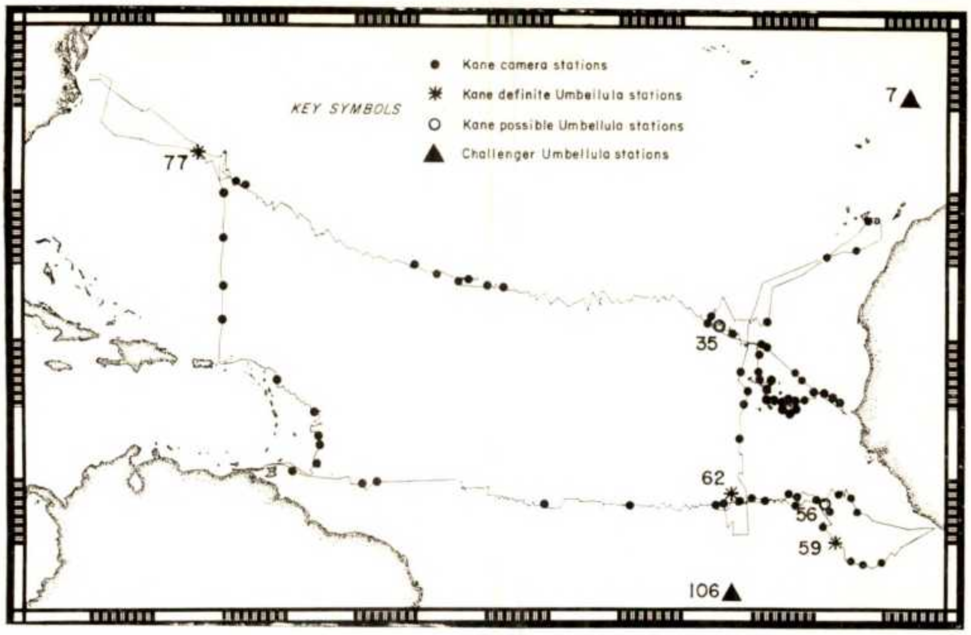

KEY SYMBOLS
● Kane camera stations
✱ Kane definite Umbellula stations
○ Kane possible Umbellula stations
▲ Challenger Umbellula stations
77
7
35
62
56
59
106

Fig. 1. Track and area covered by Kane-9 cruise. The principal characteristics of the camera stations are shown.

taken from a side view shows that the colony or specimen is leaning toward the camera at an angle of about 30°. The lean or bend probably results from the weight of the large cluster of polyps at the top. According to compass observations, the Umbellula is leaning to the west, perhaps showing bottom current movement in the area. The photograph gives no information about the current, although water movement must be present to sustain life processes.

The Kane photographs have extended the known range and concentration of the family Umbellulidae in the Atlantic.

I thank Mr L. Johnson for his help and technical assistance; Mrs R. Carson at the Smithsonian Oceanographic Sorting Center for identification; and Captain J. McCandless, the crew of the USNS Kane and participating NAVOCEANO personnel.

W. JAHN

US Naval Oceanographic Office,
Code 038—Global Ocean Floor Analysis
and Research Center,
Chesapeake Bay, Maryland.

Received November 3, 1969.

[1] Kolliker, A. V., *Reports on the Scientific Results of the Voyage of HMS Challenger, 1880*, 1.

[2] Buchsbaum, R., and Milne, L., *The Lower Animals*, 103 (Doubleday, New York, 1967).

[3] Miner, R. W., *Field Book of Sea Shore Life*, 188 (G. P. Putnam and Sons, New York, 1950).

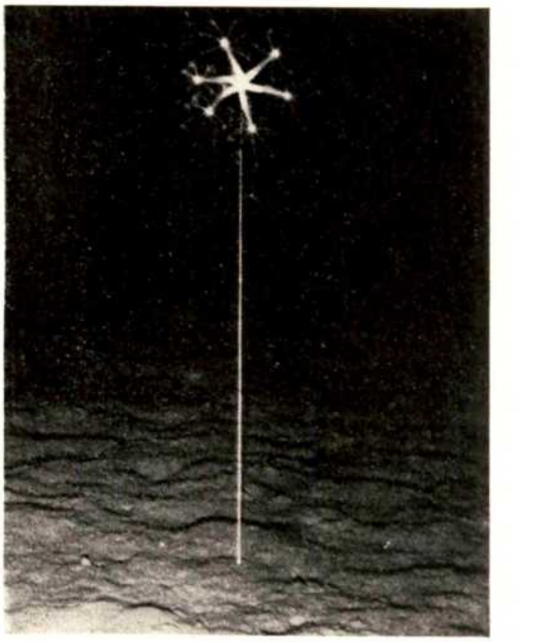


Fig. 2. Ocean bottom photograph of an organism belonging to the family Umbellulidae. Taken at station K-59, latitude 05° 08·7′ N, longitude 18° 06·5′ W, depth 5,300 m, date September 7, 1968.

Isotopic Evidence for Diminishing Supply of Available Carbon during Diatom Bloom in the Black Sea

PLANKTON samples taken during the cruise of R/V Atlantis II in the Black Sea during March and April 1969 were analysed for their $^{13}C/^{12}C$ ratios. The results showed that over a large area of the Black Sea the plankton had uniform $\delta^{13}C$ values near -23 parts per 1,000. A sample from a very pronounced diatom bloom, however, was enriched in ^{13}C by 1·5 parts per 1,000. This isotopic shift was apparently produced by the organisms' accelerated carbon fixation which had decreased the available supply of inorganic carbon.

Samples were collected at seven stations (Fig. 1) with vertical tows of an open No. 2 net (mesh aperture 0·366 mm) through the upper 100 m of water. Although the net is designed primarily for zooplankton, appreciable amounts of phytoplankton were present in all samples. The samples were treated with dilute hydrochloric acid to remove carbonate and combusted to CO_2 in a vacuum line similar to that described by Craig[1]. The isotopic analyses were carried out on a high-precision isotope ratio mass spectrometer[2]; reproducibility of the entire procedure is $\pm 0{\cdot}1$ parts per 1,000. The analytical results together with other pertinent data on the samples and stations are shown in Table 1. $^{13}C/^{12}C$ ratios are reported in the δ-notation* and are relative to the PDB standard[3].

$$^{*}\delta^{13}C\ (^{0}/_{00}) = \left(\frac{(^{13}C/^{12}C)\ \text{sample})}{(^{13}C/^{12}C)\ \text{standard}} - 1\right) \times 1{,}000$$

Table 1. HYDROGRAPHIC AND ANALYTICAL DATA

1 Sample No.	2 AII-49 station	3 Date mo/day (1969)	4 Time of collection	5 °C Temp. range 0–100 m	6 °/₀₀ Sal. range 0–100 m	7 Surface *p*H	8 Range of δ^{13}C inorg. 0–100 m	9 Z/P*	10 δ^{13}C plankton
P4	1,435	3/25	Sunset	7·5–8·0	18·30–19·26	8·04	0·0 to −0·1	10^1	−23·1
P2	1,436	3/26	Sunset	7·8–8·5	18·62–20·78	7·98	0·0 to −2·4	10^1	−23·2
P8	1,438	3/28	Noon	7·5–7·2	18·21–18·50	8·05	0·2 to −0·4	10^0	−23·1
P5	1,439	3/28	Sunset	8·3–7·8	18·49–19·69	8·00	n.d.	10^0	−23·4
P6	1,442	3/31	Sunset	7·3–7·2	18·33–18·45	8·10	0·2 to −0·1	10^{-2}	−22·0
P9	1,442	3/31	Sunset	7·3–7·2	18·33–18·45	8·10	0·2 to −0·1	10^2†	−23·3
P11	1,442	3/31	Sunset	7·3–7·2	18·33–18·45	8·10	0·2 to −0·1	10^{-4}	−21·8
P3	1,445	4/1	Sunset	7·2–8·4	18·72–20·74	7·97	n.d.	10^1	−23·7
P1	1,445	4/2	Sunrise	7·2–8·4	18·72–20·74	7·97	n.d.	10^1	−24·1
P7	1,453	4/6	Midnight	7·2–7·0	18·33–18·45	8·04	n.d.	10^2	−22·4

* Order-of-magnitude estimate of the volume ratio zooplankton/phytoplankton in the fresh sample.
† Zooplankton separated from very large sample of diatom bloom.

After a first inspection of the data it was noted: (1) There is a remarkable uniformity in the δ^{13}C values of plankton over great parts of the Black Sea. The average value near −23 parts per 1,000 is typical for plankton from cold waters of a *p*H near 8·0 and relatively high content of free CO_2 (ref. 4) if the δ^{13}C of the total dissolved inorganic carbon lies near 0 parts per 1,000 (column 8 of Table 1). (2) With the exception of the samples from Station 1442 the varying ratio of zooplankton to phytoplankton does not affect the value of δ^{13}C—this has also been observed by others in the Atlantic and Pacific Oceans[5,6]. (3) There may be a significant difference in δ^{13}C between the sunset and sunrise tows at Station 1445. The difference is in the direction to be expected from the isotope fractionation between respired carbon and cell carbon[7].

The δ^{13}C values obtained on the plankton from Station 1442 are most interesting. The total yield of this tow was approximately 100 times that of all others. Although there were no apparent signs of it at the surface, there was a strong bloom of *Chaetoceros curvisetum* in the upper 100 m. The composite sample which contained a maximum of 1 per cent zooplankton gave a δ^{13}C value of −22·0 parts per 1,000. Zooplankton alone (almost all of which was *Calanus* sp.) yielded a δ^{13}C value of −23·3 parts per 1,000, identical to most other composite samples. In contrast, pure phytoplankton from this sample had a value of −21·8 parts per 1,000.

The greater ^{13}C content of the phytoplankton can best be explained as being caused by the high demand of carbon during the bloom. Because the cells cannot directly utilize the much more abundant bicarbonate, their carbon demand must be met by the dissolved free carbon dioxide which is continuously replenished through shifts in the equilibria of the aqueous carbonate system. As the rate of carbon fixation by photosynthesis approaches and eventually surpasses the rate of isotopic equilibration in the process of generation of new carbon dioxide, the fractionations between the cell carbon and the carbon dioxide and between the carbon dioxide and the bicarbonate decrease[4]. While the total carbon supply does not limit the development of the bloom here or in any other marine situation encountered[8], these results are an example of a natural situation in which the carbon demand of a developing algal bloom significantly decreases the supply of free carbon dioxide available for photosynthesis.

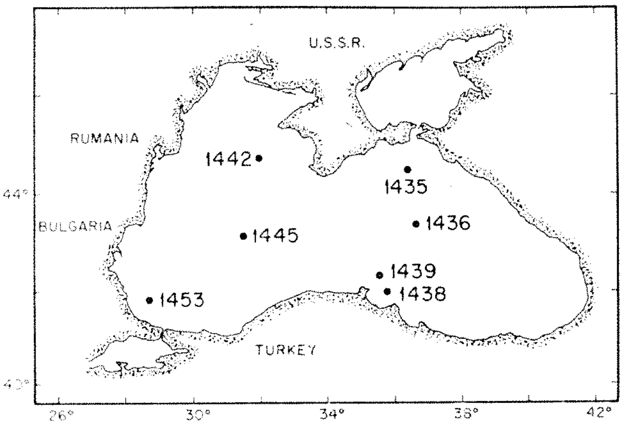


Fig. 1. Locations of plankton tows in the Black Sea.

The fact that the carbon of the zooplankton associated with the phytoplankton bloom is lighter by 1·5 parts per 1,000 can be attributed to the brevity of a diatom bloom relative to the time required for a complete metabolic turnover of the cell carbon in the zooplankton[8-10]. At the time of the sampling, the bloom had apparently not existed for a sufficient length of time to have allowed complete isotopic "assimilation" by the zooplankton. The carbon isotope content of the grazers was still similar to pre-bloom conditions typical for the Black Sea as revealed by the other samples analysed by us.

This inference was checked by an order of magnitude calculation. The carbon content of the catch was 2 g (30 per cent of dry weight). The volume of water which went through the net was estimated at 4 m^3 and contained about 160 g total inorganic carbon in solution. Of this carbon, roughly 2·5 g was free carbon dioxide (calculated using data of Alekin[11] and extrapolating values of Buch[12] and Saruhashi[13] to 10·2 parts per 1,000 chlorinity at 7·2° C and a *p*H of 8·0). The amounts of cell carbon and inorganic carbon available for photosynthesis were thus similar in the volume of water occupied by the bloom. Even with conservative estimates for the generation time of cells, in the advanced stages of the bloom the free CO_2 in the water must have been replaced at least once a day through the steps of association and dehydration or through direct dissociation of HCO_3^- ions. From values of Buch[14] one may extrapolate that it takes several minutes for these steps to proceed to 90 per cent completion in Black Sea conditions. Reaction rates for isotopic equilibria, however, are lower by orders of magnitude and the δ^{13}C of the newly formed carbon dioxide is bound to be greater than its equilibrium value of −8 parts per 1,000 at the prevailing temperature[15]. The dynamic situation of a bloom of this magnitude thus precludes isotopic equilibrium and results in an isotope content of the cells which is significantly closer to that of the inorganic carbon pool.

I thank Drs G. D. Grice and E. M. Hulburt for identifying the plankton and Dr R. R. L. Guillard for making a cell count on sample P-6. Mrs E. Ross provided valuable laboratory assistance. This work was supported by the US Office of Naval Research and by the US National Science Foundation.

W. G. DEUSER

Woods Hole Oceanographic Institution,
Woods Hole,
Massachusetts 02543.

Received October 13; revised December 9, 1969.

[1] Craig, H., *Geochim. Cosmochim. Acta*, **3**, 53 (1953).

[2] McKinney, C. R., McCrea, J. M., Epstein, S., Allen, H. A., and Urey, H. C., *Rev. Sci. Instrum.*, **21**, 724 (1950).

[3] Craig, H., *Geochim. Cosmochim. Acta*, **12**, 133 (1957).

[4] Deuser, W. G., Degens, E. T., and Guillard, R. R. L., *Geochim. Cosmochim. Acta*, **32**, 657 (1968).

[5] Sackett, W. M., Eckelmann, W. R., Bender, M. L., and Bé, A. W. H., *Science*, **148**, 235 (1965).

[6] Degens, E. T., Behrendt, M., Gotthardt, B., and Reppmann, E., *Deep-Sea Res.*, **15**, 11 (1968).
[7] Degens, E. T., Guillard, R. R. L., Sackett, W. M., and Hellebust, J. A., *Deep-Sea Res.*, **15**, 1 (1968).
[8] Strickland, J. D. H., in *Chemical Oceanography* (edit. by Riley, J. P., and Skirrow, G.), **1**, 477 (Academic Press, London and New York, 1965).
[9] Marshall, S. M., and Orr, A. P., *The Biology of a Marine Copepod* (Oliver and Boyd, Edinburgh, 1955).
[10] Lasker, R., *J. Fish. Res. Bd. Canad.*, **23**, 1291 (1966).
[11] Alekin, O. A., and Moricheva, N. P., *Dokl. Akad. Nauk SSSR*, **167** (2), 423 (1966).
[12] Buch, K., *Meerentut. Julk. Havsforsk. Skr.*, No. 151, 1 (1951).
[13] Saruhashi, K., *Pap. Met. Geophys. Tokyo*, **6**, 38 (1955).
[14] Buch, K., in *Encyclopedia of Plant Physiology* (edit. by Ruhland, W.), **5** (1), 1 (Springer-Verlag, Heidelberg, 1960).
[15] Deuser, W. G., and Degens, E. T., *Nature*, **215**, 1033 (1967).

Volatile Factor in Soil Fungistasis

SOIL fungistasis was originally described by Dobbs and Hinson[1] as a widespread phenomenon in which viable fungal spores were unable to germinate in natural soil. Although no precise and universally accepted explanation for this phenomenon is available, fungistasis is usually considered to result from biological activity in soil[2,3]. Failure to extract an inhibitory factor[5-7] has led to the nutritional theory[2,3] that the phenomenon can be explained wholly by the lack of nutrients essential for germination in the soil micro-environments of fungal spores. On the other hand, some have suggested that there are inhibitors in soil, the effects of which are nullified by the addition of suitable nutrients[4,6,8,9]. We report here evidence of a volatile factor in soil which induces fungistasis.

Three assay methods were used in a comparative study of volatile and diffusible fungistatic factors. First, the sterile 'Cellophane' agar diffusion method[10] (SCAD) was used: agar disks (8 mm in diameter and 5–6 mm thick) were placed on a sterile 'Cellophane' membrane in contact with 50 g of soil in a Petri plate. The disks were pre-activated in this manner for 24 h at 28°–29° C, transferred to another slide on a glass rod support in a sterile moist plate, and inoculated with a conidial suspension (150–200 conidia/field × 100) of a test organism. After 22–24 h at 25° C, agar disks were examined and germination of spores was recorded. Second, the soil emanation agar method (SEA) was used: the procedure was the same as the previous method except that there was no direct contact between soil and disks, which were placed on a sterile glass slide in a covered Petri dish containing 50 g of soil for pre-activation. Third, a direct assay method (D) was used: washed 'Millipore' filters were impregnated with a test conidial suspension (200–250 conidia/field, × 100) and inserted into tumblers of soil. The filters were gently removed after incubation, stained with lacto-phenol–cotton blue mounting medium, and examined for spore germination. Controls consisted of incubation of agar disks (for the first two methods) or 'Millipore' filters (for the direct method) without soil but otherwise in identical conditions. All tests were conducted in duplicate and repeated several times.

Some characteristics of soils collected from various localities in Colorado and stored air-dried before use are shown in Table 1. At the beginning of the experiment, soil was moistened to 60 per cent water-holding capacity and tests for fungistatic properties were carried out 1, 4, 7, 10 and 15 days later.

Only four of the twenty different fungi isolated from soil are discussed here, because their responses were representative of the species tested. Data obtained 10 days after soil had been moistened are omitted because results were intermediate to those collected after 7 or 15 days. Uninoculated agar disk controls in contact with 'Cellophane' (SCAD method) or on slides (SEA method) remained sterile at the end of the assay periods.

Results are given in Fig. 1. There have been conflicting views on the effect of soil *p*H on spore germination[7,10,11]. Our studies indicate a profound effect of *p*H (especially in recently moistened soils), for germination in all assays was highest in acid or neutral soils and lowest in alkaline soils.

In general, assay by the first two methods revealed a high percentage of germination in soil 1 day after moistening and declined thereafter, confirming earlier observations[1,12]. The spore germination after 1 day, as determined by the direct assay method, gives an indication of the direct effect of *p*H, for nutrients contributing to activity would be available[2,7].

Contact with soil was not necessary for inhibition of spore germination. The soil emanation agar method clearly showed reduced germination of spores, particularly when exposed to alkaline soils, thereby indicating the presence of a volatile inhibitory factor. Inhibition curves as measured by the first two methods were approximately parallel. A decline and steady state of inhibition by the volatile factor reached after 10–15 days probably resulted from the decrease and subsequent equilibrium of microbial activity in moistened soils[13]. Volatiles have been suggested[14] as fungistatic substances, but results of tests have proved negative[7]. Data shown in Fig. 1 demonstrate why this was true. Although inhibition in the soil emanation agar method was detected with all test organisms, at specific times after moistening, at some values of soil *p*H, and with certain organisms, inhibition was not evident: for example, for *P. chrysogenum* and *T. viride* in acid soils 7 and 15 days after moistening.

Particularly in alkaline clay loam soils with a low content of organic matter, little spore germination was demonstrated by the soil emanation agar method. Preliminary tests with alkaline loam and sandy loam types of soils containing low organic matter, however, indicated the presence of a volatile factor but in lower concentrations, as reflected by relatively higher germination of spores. It is not clear whether the lighter soils tested, by virtue of their physical properties, provide less favourable environments for microbial activity or lack particular organisms capable of producing inhibitory volatile substances, or whether such organisms exist but are not very active in such soils.

This research constitutes the first direct evidence of a volatile inhibitory factor[15] in soil contributing significantly to responses of fungal spores in the fungistatic phenomenon.

We thank Drs William Kreutzer, L. W. Durrell, Gary Griffin and Penelope Hanchey for advice and useful

Table 1. SOME PROPERTIES OF SOILS USED IN ASSAYING OF FUNGISTATIC FACTORS

Soil *p*H	Soil type	Organic matter (per cent)	Lime (per cent)	NO_3-N (p.p.m.)	P_2O_5 (lb/acre)	K_2O (lb/acre)	DTPA extractable micro-nutrients Zn (p.p.m.)	Fe (p.p.m.)	Mn (p.p.m.)	Cu (p.p.m.)
4·6	Loam	5·8	< 1	—	300	210	10·00	40·0	—	—
5·0	Loam	5·7	< 1	—	217	145	10·00	40·0	—	—
5·5	Loam	4·7	< 1	4·80	31	360	4·10	40·0	6·2	0·42
6·0	Loam	2·2	< 1	4·20	91	1,000	0·70	25·2	6·0	0·36
6·4	Loam	1·5	< 1	4·20	57	1,000	0·60	21·2	2·9	0·24
6·8	Clay loam	2·0	1	17·80	24	665	0·60	16·8	34·5	0·91
7·5	Clay loam	2·2	1·0	23·40	118	1,000	0·63	12·5	26·5	0·91
8·0	Clay loam	1·0	4·5	28·65	198	1,000	0·39	8·0	12·6	1·72
8·6	Clay loam	1·1	5·9	34·72	140	865	0·29	3·0	5·8	1·07
8·9	Clay loam	0·8	5·5	—	55	963	0·26	4·8	—	—

The average moisture of loam and clay loam air-dried soils was 2·2 and 4·0 per cent, respectively. DTPA, diethylenetriamine penta-acetic acid extraction method.

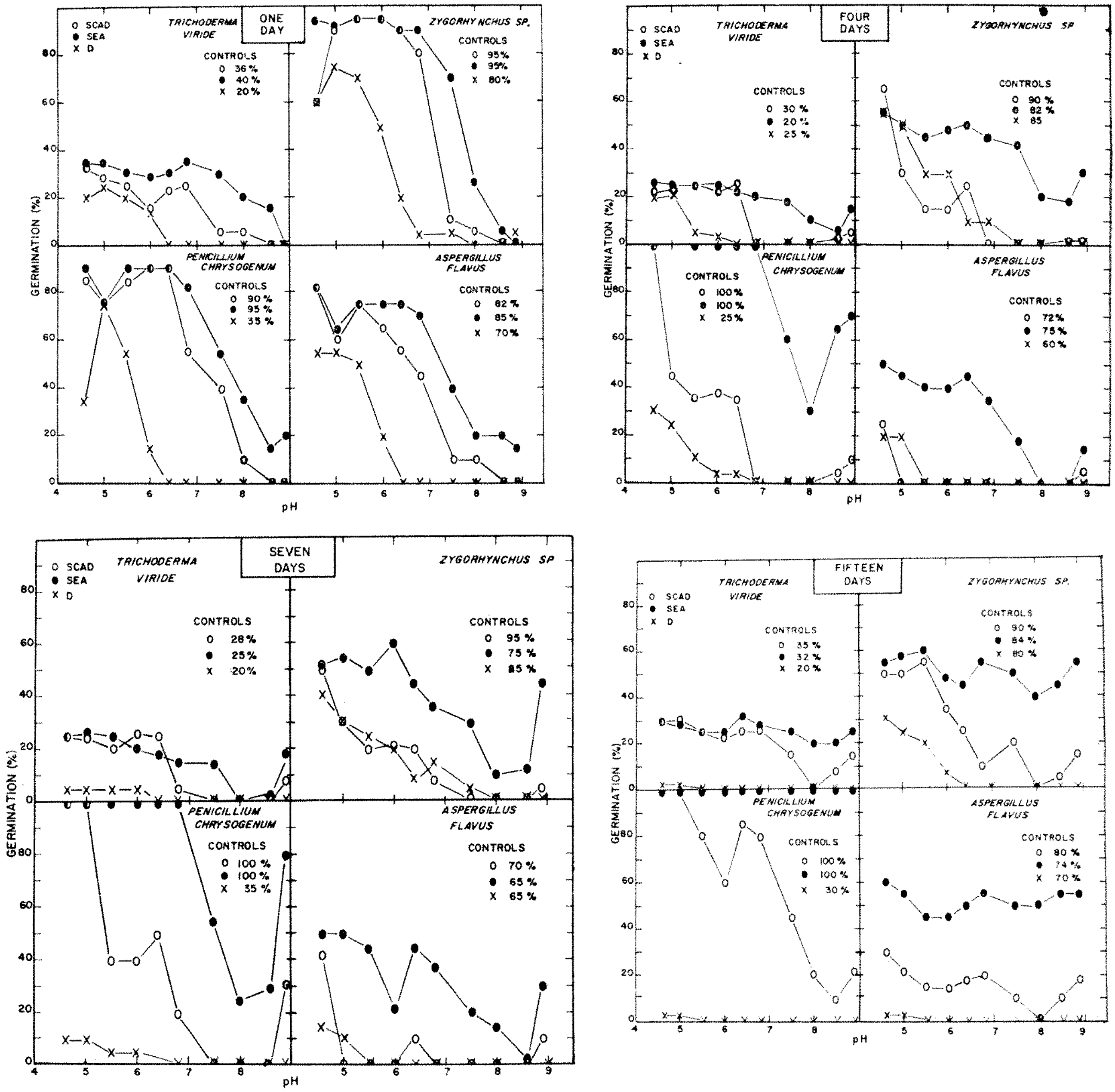


Fig. 1. Germination of conidia of four species of fungi in various fungistasis assay techniques in soils of different *p*H 1, 4, 7 and 15 days after moistening. SCAD, Sterile 'Cellophane' agar diffusion method; SEA, soil emanation agar method; D, direct method. Germination in each assay without the presence of soils is given under controls.

suggestions. This work was supported in part by the W-38 Western Regional Project.

T. S. HORA
RALPH BAKER

Botany and Plant Pathology Department,
Colorado State University,
Fort Collins, Colorado 80521.

Received November 3, 1969.

[1] Dobbs, C. G., and Hinson, W. H., *Nature*, **172**, 197 (1953).
[2] Lockwood, J. L., *Ann. Rev. Phytopathol.*, **2**, 341 (1964).
[3] Ko, W. H., and Lockwood, J. L., *Phytopathology*, **57**, 894 (1967).
[4] Baker, R., *Ann. Rev. Phytopathol.*, **6**, 263 (1968).
[5] Stover, R. H., *Canad. J. Bot.*, **36**, 439 (1958).
[6] Griffin, G. J., *Phytopathology*, **52**, 90 (1962).
[7] Lingappa, B. T., and Lockwood, J. L., *J. Gen. Microbiol.*, **26**, 478 (1961).
[8] Jackson, R. M., *J. Gen. Microbiol.*, **18**, 248 (1958).
[9] Jackson, R. M., in *The Ecology of Soil Fungi* (edit. by Parkinson, D., and Waid, J. S.), 168 (Liverpool University Press, 1960).
[10] Schuepp, H., and Green, R. J., *Phytopathol. Z.*, **61**, 1 (1968).
[11] Jackson, R. M., *J. Gen. Microbiol.*, **19**, 390 (1958).
[12] Kouyeas, V., and Balis, C., *Ann. Inst. Phytopathol., Benaki*, **8**, 123 (1968).
[13] Chase, F. E., and Gray, P. H. H., *Nature*, **171**, 481 (1953).
[14] Dobbs, C. G., and Bywater, Joan, *Great Brit. Comm. Rep. Forest Res.*, 1957, 92 (1957).
[15] Balis, C., and Kouyeas, V., *Ann. Inst. Phytopathol., Benaki*, **8**, 145 (1968).

L-Amino-acid Oxidase, a Bactericidal System

THE antibacterial activity of glucose oxidase[1-3] and xanthine oxidase[4,5] was reported twenty-five years ago. The antibacterial effect was attributed to the H_2O_2 generated during aerobic oxidation of an appropriate substrate. Considering the potency and broad spectrum of the antibacterial action of these enyzmes, it is surprising

Table 1. BACTERICIDAL ACTIVITY OF L-AMINO-ACID OXIDASE AGAINST PATHOGENIC STRAINS

Amount of enzyme* (μg)	Per cent lethal action *M. aureus*	*Salmonella typhimurium*	*Salmonella St Paul*	*Salmonella typhosa*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Serra tia marcescens*	*Escherichia coli*
50	100	100	98	100	100	100	88	50
20	100	93	73	95	100	100	47	0
10	100	63	26	53	100	91	0	0
5	100	14	0	0	95	70	0	0
2	100	0	0	0	33	12	0	0
1	98	0	0	0	0	0	0	0
0·5	26	0	0	0	0	0	0	0
0·2	0	0	0	0	0	0	0	0
Estimated LD_{50} (μg)	0·7	9·0	15·0	10·0	3·0	3·5	22·0	50·0

* In 1 ml. phosphate buffered saline (pH 6·9) containing mM L-leucine.

that attention has not been focused on them or on other flavoproteins as agents in host defence. In this report, the bactericidal action of L-amino-acid oxidase is examined.

L-Amino-acid oxidase (from *Crotalus adamanteus*), purified according to Wellner and Meister[6], D-amino-acid oxidase (electrophoretically purified), and catalase were purchased from commercial sources. Stock solutions of enzymes and certain other reagents were prepared in 5 mM phosphate buffer saline at pH 6·9 and sterilized by filtration through 0·45 μm membranes. All bacterial strains were recent clinical isolates; they were maintained on trypticase-soy agar before subculture in trypticase-soy broth. The bactericidal assay used in these studies is described elsewhere[7]. The bactericidal activity of the L-amino-acid oxidase system was evident to varying degrees against all strains tested. The bottom line in Table 1 gives an extrapolated estimate of the LD_{50} for each bacterial strain.

Other data demonstrate that the oxidase is labile to heating at 60° C, the optimum pH is between 6·5 and 7·0; 3 μg of the oxidase is capable of killing up to 10^6 staphylococci. Several experiments were tried to demonstrate the bactericidal effect by a D-amino-acid oxidase system. Different batches of the enzyme in amounts ranging from 1–100 μg were incubated with bacterial strains in the presence of 10^{-2} to 10^{-6} M concentrations of D-serine; no antibacterial activity was noted. The addition of either D-leucine or D-methionine (10^{-4} to 10^{-6} M) to the system also did not elicit an antibacterial effect.

Certain characteristics of L-amino-acid oxidase as determined by bactericidal assay (Table 2) were in accord with the established behaviour of the enzyme in conventional assays with amino-acid substrates. The enzyme was heat-labile, insensitive to 10^{-3} M KCN and inhibited by excess substrate (L-leucine). Catalase effectively inhibited the antibacterial activity, most probably by destruction of H_2O_2, the actual lethal agent. A small synergistic effect is noted when amounts of exogenous H_2O_2 are added to the oxidase at levels where each agent alone is not very lethal.

Table 2. EFFECT OF SOME REAGENTS ON THE L-AMINO-ACID OXIDASE BACTERICIDAL SYSTEM

Reagent	Amount or concentration	Per cent lethal action *M. aureus* (2 μg oxidase)	*S. typhimurium* (20 μg oxidase)	
None		100	94	
Catalase	1·0 μg	0	0	
	0·2 μg	21	0	
	0·1 μg	86	32	
	0·01 μg	100	82	
60° C; 30 min	1·0 μg	100	96	
KCN	10^{-3} M	85	93	
L-Leucine*	10^{-1} M	0	0	
	10^{-2} M	100	99	
	10^{-3} M	100	93	
H_2O_2	None	28		0·5 μg oxidase in this experiment
	10^{-5} M	96		
	10^{-6} M	36		
	10^{-5} M	24		H_2O_2 controls
	10^{-6} M	0		

* L-Leucine at the indicated concentrations was also incorporated into the agar medium for overnight incubation.

The fact that the L-amino-acid oxidase system produces a bactericidal effect, while the D-amino-acid oxidase system does not, raises the question of the site of action of the H_2O_2 produced during oxidation of amino-acid substrates. Because staphylococci produce catalase but are quite susceptible to the L-amino-acid oxidase system, an intracellular site of the action of H_2O_2 seems unlikely. Furthermore, the marked inhibition of killing by small quantities of exogenous catalase argues against an intracellular site. The tendency of D-amino-acid oxidase to polymerize[8] may interfere with its ability to approach sufficiently close to the cell to obtain a lethal concentration of H_2O_2, which suggests that peroxide sensitive loci exist on or near the cell membrane. Similarly, the lesser sensitivity of all smooth enteric strains to L-amino-acid oxidase may relate to a greater difficulty of the enzyme to penetrate through surface lipopolysaccharides. An alternative possibility would be that the bactericidal flavoproteins produce a toxic intermediate, that is a reduced form of oxygen other than H_2O_2, which is not produced in the non-bactericidal D-amino-acid oxidase system.

Iyer and colleagues[9] suggested that the formation of H_2O_2 within phagocytic cells may represent an important antibacterial defence mechanism. These authors and others[10] reported significant increases in H_2O_2 production during phagocytosis. The lack of increased H_2O_2 formation in phagocytosing granulocytes from patients with chronic granulomatous disease[11] may account, in part, for the inability of these phagocytes to kill certain bacterial strains[12,13].

Rossi and Zatti[14] reported a stimulation of an NADPH-oxidase activity in leucocyte granules following phagocytosis. Such a granule bound oxidase may constitute an important bactericidal system in which the enzyme is delivered directly to the phagocytic pouch containing bacteria. This mechanism would exclude inhibition of bactericidal action by catalase, a cytoplasmic enzyme[15]. Continuing studies indicate that rabbit neutrophilic leucocytes do contain an oxidase system with bactericidal properties.

This investigation was supported by a US Public Health Service research grant from the US National Institutes of Health.

ROBERT C. SKARNES

Worcester Foundation for Experimental Biology,
Shrewsbury,
Massachusetts.

Received September 29; revised December 8, 1969.

[1] Coulthard, C. E., Short, W. F., Michaelis, R., Sykes, G., Skrimshire, G. G., Standfast, A. F., Birkenshaw, J. H., and Raistrick, H., *Nature*, **150**, 643 (1942).

[2] Kochalaty, W., *Arch. Biochem. Biophys.*, **2**, 73 (1943).

[3] Roberts, E. C., Cain, C. K., Muir, R. D., Reithel, F. J., Gaby, W. L., van Bruggen, J. T., Homan, D. M., Katzman, P. A., Jones, R. L., and Doisy, E. A., *J. Biol. Chem.*, **147**, 47 (1943).

[4] Green, D. E., and Pauli, R., *Proc. Soc. Exp. Biol. and Med.*, **54**, 148 (1943).

[5] Lipmann, F., and Owen, C. R., *Science*, **98**, 246 (1943).

[6] Wellner, D., and Meister, A., *J. Biol. Chem.*, **235**, 2013 (1960).

[7] Skarnes, R. C., *Nature*, **216**, 806 (1967).

[8] Meister, A., and Wellner, D., in *The Enzymes* (edit. by Boyer, P., Lardy, H., and Myrbäck, K.), ch. 26 (Academic Press, New York, 1963).

[9] Iyer, Y. G., Islam, D. M., and Quastel, J. H., *Nature*, **192**, 535 (1961).

[10] Paul, B., and Sbarra, A. J., *Biochim. Biophys. Acta*, **156**, 168 (1968).

[11] Holmes, B., Page, A. R., and Good, R. A., *J. Clin. Invest.*, **46**, 1422 (1967).

[12] Quie, P. G., White, J. G., Holmes, B., and Good, R. A., *J. Clin. Invest.*, **46**, 668 (1967).

[13] Kauder, E., Kahle, L. L., Moreno, H., and Partin, J. C., *J. Clin. Invest.*, **47**, 1753 (1968).

[14] Rossi, F., and Zatti, M., *Experientia*, **22**, 758 (1966).

[15] Evans, W. H., and Rechcigl, M., *Biochim. Biophys. Acta*, **148**, 243 (1967).

Altered Cell Walls of *Staphylococcus aureus* resistant to Methicillin

METHICILLIN-resistant (MR) strains of *Staphylococcus aureus*, those capable of multiplying in the presence of 12·5 μg of methicillin/ml. (eight or more times that tolerated by methicillin-sensitive (MS) strains), are also resistant to other commonly used antibiotics, including other semi-synthetic penicillins and cephalosporins[1–7]. They can cause major disease[6–11] and thus may pose a serious therapeutic problem.

The mechanism of resistance to methicillin seems to be independent of penicillinase (β-lactamase) because MR strains grow in the presence of high concentrations of methicillin[7,12], and because variants which have lost the ability to synthesize penicillinase remain MR[10,13]. Thus the mechanism is grossly different from that by which most strains of *S. aureus* resist benzylpenicillin—by means of penicillinase[14–16]. For this reason, and because penicillins are known to act by interfering with normal cell wall formation[17–19], the possibility that methicillin-resistance might be associated with some alteration in cell wall composition was considered.

Table 1. SUSCEPTIBILITY OF *Staphylococcus aureus* TO LYSOSTAPHIN (L) AND METHICILLIN

Strains	Proportion MR	Rate of lysis by L, (Δ% T/h)	Ratio of rates of lysis, parent/MR clone	*MIC* of methicillin, (μg/ml.)
MR 1*a*	$4 \cdot 2 \times 10^{-5}$	45–287	5·7–8·4	⩾1,600
MR 1*b*	0·57–0·73	14–34		⩾1,600
MR 2*a*	$3 \cdot 1 \times 10^{-7}$	99–328	3·7–6·3	800
MR 2*b*	0·57	20–83		800
MR 3*a*	5×10^{-5}	85	3·1	800
MR 3*b*	0·37	27		⩾1,600
MR 4*a*	$6 \cdot 3 \times 10^{-5}$	56	2·9	⩾1,600
MR 4*b*	0·37	19		⩾1,600
MR 5*a*	$4 \cdot 5 \times 10^{-6}$	72	2·8	800
MR 5*b*	0·61	26		⩾1,600
MR Col. *a*	0·06	35	2·5	⩾1,600
MR Col. *b*	0·75	14		⩾1,600
60/1	0	53–165		6·2
Oxford 167	0	396		3·1

MR, Methicillin-resistant, naturally occurring; *a*, clone from antibiotic-free agar (parent strain); *b*, clone from agar containing 50 μg/ml. of cloxacillin; 0, none detected, that is $< 10^{-8}$ of cells in the culture; T, transmission of light at 500 or 620 nm; *MIC* = minimum inhibiting concentration after 48 h at 37° C.

Cell walls of naturally occurring MR *S. aureus* were compared with MS cells with respect to amino-acid composition and susceptibility to lysostaphin (supplied by Dr P. Tavormina, Mead Johnson Research Center, Evansville, Indiana). The amino-acids of acid hydrolysates of cell walls of populations of predominantly (37–75 per cent) MR cells were found to have essentially the same molar ratios of glutamate, lysine, alanine and glycine as those of MS cells.

The predominantly MR cell populations were obtained by cloning three different clinical MR isolates in the presence of 50 μg/ml. of cloxacillin. Eight different MS cell populations were studied: two naturally occurring MS strains and six parent strains from which the MS cell populations were cloned and which originally contained only 6 in 10^2 to 3 in 10^7 highly resistant cells, or colony forming units (c.f.u.). The proportion of "highly resistant" c.f.u. was determined by comparing the total colony count on medium free antibiotic with that on medium containing 50 μg/ml. of cloxacillin. The cells were grown for 19 h at 37° C in Difco brain–heart infusion (BHI), washed, disrupted with the MSK mechanical cell homogenizer using 0·1 mm glass beads[20]. The cell walls were separated by differential centrifugation[21,22] and hydrolysed with 6 M HCl at 105° C. Amino-acids in the hydrolysates were analysed in the Phoenix (Philadelphia) amino-acid analyser.

Rates of lysis of MR and MS strains of *S. aureus* by lysostaphin were compared by observing changes in light transmission at 500 or 620 nm of intact bacteria suspended in *p*H 7·4 Tris–NaCl buffer[23]. "Parent" strains of naturally occurring MR *S. aureus* contained only a minority (6 in 10^2–3 in 10^7) of highly resistant c.f.u.; they were lysed by lysostaphin 2·5 to 8·4 times as rapidly as were clones derived from them, which contained 37–75 per cent highly resistant c.f.u. (Table 1). This consistent difference in rate of lysis was taken as evidence that the cell walls or surfaces of MR cells were different from those of MS cells because the wall is the substrate for the lysostaphin[24,25].

The fact that no difference in amino-acid composition of MS and MR cells was detected by the method used suggests that the difference in the walls is not attributable to different peptide composition. But the data presented could be accounted for by difference in amino-acid sequence, or the presence of some non-peptide component that prevented access of enzyme to its substrate, the glycyl–glycyl bonds[24,25].

The observed difference in the cell wall or surface could be either the cause of the resistance to methicillin, the result of the resistance mechanism, or a coincidentally linked trait.

Methicillin-resistance *per se* is not invariably associated with decreased susceptibility to lysostaphin. Two MS strains which were "trained" to methicillin resistance by serial transfers in increasing concentrations of the antibiotic until they grew in 100 μg/ml. underwent little or no change in susceptibility to lysostaphin. Similarly, a strain of MS *S. aureus* selected for resistance to lysis by lysostaphin showed no significant change in its resistance to semi-synthetic penicillins.

L. D. SABATH
C. D. LEAF
D. A. GERSTEIN
M. FINLAND

Thorndike Memorial Laboratory
(Channing Laboratory),
Harvard Medical Unit,
Boston City Hospital
and
Department of Medicine,
Harvard Medical School,
Boston, Massachusetts.

Received September 9, 1969; revised January 6, 1970.

[1] Barber, M., in *Resistance of Bacteria to the Penicillins*, 89 (Churchill, London, 1962).

[2] Chabbert, Y. A., and Baudens, J. G., *Ann. Inst. Pasteur*, **103**, 222 (1962).

[3] Bulger, R. J., *Lancet*, i, 17 (1967).

[4] Bastin, R., Worms, R., and Acar, J. F., *Pathol. Biol.*, **15**, 1205 (1967).

[5] Churcher, G. M., *J. Clin. Pathol.*, **21**, 213 (1968).

[6] Benner, E. J., and Kaysor, F. H., *Lancet*, ii, 741 (1968).

[7] Barrett, F. F., McGehee, jun., R. F., and Finland, M., *New Engl. J. Med.*, **279**, 441 (1968).

[8] McHenry, M. C., Gavan, T. L., Farmer, R. G., and Evarts, C. M., *Cleveland Clinic Quart.*, **36**, 9 (1969).

[9] Borowski, J., Kamienska, K., and Rutecka, I., *Brit. Med. J.*, **1**, 983 (1964).

[10] Dyke, K. G. H., Jevons, P., and Parker, M. T., *Lancet*, i, 835 (1966).

[11] Chabbert, Y. A., Baudens, J. G., Acar, J. F., and Gerband, G. R., *Rev. Franc. Etud. Clin. Biol.*, **10**, 495 (1965).

[12] Sabath, L. D., Barrett, F. F., Gerstein, D. A., and Finland, M., *Antimicrobial Agents and Chemotherapy-1968*, 302 (1969).

[13] Seligman, S. J., *Nature*, **209**, 994 (1966).

[14] Barber, M., in *Drug Resistance in Micro-organisms*, 282 (Churchill, London, 1957).

[15] Pollock, M. R., *Drug Resistance in Micro-organisms*, 78 (Churchill, London, 1957).

[16] Barber, M. J., *Pathol. Bact.*, **59**, 373 (1947).

[17] Lederberg, J., *J. Bact.*, **73**, 144 (1957).

[18] Wise, E. M., and Park, J. T., *Proc. US Nat. Acad. Sci.*, **54**, 75 (1965).

[19] Tipper, D. J., and Strominger, J. L., *Proc. US Nat. Acad. Sci.*, **54**, 1133 (1965).

[20] Bleiweis, A. S., Karakawa, W. W., and Krause, R. M., *J. Bact.*, **88**, 1198 (1964).

[21] Salton, M. R. J., *The Bacterial Cell Wall*, 58 (Elsevier, Amsterdam, 1964).

[22] Nathenson, S. G., and Strominger, J. L., *J. Pharm. Exp. Therap.*, **131**, 1 (1961).

[23] Zygmunt, W. A., Harrison, C. F., Browder, N. P., and Tavormina, P. A., *Appl. Microbiol.*, **16**, 1174 (1968).

[24] Browder, H. P., Zygmunt, W. A., Young, J. R., and Tavormina, P. A., *Biochem. Biophys. Res. Commun.*, **19**, 383 (1965).

[25] Tipper, D. J., and Strominger, J. L., *Biochem. Biophys. Res. Commun.*, **22**, 48 (1966).

Book Reviews

WHITHER THE BRITISH LANDSCAPE?

New Lives, New Landscapes

By Nan Fairbrother. Pp. viii + 397. (Architectural Press: London, February 1970.) 75*s*.

THIS book is about the changing environment, though chiefly about the countryside. In it, the author projects a mass of information about ecology, geology, sociology, geography, agriculture, land economics, planning and associated law, and a few other disciplines. Some of this is relevant and in context, some not. Some is accurate, some probably accurate, and some not so. The information supports a pragmatic and socially orientated plea for better landscape planning, and, as such, is more persuasive than aesthetic arguments only. The style of writing is varied. Some passages are scholarly and thought provoking, some casual and entertaining, some chatty and irritating. The mass of information, and the presentation, tend to obscure the essentials of the argument: certainly from the point of view of the professional and the administrator.

The text is interwoven (usually in bracketed asides) with a synopsis of the author's life history, presumably designed to demonstrate that she knows how most of the groups in society think about the environment. Poor little girl from urban slum goes to university, and holidays pre-war in the remoter hills and coastal regions of the UK. Wartime evacuee mum lived in uncomfortable muddy countryside, but now has a house in London, one in the country, and fits in holidays in the United States with the widening interests of a professional, but not practising, landscape architect. This may of course be more typical of modern society than I suspect. She is certainly methodical. Her card index of suitable quotes from other writers must be voluminous, and if she does not keep an index of "ideas about the countryside, and so on" (her style, not mine), she must have an excellent memory, for nearly everybody I have ever heard, good, bad or madcap, appears somewhere. Some sentences are positively arresting. "In popular (recreation) areas, however, the natural vegetation is no longer forest but people and cars. . . ."

In part one, "A New Society in an Old Setting", the history of our changing environment is reviewed extremely well. Though this lacks the scholarship of writers like W. G. Hoskins, the interpretation is rewarding in its simplicity, its realism and a freedom from nostalgia. As an explanation of why the environment is like it is and how we, the people of Britain, have made it so, it provides excellent background reading for those developing an interest in the environment.

Part two, "Landscapes for an Industrial Democracy", reiterates some of the arguments of the first part. It presents a thesis that in Britain we have moved into an era where there are four basic environments: "Built-Up Urban", "The Green-Urban", "The New Farming", and "The Man-Made Wild". Will this classification make an impact and come into general use? Has it the merit of more simplicity than "Urban", "Rural" and "Urban-Rural Fringe"?

Part three, however, "A Four-Point Plan for a New Landscape Framework", presumably the essence of the sermon, is unconvincing and naïve. Our exploding cities are to have an aesthetic "cordon sanitaire" of tree belts used chiefly for recreation. Elsewhere, in landscape regions identified on ecological criteria, we are to convince all those concerned with managing land to plant the truly native tree and shrub species, thereby achieving beauty and regional character. Of course, states Miss Fairbrother, this will not cost very much.

I find this an irritating book. Perhaps because this is European Conservation Year when interest in the environment should stimulate the market for books about it, the Architectural Press, or the author, or both, hurried too much. Whatever the intended market, the book would have been better if half the length, and with the chatter, repetition and biography edited out.

I also find it a disturbing book. The simplicity of the ideas is such that they do not leave the mind. Presumably few authors would ask for more, but the feeling remains that the book fails. Without part three and with a slight redraft of part two, it could be accepted as a popular interpretative work. With some ruthless editing, enrichment of the nature conservation content, a deeper understanding of land use and recreation planning and its organization (particularly in the context of new legislation and the proposed reorganization of local government), it could have been a more challenging and influential work.

Nevertheless it makes interesting reading. Nan Fairbrother quotes Frank Fraser Darling: "We can be of little service to our fellows until we become disillusioned without being embittered". Nobody reading this book can conceive that she is disillusioned; optimism and hope so dominate the presentation that she may be criticized for being too ready to accept change. Moreover, when a writer has sufficient humour, imagination, and indeed commonsense, to propose a society "For enjoying the countryside without feeling virtuous", readers may be sure that there is no evidence of bitterness in her work.

R. J. S. HOOKWAY

CHOICE IN EDUCATION

Decision Models for Educational Planning

By Peter Armitage, Cyril Smith and Paul Alper. (LSE Studies on Education, from the Higher Education Research Unit of the London School of Economics and Political Science.) Pp. ix + 124. (Allen Lane (The Penguin Press): London, December 1969.) 75*s*.

EDUCATIONAL planning is a fashionable exercise. It arises from the fact that education takes a big part of the national income—roughly 6 per cent of the gross national product in the United Kingdom—and all the signs suggest that it will continue to take a rising proportion of the GNP. This large and rising proportion of the GNP ought to be allocated in order to get the best results. In some ways, in the private sector, gross profit rates are the criterion that can be used for such allocation; in some public sectors, of which roads are an instance, an approximation to gross profit rates can be reached by the use of so called cost–benefit techniques. In other areas, of which education and health are instances, the criteria are far less quantitative. It is difficult to see why £100 million should go to universities rather than to primary schools.

In actual fact, big decisions like that rarely have to be taken. In any one fiscal year most expenditure is predetermined because the teachers and buildings are there and have to be paid for. Such margins as there are tend to be committed to certain overwhelming objectives of which the most important is extra pupils who just turn up and have to be taught.

But over the longer term strategic planning is possible. It is not necessarily inevitable to raise the school-leaving age. We are not obliged by ineluctable forces to offer a place in higher education to all who ask for one. These are, to some extent, affective choices.

On what basis, other than sheer whim or preference, can these choices be made? This book does not answer that question. What it does is to show how interrelated the educational system is and the range of choices that are restricted by making any one decision. They offer a range of choices to those who are seeking to influence policy.

The trouble is that our political processes do not favour ranges of choice. As I have said elsewhere, in connexion with this excellent book, which is clearly written and helpful, in a system where the Department chooses the highest figure and the Treasury picks the lowest figure, rational decision is hindered. But the techniques suggested here will be used more and more and ultimately will prove their own case.

JOHN VAIZEY

EDUCATION MANIFESTO

Education for Democracy

Edited by David Rubinstein and Colin Stoneman. (Penguin Education Special.) Pp. 220. (Penguin Books: Harmondsworth, Middlesex, February 1970.) 6*s*.

EVERYBODY, it seems, wants to hear how new techniques in education are producing a generation of illiterates. The Black Papers, arguing for a return to an unashamedly élitist education system, to preserve the standards which they allege are fast slipping away, have had most of the press coverage while publications defending progressive techniques have passed almost without comment. Such a fate is, however, unlikely to befall this book. If the impressive list of contributors does not ensure a wide audience for this manifesto on progressive education, its sheer readability should do so.

The book is a collection of essays bound together by the common belief that education should be designed to cater for all children, and that a system designed to produce élites is both socially unacceptable and educationally unsound. Most of the contributors make no secret of the fact that they see education as a means of reforming society. Arthur Rowe, for example, makes the point that the present education system, being closely tied to class structure, can only perpetuate a class ridden society, and this theme is constantly echoed in other essays. Another fundamental belief that keeps cropping up is a rejection of the Black Papers' thesis of innate intelligence. Lewis Cohen and Colin Stoneman, in a carefully argued essay, outline a theory of intelligence based on several independent factors, some, if not all, of which may be genetically determined but which need to be developed to their full. They argue that labelling 80 per cent of British children failures, and treating them as if they are unlikely ever to excel in anything, will certainly not develop their potential.

In the essays on primary education, the contributors closely follow the progressive themes of freedom and of learning through experience rather than through formalized teaching methods. John Mitchell argues that traditional authoritarian teaching methods are likely to suppress any desire in the child to find out things for himself. The child will consequently lack any originality of expression, and the whole learning process will become a chore.

The section devoted to secondary education is, of course, bitterly opposed to selection of any sort at eleven-plus. In particular, Peter Mauger argues that the present system came into being based on pseudo-psychological grounds that were not even held at the time it was introduced, and, because middle class children have an overwhelming advantage in the selection process, the system is class biased, élitist and anti-democratic. But, although Brian Simon presents a good, rational argument against streaming, this section lacks any thorough discussion of alternatives.

Higher education does not fare so well in this book. Although there is a plea for higher education for all who want it, and various allusions are made to the educational absurdity of the binary system, little direct discussion of the structure of the present system is given. But this may be more a product of the length of the essays than their intention. Throughout the book, brevity occasionally takes precedence over depth of argument, with the result that the essays sometimes seem rather superficial. But loss of depth is matched by readability and immediate assimilation, which should ensure that the progressive message should get across to a wide cross-section of people. In any case, it is a book which every serving or aspiring teacher can ill afford to miss.

COLIN NORMAN

BOYLE'S LIFE

The Life of the Honourable Robert Boyle, F.R.S.

By R. E. W. Maddison. Pp. xxii + 332 + 40 plates. (Taylor and Francis: London, October 1969.) 190*s*.

NOTED for his law of which he was not sole discoverer, and associated with an apparatus he did not invent, Robert Boyle is among the few scientists of the seventeenth century still popularly known. Having connexions in both of the warring parties in the Civil War, but of an apolitical cast of mind, he quickly became absorbed in the philosophical and utilitarian designs of the circle of men which revolved about Samuel Hartlib. This was not, however, to be his single absorbing interest; charitable work and religious contemplation were of equal, if not greater, importance, and should not be forgotten in the assessment of his scientific work.

His life divides conveniently into the three periods of prolonged residence at Stalbridge, Oxford and London; it was largely uneventful. In spite of the reduction of his time by persistent ill-health, by the demands for entertainment of a stream of foreign visitors (he seems to have been considered a scientific sight of London), and by a number of public appointments, Boyle produced a stream of religious and philosophical works which were constantly esteemed and acclaimed. He could usually be relied on by the Royal Society for an entertaining experiment and, with John Ray, was one of the most frequent contributors to early numbers of the *Philosophical Transactions*.

Of Boyle's importance there is no doubt. As John Freind put it in 1712, it rests in the fact that he "has not so much laid a new foundation of Chymistry, as he has thrown down the old". It would be foolish, however, to look to this book for an explication of this, for this is not Maddison's purpose. This is strictly and limitedly defined. In the absence of any study of Boyle based on the original sources since Thomas Birch published his in 1744, and from which all others derive, Maddison has set out to recount accurately the events of Boyle's life as a supplement to Birch, and to offer a guide to his diverse interests. The book is scholarly and antiquarian and is, as such, an unfortunate rarity in the history of science. It draws together a great amount of esoteric information on various aspects, and sets out the state of discussion, together with the available facts, at controversial points.

It is a pity the publishers have not bothered to attain the standards of printing accuracy, illustration reproduction and typographical design of many cheaper works. Ugly is the only word appropriate to its physical appearance. This, of course, does not affect its value as a quarry for other scholars (and not simply those interested in science), but illustrations properly integrated with the text would have increased its usefulness considerably. The general reader may well find it heavy going, especially the opening chapter, where the weight of Maddison's annotation will defeat all but the most indefatigable. Elsewhere, however, one wishes for rather more: the book's scholarly usefulness would have been surely greater if references to correspondence printed by Birch had also indicated the manuscript source where this is extant. Equally one sometimes wishes for more extended treatments of particular aspects (especially that of the Invisible College). Maddison assumes a great deal of background information in the reader of what is, none the less, a basic work.

A. J. TURNER

SOLVENT CHEMISTRY

Non-Aqueous Solvents

By T. C. Waddington. (Studies in Modern Chemistry.) Pp. vii+88. (Nelson: London, November 1969.) 40*s* boards; 20*s* paper.

THE book is short, comprising six chapters covering solute–solvent interaction, protonic solvents, liquid oxide solvents, oxyhalide solvents, liquid halides and fused salts. The first chapter is deservedly the longest, dealing with the energetics and mechanisms of solvation and solvent self-ionization. In this field it is refreshing to find recognized a few non-aqueous solvents, for example, acetone, which are not noted for self-ionization or participation in reactions. It is also encouraging to find solvation differentiated from ligand coordination. All the solvents discussed are adequately documented by their physical data. Each chapter ends with a short bibliography and some revision exercises.

The sections on particular solvent types are excellently furnished with explicit stochiometrical equations, which makes for smooth reading. The book is suitable as a rapid sketch of the field for a student, but some additional background explanation would improve it in this respect. Thus, for example, Lewis acids and the Lux–Flood theory are rather taken for granted. Similarly, while the electrochemical aspects of the various solvents are well recognized, apart from a reference to the Shedlovsky equation the theoretical bases of Λ_0 and K_A are left undefined. On page 44 the author identifies chemical affinity with exothermicity and it is to be hoped that he will subsequently correct this general impression even if the case in point seems an approximate justification.

S. R. C. HUGHES

MODULAR FORMS

Modular Forms and Dirichlet Series

By Andrew Ogg. (Mathematics Lecture Notes Series.) Pp. xviii+173. (W. A. Benjamin: New York and Amsterdam, 1969.) n.p.

THESE notes cover the basic properties of Hecke operators on the modular group and its principal congruence subgroups, and of Dirichlet series with Euler products and functional equations. Chapter one is an account of Hecke's work on Dirichlet series with signature (λ,k,C). Chapter two discusses Hecke operators on the full modular group, treated as correspondences on a set of lattices, completed in chapter three using the Petersson inner product. Chapter four extends the theory to congruence subgroups, including the explicit construction of Eisenstein series. Chapter five gives Weil's characterization of forms on $\Gamma(N)$ by means of functional equations for associated Dirichlet series, and chapter six gives a brief account of theta series based on quadratic forms following Hecke and Schoeneberg.

The book covers material not readily available elsewhere, because the literature is diffuse even for readers of German. Hecke's own papers do not use the Petersson inner product, and Petersson's refer remorselessly back to Hecke for basic results, as well as being too general for a first reading. The author's style is discursive, with frequent digressions and anticipations, but this is an advantage for didactic purposes. A disadvantage for the novice is his cavalier treatment of the main definition: a "modular form" is defined three times (II-2, III-5, IV-3), each relevant for its immediate context, but the reader should be told that other writers might allow poles but disallow any subgroup of $GL^+(2,\mathbb{R})$. Similarly the author's definition of "dimension" agrees with those of Klein, Hecke and Petersson, but his notation (k for $-k$) does not; and "level" is not always confined to congruence subgroups. Finally, the author's convention (I-32 *et seq.*) that $-1/\tau+1$ means $-1/(\tau+1)$ really is peculiar to himself, and he should warn the reader in advance. But these are minor issues which do not arise if the book is used as a text for a lecture course, and the author's treatment of the subject is largely both logical and readable.

It is therefore unfortunate that the presentation and appearance of the book do not do the text justice. The book was printed directly from a typescript, in the interests of rapid publication. The typing is neatly done, though inevitably not as clear as print when so many suffixes and indices are used, but there are some needless visual difficulties. The text runs on continuously, broken only by chapter headings and the underlining of definitive words, so that the main results do not stand out. It is often hard to see when the statement ends for a theorem proved in advance, or the proof for one proved in arrear. These faults, however, can largely be remedied with a pen and ruler, and do not detract seriously from a useful addition to the literature.

A. O. L. ATKIN

MATHEMATICAL LOGIC

Mathematical Logic and Hilbert's ϵ-Symbol

By A. C. Leisenring. (University Mathematical Series.) Pp. ix+142. (Macdonald (Technical and Scientific): London, October 1969.) 60*s*.

THIS book on mathematical logic is very much devoted to Hilbert's ε-symbol, a subject that normally is not given prominence in textbooks. The author has tried to make the book self-contained and hopes that it will be useful as supplementary reading in an undergraduate course. The ε-symbol has some advantages in formal set theory; only time will tell whether it should have a higher status as a topic in mathematical logic—my opinion is that its status will not change very much.

The book is well written, but the reader will need to start with some background knowledge of logic. The content of the book is sufficiently distinctive for it to be recommended as supplementary reading to any course on logic, and the book would be a useful addition to any library.

The text begins with an eight page introduction that is readable and worth reading. The first chapter introduces a formal language and studies the semantics of this language. Deductive properties of systems based on the formal language are studied in the second chapter. Some knowledge of a standard text may be helpful to put in perspective the author's stress on the ε-calculus and its advantages over the predicate calculus. In chapter three Hilbert's ε-theorems (which say, very roughly, that the ε-calculus and predicate calculus are equally powerful) and some related results are proved, and in chapter four it is shown that the ε-calculus is useful in formal set theory and in proofs of consistency. In the last chapter, a Gentzen type system that incorporates the ε-symbol is introduced and a cut elimination theorem is proved.

The book is well produced and ends with a four page bibliography and eight pages of indexes.

R. M. DICKER

PLANT ANATOMY

Anatomy of the Monocotyledons

Vol. 3: Commelinales–Zingiberales. By P. B. Tomlinson. Pp. xx + 446. (Clarendon Press: Oxford; Oxford University Press: London, February 1969.) 105*s*.

Vol. 4: Juncales. By D. F. Cutler. Pp. ix + 357. (Clarendon Press: Oxford; Oxford University Press: London, October 1969.) 100*s*.

THESE two volumes make further substantial progress in the serial publication of the *Anatomy of the Monocotyledons*. The editor, Dr Metcalf, has now retired from the Royal Botanic Gardens, Kew, but he will continue to edit the series; and both he and the authors of these volumes are to be congratulated on the excellent progress which is being made.

Volume three is in two sections. In the first, Dr Tomlinson deals with the eight families which form Engler's order, the Farinosae, including the Bromeliaceae. The second covers another series of eight families in the order Zingiberales, including the Musaceae. The account of each family begins with several pages of general anatomical description (though, regrettably, a formal description of the family is not given); this is followed by sections, of variable length, on the individual anatomical features. The literature is well covered, and there are many clear diagrams and drawings. The text is chiefly designed for reference, but it is well written and set out, so that it is comparatively easy to gain an impression of the anatomical features of a family. There are some special sections, such as that on the ecological anatomy of the Bromeliaceae, which are brilliantly done. There are short appendices on the distribution of diagnostic features in the families, which will be of use in identification, and there is also an interesting short discussion on stomatal ontogeny. Some taxonomic problems, in both orders, are discussed in the light of the new anatomical evidence available, and the weight of this evidence is assessed. The amount of original work presented is large, and the volume represents a major contribution to our knowledge of tropical monocotyledons.

Volume four differs in several ways from volume three. Dr Cutler deals with only a single order, the Juncales, with six families, in a book which is three-quarters the length of volume three; and the treatment, which is of a high standard in both text and illustrations, is accordingly much more detailed. In particular, 215 pages are devoted to the Restionaceae, a family of the southern hemisphere. This account consists of a general introduction (but again, there is no formal description), followed by an artificial key to the twenty-four genera. The anatomy of each genus is then described, the genera being arranged alphabetically. Some of the descriptions are very detailed; thus 50 pages are devoted to the genus *Restio*, of which eighty-four species have been examined anatomically by the author. This very thorough treatment is not only important for identification and morphological study; it has considerable potential value as a means of elucidating the taxonomy of the group in relation to its geographical distribution. While this aspect of the matter is not omitted in the author's account, it is not, in my view, given sufficient prominence, nor are the threads drawn together as neatly as they might be. Thus, three somewhat inconsistent statements are made (p. viii, p. 116 and p. 296) about the South African and Australian species of *Restio* and whether they should continue to be regarded as being within the compass of a single genus. This is a question of great phytogeographical interest. It is unlikely that anybody knows more about it than Cutler; and it is a pity that he has not been able to devote a few pages to a critical discussion and a decision.

Both volumes are well printed and indexed and are reasonably priced. D. H. VALENTINE

GREENFINGERS IN AMERICA

A Short History of Botany in the United States

Edited by Joseph Ewan. Pp. ix + 174. (Hafner: New York and London, 1969.) $7.50.

HOST countries to international botanical congresses in recent years have provided participants with a brief guide to their country's contribution to the development of botanical science. The proceedings of the eleventh International Botanical Congress, which was held in Seattle in August 1968, are reported in this volume. It outlines the growth of knowledge of the floral richness of the United States, and the important role of American botanists in the advance of botanical research. The type of presentation, a volume of essays by experts in their respective fields, is not ideal, but the method was obviously dictated by the short space of time available for the volume's production. Professor Joseph Ewan, one of the leading historians of biological science in the United States, has contributed two essays and edited the other contributions. His essay, "Early History", opens the door to the fascinating story of the gradual discovery of the natural wealth of America. Many of the early naturalists came from Great Britain and later in the early nineteenth century a number of British plantsmen conserved and enhanced the floral wealth of the USA. This contribution was in no way one-sided, for many of the garden plants and trees loved today in Great Britain came from America in the seventeenth and eighteenth centuries. Britain's countryside and the gardens of many famous country houses were also greatly enhanced at that period by the extensive planting of American trees.

The other essays deal largely with post-World War II advances. The individual treatment of these subjects is unavoidably uneven, but they present a useful record. The essays could not be more concise. The editor has wisely omitted references to the published work of the various botanists mentioned. This detail can, however, be obtained from consulting the items given in the list of "Recommended Reading". The "Calendar of Events", a chronological list of important American events, discoveries and publications of botanically significant works, is a unique feature which with the "Index of Persons" giving birth and death dates must have been a labour of love on the part of the editor. I have noted one error in the "Calendar"—Schweintz, L. D. *de Snyopsis fungorum Carolinae* . . . is listed as being published in 1818 whereas it should have appeared under the date 1822.

This book will prove a valuable, up to date, quick reference to the development of botanical science in general and the achievements of American botanists in particular. The editor and the publishers are to be congratulated on producing an attractive publication which should be in every botanical library. PHYLLIS I. EDWARDS

AUSTRALIAN GEOLOGY

Stratigraphy and Palaeontology

Essays in Honour of Dorothy Hill. Edited by K. S. W. Campbell. Pp. xxiii + 390. (Australian National University Press: Canberra, October 1969.) $A17.50.

THIS volume consists of twenty papers of varying length distributed into four groups: interpretative palaeontology, biostratigraphy and biogeography; descriptive palaeontology and palaeoecology; geomorphology; and sedimentation.

In the first group, Bulman demonstrates that protheeal folds originated more than once among graptolites and that *Dicellograptus* is probably polyphyletic. Wells indicates that details of the actual growth of dissepiments have not been directly observed in recent corals, but the growth patterns support the idea that it is centripetal.

Sutherland and Haugh give a detailed account of the structure and development of a discoid rugose coral, *Gymnophyllum*, from the Westphalian of Oklahoma. Birenheide adds to knowledge of a stromatophore from the Oligocene of Germany. Jell, in a study of the septal microstructure of the Phillipsastraeidae, indicates the polyphyletic nature of many of its genera and also suggests that ultimately fasciculate, cerioid, thamnasterioid and aphroid species with similar internal structure may come to be regarded as congeneric and that the corallum form is unlikely to be of suprageneric significance. I would point out that this is a state of affairs similar to that in the Graptoloidea.

The first paper in the second group is by Campbell and McKellar on the Eastern Australian Carboniferous faunas. These are of general interest because they are associated with glacial deposits in New South Wales and recent palaeomagnetic studies have provided a stimulus to the consideration of the palaeoclimatic and palaeolatitudinal distribution of these faunas. A zonal scheme based largely on brachiopods is given. It is claimed that, for Australia, the palaeomagnetic evidence summarized by Irving and the geological data here summarized are remarkably consistent. The authors, on the other hand, are critical of the data so far known from other continental areas for the same period. Jull on the Lower Carboniferous corals of Eastern Australia has important comments on the taxonomy of *Lithostrotion* and *Orionastraea*. Cretaceous faunas are treated in two papers by Day, Dettmann and Playford from the point of view of the palaeogeography and correlation especially by palynology which has received much attention and proved its worth in relatively detailed correlation. Two papers by Dickins, Gostin, Runnegar and Whitehouse summarize notable advances in various aspects of the correlation, palaeoclimatology and faunal distribution of the Permian of New South Wales and Queensland. Pursuing the same general theme Wass, in a study of Permian Polyzoa, shows that there is greater similarity between Eastern and Western Australian forms than suspected hitherto and that the distribution routes probably lay to the north rather than to the south of the continent.

The third group of papers is descriptive. Bartholomai describes a Cretaceous elopid fish and Fleming adds to the relatively meagre knowledge of uppermost Carboniferous faunas by describing that from the Neerkol Formation of Queensland. Runnegar, in a paper on Permian fossils from the southern extremity of the Sydney basin, shows that current interpretations of the palaeogeography of the basin are in need of revision, and Dear describes a new linoproductid from the Upper Permian of Queensland whose closest comparison is with forms from the Kazanian of the Taimyr Peninsula of north USSR. Strusz in a description of certain Devonian cystiphylloid corals demonstrates their close affinity with species from Europe and less so with those of North America.

In the last group of papers, Ferguson gives an account of two Cainozoic erosion surfaces, one pre-volcanic and one lateritic, in south Queensland. Driscoll provides an interesting study of animal sediment relationships of Mississipian formations in Michigan and successfully applies recently published concepts from studies by oceanographers of present day conditions. The last paper is a valuable summary by Maxwell of the structure and development of the Great Barrier Reef. The complexity of the factors involved is well indicated, and it is to be hoped that this account will help in the efforts currently being made to conserve this extraordinary and unique feature of the Earth for the research and enjoyment of future generations.

The volume is well produced and the illustrations, both line and half tone, are excellent. It gives an indication of the enormous recent advances in the knowledge of Australian palaeontology and stratigraphy. It is a worthy tribute to Professor Dorothy Hill, whose research and teaching have inspired many of these papers and whose efforts to interest authority in the wealth of material in Australia are now meeting, in some measure, the support they deserve.

J. SHIRLEY

COMPARATIVE ZOOLOGY

Principles of Comparative Anatomy of Invertebrates

By W. N. Beklemishev. Translated by J. M. Maclennan. Edited by Z. Kabata. Vol. 1: Promorphology. Pp. xxx + 490. Vol. 2: Organology. Pp. vii + 529. (Oliver and Boyd: Edinburgh, February 1970.) 200*s* the set.

IT is difficult to do justice to this intellectual *tour de force* in a short space. My first impression of the work was that it would have gladdened the heart of McBride. On further acquaintance, I still approach it with some reservations. It should be made clear at the outset that this is not a book for the beginner. Rather, it should be read by those who have been devoted to invertebrates for some time and have a considerable knowledge of invertebrate structure and systematics. To give but one example: while the fate of the rosette cells resulting from spiral cleavage is discussed, there is no explanation of how $1a^{111}$–$1d^{111}$ are derived. Great stress is laid on such details, and terms such as homoquadrant may confuse the average student. Acceptance of *Polygordius* as showing a "primitive" type of development will not appeal to all.

The book is published in two volumes separately paged but with all the references and an index at the end of the second volume. It is a translation of the third (1964) edition. In the same year, Oxford University Press published Professor R. B. Clark's *Dynamics of Metazoan Evolution*. It would be hard to find a more contrasted approach. Beklemishev's is strictly classical. Yet, when so many new books present fewer and fewer facts, fewer arguments and more conclusions, those of us who still believe that comparative morphology is of vital importance in understanding the way in which animals have evolved can but welcome the translation of this outstanding work. On the other hand, there is much with which one must disagree. And it is for this reason that the book will perhaps best be appreciated by those with some knowledge of invertebrates. Sedgwick's, and later Remane's, views on the origin of the coelom and the mesoderm are stressed. The ctenophores thus occupy a special place, and while the book does bring together so many diverse facts in a well ordered manner, it is surprising to find serious comparisons made between the tentacles of *Rhabdopleura* and of ctenophores. Hadzi's book is not mentioned; nor, for that matter, is Sharov's—though perhaps this is a blessing.

Personally, I do not like such terms as "promorphology", and "architectonics". There are also some errors of judgment on the part of the translators, though these are few and the translation for the most part is excellent. Annelid "septa", for example, are unfortunately translated as "dissepiments", while (page 256, vol. 1) septum is used to describe the thoracic collar of serpulids. The nereid peristomium is said to have three segments (page 243, vol. 1) but elsewhere (page 258, vol. 1) only two. But I have found few statements likely to cause confusion.

The bibliography is remarkably good, yet there are omissions. Some have been mentioned already. Beklemishev was clearly acquainted with Manton's work, and also that of Tiegs, yet Tiegs and Manton's important paper[1] is not mentioned. Nor, of course, when this edition appeared, was Anderson's work[2] or that of Åkesson[3] on the *Lopadorhynchus* larva known. I would like to think that less stress would have been laid on the earlier work of Kleinenberg and of Meyer and on the importance that other zoologists attached to their work at that time if the author had known of this new work. Similarly, it is a pity that Ivanov's views on the germ layers and his concept of "primary" and "secondary" segments which cannot now be accepted should occupy such important corners in Beklemishev's argument. He therefore uses terms such as "kinetoblast" and "phagocytoblast".

Yet there is much to be gained from a reading of these volumes. The comparative approach to the muscular systems, circulatory systems, reproductive organs and to the development of the colonial habit in diverse phyla is stimulating in the detail in which each comparison is made.

This is a book which every zoology department should have in its library. It is a mine of information, much of which is not readily available except in specialist texts or papers. It is well printed and produced, and the publishers are to be congratulated on making this work available in English. R. P. DALES

[1] Tiegs, O. W., and Manton, S. M., *Biol. Rev.*, **33**, 255 (1958).

[2] Anderson, D. T., *Acta Zool.*, **47**, 1 (1966).

[3] Åkesson, B., *Ark. Zool.*, **20**, 55 (1967).

STUMPTAIL BEHAVIOUR

The Behavioral Repertoire of the Stumptail Macaque

A Descriptive and Comparative Study. By Mireille Bertrand. (Bibliotheca Primatologica, No. 11.) Pp. xi+273. (Karger: Basel and New York, 1969.) 122*s*; $14.65.

THIS monograph describes in detail the behavioural repertoire of *Macaca speciosa*. Most of the information was obtained from observations of captive animals—usually ones living in groups in large cages. A brief field study allowed a few comparisons to be made with the behaviour of wild animals, and in some cases comparisons are made with the behaviour of other primate species.

The first two chapters deal with the taxonomy and distribution of stumptails, and with the materials and methods used in the study. The next chapter presents quantitative data on the proportion of time spent in various occupations (for example, feeding, grooming, locomotion) by the groups of captive animals. The group structure was manipulated so that the behaviour of non-adults alone could be compared with that of non-adults in the presence of adults and vice versa. Not surprisingly there were differences, and furthermore a few observations on a wild group suggested that they differed from the captive ones.

The following seven chapters each contain detailed descriptions of different types of behaviour. It is unfortunate that the criteria for the assignment of patterns or groups of patterns between these chapters are not consistent. For instance, play is assigned as follows: locomotory play is with locomotion because some of the same motor patterns are involved (a descriptive criterion); rough-and-tumble play is with agonistic and friendly behaviour because of its comparability with agonistic behaviour and because of a similarity in function in establishing dominance (descriptive and functional criteria); investigatory play is with watchful and alarm behaviour because they are elicited by the same kinds of environmental stimuli (a causal criterion). The behavioural category "play" is undoubtedly too broad to be specially useful, particularly when function is being considered, but the subdivision of this or any other such category on variable criteria is no more than a hindrance.

The observations are, however, of high quality, and the details of the postures, facial expressions and vocalizations, and of the contexts in which they occur, provide a solid basis for comparison with other species. Furthermore, anybody who has the opportunity to study the species in the wild will be much helped by the descriptions in the book. The descriptions are well supported by good photographs, including sound spectrographs, and line drawings. There is a good bibliography but, unfortunately, no index. Y. SPENCER-BOOTH

Obituaries

Earl Russell

EARL RUSSELL, OM, FRS, died on February 3, 1970, at the age of 97. As a philosopher in the widest and oldest sense of the word, he had the rare ability to communicate lucidly with layman and professional philosopher alike, and to extend the range of his interests consistently from symbolic logic to social involvement. His intellectual scope included politics, the history of philosophy, mathematics and, not least, science.

Bertrand Russell's philosophy of science was very much a product of his wider philosophical and logical interests. After his pre-1900 abandonment of idealism, Russell became a logical realist and a pluralist. His analysis of science was dominated by two powerful maxims. The first was a determination to pursue a robustly realistic "common sense" attitude to those objects, both concrete and abstract, with which science deals. The "real" things in the world were not inextricably mental—the pervading orthodox view—but independent, individual things whose discovery and description required an analysis of human belief in order to find their correct form. These forms were to be expressed in simple or "atomic" propositions.

Pursuing this analysis in *The Principles of Mathematics* (1903) Russell was to find an overabundance of "real" things: "A man, a moment, a number, a class, a relation, a chimaera or anything else that can be mentioned"—such an excess constituted a challenge to an orderly and disciplined mind. Nevertheless, his pursuit of this analysis placed and kept him firmly in the British empiricist tradition.

In the writing of his *magnum opus*, the celebrated *Principia Mathematica*, Russell developed the techniques of "logical construction" of one entity from another which were to support his second maxim: "Whenever possible, substitute constructions out of known entities for inferences to unknown entities". Numbers become classes of classes of objects and later, in *The Analysis of Matter* (1927), objects became classes of events, which were constructed from classes of sense impressions.

Although a good deal of Russell's analysis of the content and validity of our knowledge of the world is conducted at an abstract level (as, for example, in *Our Knowledge of the External World*, 1914) he did not hesitate to plunge where necessary into a detailed analysis of current scientific theories. An example of this was *The Analysis of Matter*, in which he discussed the theory of quanta, special relativity, geodesics, invariants, differential equations, the theory of measurement and of space and time.

Perhaps his most direct influence on the philosophy of science has been in these applications of logical construction and other symbolic techniques which first appeared in the *Principia*. Their effect on modern logic (and mathematics) has been incalculable. The success of this analytic approach moved many other philosophers, including the Vienna Circle group of logical positivists, to attempt similar projects in the physical sciences.

Much later in his career (*Human Knowledge: Its Scope and Limits*, 1948) Russell turned again to a sustained attack on the problems specifically related to science and provided a more modern analysis of a number of basic scientific concepts. In this work he presented his analysis of two problems that have been prominent in the philosophy of science: namely, the difficulty of providing a formally correct and materially adequate definition of the nature of probability statements, and the provision of a theory of non-deductive inferences as they are used in the sciences. In 1950 Russell was a founder member of the British Society for the Philosophy of Science.

Characteristically, Russell had no hesitation in attempting the difficult task of bridging the ever widening gap between philosophy and science. In writing *The ABC of Quanta* (1923) and *The ABC of Relativity* (1924) he presented, perhaps somewhat idiosyncratically, the fruits of modern science predigested for philosophers, and demonstrated the benefits of analytic philosophy in a manner palatable to scientists. A Herculean task!

Beyond his philosophy of science, Russell was an indefatigable popularizer of science and an exponent of the application of scientific method to the solving of social, political, moral and philosophical problems. *Our Knowledge of the External World* was subtitled "As a field for Scientific Method in Philosophy". He was undoubtedly among the pioneers who called for social responsibility on the part of scientists and he acted accordingly, in repeatedly analysing the impact of science and technology on society.

Perhaps some insight into the source of Russell's continuing influence is found in the concluding paragraph of *Human Knowledge*. "In this sense, it must be admitted, empiricism as a theory of knowledge has proved inadequate, though less so than any other previous theory of knowledge. Indeed, such inadequacies as we have seemed to find in empiricism have been discovered by strict adherence to a doctrine by which empiricist philosophy has been inspired: that all human knowledge is uncertain, inexact and partial. To this doctrine we have not found any limitation whatever."

Correspondence

Can a Scientific Article be Libellous?

SIR,—In a case before the Court of Appeal, reported in *The Times* (February 13, 1970), a dental surgeon was given leave to take libel action against the British Medical Association and others. The Court held that "a scientific paper in the *British Medical Journal* which was critical of a technique for dental anaesthesia introduced, used and recommended [previously in the same journal] by a named dental surgeon was held by a majority of the Court to be capable of bearing a meaning defamatory of the surgeon in the way of his profession and that it should not therefore be struck out *in limine* as disclosing no reasonable cause of action". While the judges avoided implications as to the likely outcome of such action in court, they maintained that a suit for libel was justified. As suggested in the dissenting judgment by the Master of the Rolls, Lord Denning, the ramifications of this decision on the scientific world could be serious.

The central issue in the majority judgment was expressed by Lord Pearson, who was reported as saying that "the author's name, practice and reputation have been and were closely associated with the technique in question". Therefore, an attack on the technique held forth the possibility of being an attack on the author and must be entered to jury-decision. In suits claiming libel, the defendant must prove to a jury's satisfaction that his actions did not constitute a personal, subjective defamation of the plaintiff. However, the cost in time and funds to prepare a case to the satisfaction of a lay-jury could be prohibitive to many research efforts and personally ruinous to the defendant.

The Master of the Rolls was reported as expressing that "it would be a sorry day if scientists were to be deterred from publishing their findings for fear of libel actions. So long as they refrained from personal attacks, they should be free to criticize the systems and techniques of others. It was in the interest of truth itself. Were it otherwise, no scientific journal would be safe". On the basis of the judgment in this case, allowing the libel action to proceed, there is clearly cause for concern for all of us. Within the scientific community one accepts that criticism is given and taken. While reputations may be enhanced or damaged in this manner, it rarely becomes a legal issue. However, individual criticism of any research involving commercial interests would appear now to be open to the risk of expensive libel action.

Yours faithfully,

JOHN R. LEWIS
JOHN S. GRAY
LELAND W. POLLOCK
P. GEOFFREY MOORE

Wellcome Marine Laboratory,
University of Leeds,
Robin Hood's Bay,
Yorkshire.

Should Slides be Seen Blind?

SIR,—The suggestion is in the wind that, in the safety evaluation of drugs and potential food additives, histopathological material derived from studies on animals should be examined "blind"; that is to say, by a pathologist who is not told which material is derived from treated animals and which from untreated controls. If this suggestion is being seriously put forward, and if it applies to the first time that slides are to be examined, then we believe it to be ill-advised.

The pathologist's appraisal of toxicity should begin with a consideration of the overall design of the experiment and of details of mortality and morbidity in animals subjected to each form of treatment. Next he should acquaint himself with the clinical history of each animal. Central to his appraisal will be observations made by the naked eye at necropsy. Subsequent microscopic examination of material taken for histopathology may or may not add to the information already gained; but it is more likely to do so if detailed clinical and post mortem reports are available to the pathologist at the time he examines the slides. In any case, unless he has these reports before him, he cannot be sure that the microtome knife has found all the lesions seen macroscopically, and he may be in doubt as to the exact location and size of lesions. This description is nothing more than a statement of the general principles of sound pathological practice as they apply to the special problems associated with the safety evaluation of drugs and other agents.

Many variables face the pathologist as he examines histological material from toxicity studies. It would be impracticable for him to consider all of them. Awareness of the nature of the experiment, the clinical and post mortem findings, and other pharmacological or toxicological information must inevitably direct his attention to relevant features which might otherwise be overlooked.

Once a preliminary survey of the slides from a safety-evaluation experiment has been made, there may be a case for the slides from selected tissues to be re-examined to confirm the presence of a qualitative change in response to treatment, or to measure an apparent quantitative difference between material from treated and control animals. This may be the point at which it would be prudent to ask the same, or a second, pathologist to examine slides "blind". Perhaps the suggestion that is in the wind concerns only such selective re-examination of material. But if primary "blind" examination is proposed, it might as well be done by a blind pathologist !

Yours faithfully,

F. J. C. Roe
R. L. Carter

Department of Experimental Pathology,
Chester Beatty Research Institute,
Institute of Cancer Research,
Fulham Road,
London SW3.

E. Cotchin

Department of Pathology,
Royal Veterinary College,
Royal College Street,
London NW1.

G. M. Bonser

Department of Experimental Pathology
and Cancer Research,
Annexe,
171 Woodhouse Lane,
Leeds LS2 3AR.

Understanding Media

Sir,—On February 15, 1969, *Nature* published a communication from Drs R. G. Edwards, B. D. Bavister and P. C. Steptoe entitled "Early Stages of Fertilization *in vitro* of Human Oocytes matured *in vitro*". To a biologist, the results seemed to be in the nature of a sound record of work in progress, more likely than not to be consistent with a true fertilization *in vitro*, and further communications were awaited with great interest. But there was at that time no evidence of the critical final stages of the fertilization process, and no development to the stage of an embryo. The results could not certainly be distinguished from those to be expected from abortive parthenogenesis, a phenomenon that bedevilled much earlier work on animals before fertilization *in vitro* was conclusively demonstrated in the rabbit by M. C. Chang and others. There was incontrovertible evidence of gross maldistribution of chromosomes in many eggs. All this was made explicit or given due reference in the paper itself.

Shortly after, if my sense of public opinion is correct, large numbers of people came to believe in some or all of the following propositions: (1) that an absolute, unequivocal proof of fertilization of the human egg *in vitro* was available; (2) that this was the first experiment on fertilization *in vitro* in man; (3) that human foetuses had for the first time been cultured *in vitro*; (4) that "life" had been "created".

Since the authors had claimed none of these things, and since all the propositions seemed at that time false, or, at best, arguable, it seems—unless my understanding of public opinion is at fault—that certain misconceptions had spread throughout the world on a large scale. Some still appear to be current. Embarrassment must certainly have been caused to the authors as well as to all others who share with them a regard for the good name of reproductive biology.

The public must necessarily derive its information on technical matters largely from the popular media of mass-communication. It might be opportune for formal enquiry to be instituted into the extent to which these media do actually succeed in giving the public a correct version of scientific material. It would be easy to blame the public itself for its misunderstandings—but perhaps it is the lines of communication that are at fault.

Yours faithfully,

R. A. Beatty

Department of Genetics,
University of Edinburgh,
Scotland.

Capacity for Misunderstanding

Sir,—An important question of terminology arises in the teaching of thermodynamics. In this subject the word "heat" means energy transmitted because of a temperature difference. It very often happens that this is confused with internal energy, as a result of which serious misunderstanding arises. A recent test showed that not one of 148 university entrants in science and engineering knew the scientific meaning of heat. Consequently the first law of thermodynamics and even such an elementary concept as an adiabatic process must be meaningless to them. A contributory factor in this widespread misunderstanding may well be the use of the misleading term "heat capacity". The word capacity conveys the idea of containment, hence this term falsely suggests the containment of heat in matter. Unfortunately the equally misleading term "specific heat capacity" is coming into use.

I suggest that there would be a great educational advantage in discontinuing the use of the word "capacity" in this connexion. It would be better to use some such word as "acceptance". The "heat acceptance" of a body would mean the heat added divided by the consequent temperature rise. Heat is usually partly converted into internal energy in the body and partly into some other form of energy by means of external work. The word acceptance avoids the false implication that heat as such is contained in the body.

Yours faithfully,

J. W. Warren

Department of Physics,
Brunel University,
London W3.

Appointments

V. A. Kotel'nikov, Institute of Radio Engineering and Electronics, Moscow, has been elected to the position of vice-president of the **Academy of Sciences of the USSR.**

Dr Mahlon B. Hoagland, Dartmouth Medical School, has been appointed director of the **Worcester Foundation for Experimental Biology.**

The Secretary of State for Education and Science, **Mr Edward Short,** has appointed four new members of the **Council for Scientific Policy:** Professor G. S. Brindley, London Institute of Psychiatry; Professor A. H. Bunting, University of Reading ; Dr H. Morrogh, British Cast Iron Research Association ; Dr R. G. West, University of Cambridge.

Announcements

The Grand Prix Technique de la Ville de Paris has been awarded to **Professor Max Serruys.**

Dr Richard D. Deslattes has received an **Arthur S. Flemming award,** for his direction of research which led to the successful design, construction and demonstration of the first device combining an X-ray and optical interferometer.

The **International Commission on Radiation Units and Measurements** invites nominations for the award of the **Gray medal.** The medal will be awarded for outstanding contributions in a field of interest to the Commission, and will probably be presented during the ICRU meeting in 1971. Further details can be obtained from W. R. Ney, ICRU, 4201 Connecticut Avenue NW, Washington, DC 20008.

Between six and nine fellowships in microbiology, each lasting nine months, will be sponsored jointly by **Unesco** and the **International Cell Research Organization** to enable young scientists from developing countries to be trained in research laboratories abroad. Further information can be obtained from Unesco, Place de Fontenoy, 75 Paris 7.

ERRATUM. In the article "Effect of β-Adrenergic Blockade and Alcohol on Simulated Car Driving" by V. Goldman, B. Comerford, D. Hughes and G. Nyberg (*Nature*, **224**, 1175; 1969), the measurements of alprenolol levels in plasma in Table 2 should be in nanograms (ng) rather than micrograms (μg).

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Wanted: 4-bedroomed accommodation for English university lecturer and family in Boston, Massachusetts area (Natick or Framingham preferred), from April 1 to December 31, 1970. Please contact Dr F. Jones, Department of Colour Chemistry and Dyeing, The University, Leeds LS2 9JT (telephone Leeds 31751, extension 6125).

Wanted: Furnished house or apartment (2 bedrooms will do) for American professor, wife and two school-age daughters in London (or convenient transport to Imperial College) from late July to December 31, 1970. Will exchange for 3-bedroom furnished house with garden within walking distance of University of California, Berkeley, available July 1 to December 31, 1970. Please contact Dr J. G. Hancock, 2225 Ward Street, Berkeley, California 94705, USA.

Wanted: 1–2 bedroomed, furnished house or flat, preferably in the London WC1 area, for visiting fellow and his wife, from September 1970 for 12 months or more. Please contact Dr G. Gregoriadis, Department of Medicine, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA.

Vacant: From September 1970 to September 1971, detached, furnished house, 3 bedrooms, central heating, garage, gardens. Quiet location in Congleton, Cheshire, close to Jodrell Bank telescope; also good access to Manchester and Keele Universities. Please contact Dr T. J. Franklin, 23 Birch Road, Congleton, Cheshire (telephone Congleton 3796).

Vacant: From July 6 to September 26, 1970, 3-bedroom furnished house in Aberdeen. Central heating; adjacent to University. Please contact Professor P. C. Sylvester-Bradley, Department of Geology, the University, Leicester.

British Diary

Monday, March 16

Measuring Man's Needs (5.30 p.m.) Dr A. J. O'Hara-May, British Society for the History of Science, in the Lecture Theatre of the Science Museum, Exhibition Road, London SW7.

Sonar (5.30 p.m.) Mr T. N. Reynolds, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Choice of Industrial Drives up to 100 hp (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Parliamentary and Scientific Committee (5.30 p.m.) Mr Roger Gresham Cooke, MP, Royal Institution, Library Circle, at 21 Albemarle Street, London W1.

Tuesday, March 17

Aspects of Fertilizer Usage (10.30 a.m.) Society of Chemical Industry, Agriculture Group; and the Fertilizer Society, at 14 Belgrave Square, London SW1.

Deformation and Fracture of Soft Materials (3): Strength of Adhesive Bonds (4.30 p.m.) Professor A. N. Gent, University of London, at Queen Mary College, Mile End Road, London E1.

Differential Thermal Analysis (2.30 p.m.) Society for Analytical Chemistry, East Anglia Section, at Spillers Ltd, Technological Research Station, Station Road, Cambridge.

Electrical Methods of Machining, Forming and Coating (three-day conference) Institution of Electrical Engineers, at Savoy Place, London WC2.

Management and Economics in the Electronics Industry (four-day international symposium) Institution of Electronic and Radio Engineers; and the Institution of Electrical Engineers, at the University of Edinburgh.

Noise Reduction in Manipulative Machinery (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Rubber and Plastics for Use in Hovercraft (7.30 p.m.) Institution of the Rubber Industry, at Retford.

The Nature and Hazards of Nuclear Fallout (7.30 p.m.) Dr R. E. Ellis, British Society for Social Responsibility in Science, at the Blackhorse, Rathbone Place, London W1.

UNIDO and Its Work in the Petrochemical and Plastics Field in Developing Countries (6 p.m.) Mr H. May, Society of Chemical Industry, at 14 Belgrave Square, London SW1.

Wednesday, March 18

Chromosomal Abnormalities in Blood Diseases (2 p.m.) Dr Sylvia D, Lawler, University of London, at the Royal Postgraduate Medical School, Du Cane Road, London W12.

Computer Typesetting (7 p.m.) Mr R. Chapman, Institution of Electronic and Radio Engineers; and the British Computer Society, at the University of Bristol.

Electronic Engineering in the Solution ot Harbour Approach Problems for Large Ships (6 p.m.) Mr T. W. Welch, Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Filtration (9.30 a.m. symposium) Institution of Chemical Engineers, at Victoria Halls, Bloomsbury Square, London WC1.

Foams (2 p.m. symposium) Plastics Institute, at the Great Western Royal Hotel, Paddington.

Gas Turbines—Combustion Chambers—One Large or Many Small? (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Is It Strong Enough? (1.10 p.m.) Professor M. L. Meyer, City University, at the Graduate Business Centre, Gresham College, Basinghall Street, London EC2.

Manoeuvre Demand Control for Aircraft (5 p.m.) Mr G. T. Shanks, Institute of Navigation, at the Royal Institution of Naval Architects, 10 Upper Belgrave Street, London SW1.

Modern Dairy Science (2.30 p.m.) Mr E. L. Crossley, Royal Society of Arts, at John Adam Street, London WC2. (E. F. Armstrong Memorial Lecture.)

Recent Advances in Diagnostic Agents and Techniques (10 a.m.) Nuclear Medicine Committee, Middlesex Hospital Medical School, at the British Institute of Radiology, 32 Welbeck Street, London W1.

Thursday, March 19

Automation in Production, Sampling and Testing of Silicate Materials (9.30 a.m.) Society of Chemical Industry, Road and Building Materials Group, at Victoria Halls, Bloomsbury Square, London WC1.

Clay and Colloid Particles: Size, Shape and Dispersion (two-day spring meeting) Mineralogical Society; jointly with the Basic Science Section of the British Ceramic Society, at the Department of Agricultural Science, Downing Street, Cambridge.

Electronic Aids to Night Vision (4.30 p.m.) Mr P. Schagen, Royal Society, at 6 Carlton House Terrace, London SW1.

High Energy Ignition on Jet Aeroengines—a Survey of Current Techniques and Future Trends (7 p.m.) Mr B. R. Clifford, Institution of Electronic and Radio Engineers, at Medway College of Technology, Chatham, Kent.

Inducers and Supercavitating Pumps (6 p.m. discussion) Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1.

Influence of Stricture on the Base Metal Deposits of Southwest Sardinia, Italy, Mr J. C. McM. Moore; **Genesis of the Forari Manganese Deposit, New Hebrides,** Mr A. J. Warden (5 p.m.) Institution of Mining and Metalurgy, at the Geological Society, Burlington House, Piccadillly, London W1.

Laser Applications in Electronics (7.30 p.m.) Professor W. A. Gambling, Institution of Electronic and Radio Engineers, in the J. J. Thomson Physical Laboratory, University of Reading.

Neutron Therapy (5.45 p.m. discussion); **Tissue Heterogeneity and Treatment Planning** (7.15 p.m.) Dr A. H. Laing, Mr G. Innes and Dr T. E. Burlin, British Institute of Radiology, at 32 Welbeck Street, London W1.

Paint User Test Methods (half-day conversation) Oil and Colour Chemists' Association, in the New Engineering Block, University College, London, Gower Street, London WC1.

The Code of Practice for Cathodic Protection (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Friday, March 20

Automatic Methods in Analysis (6.30 p.m. discussion) Society for Analytical Chemistry, Western Section, at the George Hotel, Chepstow.

Cosmetics—Odour and Colour (6.30 p.m.) Mr J. P. Slater, Oil and Colour Chemists' Association, at the Chamber of Commerce House, 75 Harbourne Road, Birmingham 15.

European Conservation—Action Now (1.15 p.m.) Mr R. J. S. Hookway, Institute of Rural Life at Home and Overseas, at Friends International Centre, Courtauld House, Byng Place, London WC1.

Microwave Filters (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Proteolytic Enzymes (6.30 p.m.) Dr R. G. W. Spickett, Society of Chemical Industry, at 14 Belgrave Square, London SW1.

Technological Forecasting and Corporate Long-range Planning (5.30 p.m.) Dr B. C. Lindley, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Mechanism of Visual Receptors (9.45 a.m. all-day meeting) British Photobiology Society, at the Institute of Ophthalmology, Judd Street, London WC1.

Saturday, March 21

Village Craftsmen of Buganda (3.30 p.m.) Mr Bruce Kent, Inner London Education Authority, at the Horniman Museum, London Road, Forest Hill, London SE23.

Monday, March 23

Electrical Aspects of Spacecraft (7.30 p.m.) Mr H. J. H. Sketch, Institution of Electrical Engineers, at the J. J. Thomson Laboratory, University of Reading, Whiteknights Park, Reading.

Integrated Management Systems in Manufacture (5.30 p.m.) Mr F. N. Stephenson, Institution of Electrical Engineers, jointly with the Institute of Measurement and Control and the I.Mech.E., at Savoy Place, London WC2.

Memorial Service for Lord Jackson of Burnley, FRS (12 noon) at Holy Trinity Church, Prince Consort Road, London SW7.

Newer Polymers (10 a.m. symposium) Society of Chemical Industry, Plastics and Polymer Group, in Physics Lecture Theatre 1, Imperial College, London SW7.

Overhead-line Oscillations and the Effect of Aerodynamic Dampers (5.30 p.m.) Mr J. C. R. Hunt and Mr D. J. W. Richards, Institution of Electrical Engineers, at Savoy Place, London WC2.

Structural Dynamics (three-day symposium) Loughborough University of Technology, in association with the British Acoustical Society; the Institution of Civil Engineers; the Institution of Mechanical Engineers; and the Royal Aeronautical Society, at the University.

The Biochemistry of Steroid Hormone Action; Cerebral Enzymes and Isoenzymes; Membrane-bound Enzymes; Mitochondria and Calcium Transport, Professor A. L. Lehninger (Fifth Jubilee Lecture) (two-day meeting) Biochemical Society, at University College, London, Gower Street, London WC1.

The Dielectric Properties of Heterogeneous Systems including Biological Materials (three-day meeting) Dielectrics Discussion Group, at the University College of Wales, Aberystwyth.

The Shape of Things: General Conceptions of the Nature of Things (7.45 p.m.) Mr Ian Ross, H. G. Wells Society, in the Common Room of the Electrical Engineering Department, Imperial College, Exhibition Road, London SW7.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

UKAEA Research Group Report 6212: Radioactive Fallout in Air and Rain—Results to the Middle of 1969. By R. S. Cambray, Miss E. M. Fisher, W. L. Brooks and D. H. Peirson. Pp. 46. (Harwell: Atomic Energy Research Establishment, 1969. Available from HM Stationery Office.) 7*s*. [1812

Ministry of Technology. Computer-aided Design Committee—Second Annual Report, 1968–1969. Pp. xiii + 44. (London: Ministry of Technology, 1969.) [1912

Chelsea College of Science and Technology, University of London. Annual Report 1968/1969. Pp. 65. (London: Chelsea College of Science and Technology, 1969.) [1912

Department of Education and Science. Further Review of Certain Persistent Organochlorine Pesticides Used in Great Britain. (Report by the Advisory Committee on Pesticides and other Toxic Chemicals.) Pp. iii + 148. (London: HM Stationery Office, 1969.) 12*s* 6*d* (62.5*p*) net. [1912

Food Aid and Britain. By Hal Mettrick. Pp. 124. (London: Overseas Development Institute, Ltd., 1969.) 30*s*. [1912

Universities Research Reactor—Annual Report 1968/1969. Pp. vii + 34. (Risley, Warrington: Universities Research Reactor, 1969.) [1912

The Growth of Cancer Cells. By Professor E. H. Cooper. (Inaugural Lecture delivered in the University of Leeds on 17 March, 1969.) Pp. 22. (Leeds: Leeds University Press, 1969.) 3*s* 6*d*. [2212

Chemistry and Industry, 1970 Buyers' Guide: Chemicals, Chemical Plant and Laboratory Equipment. Pp. 127. (London: Society of Chemical Industry, 1969.) 20*s*. [2212

Department of Employment and Productivity. Training Research Register 1969. Pp. 144. (London: HM Stationery Office, 1969.) 12*s* 6*d* (62.5*p*) net. [2212

Your Environment, No. 1, Winter 1969. Pp. 1–32. Subscription: 30*s* for four issues. (London: *Your Environment*, 10 Roderick Road, NW3.) [2312

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 265, No. 1165 (18 December, 1969): Solution of Laplace's Equations for the M_2 Tide in the World Oceans. By C. L. Perkeris and Y. Accad. Pp. 413–436. 17*s*; $2.20. Vol. 265, No. 1166 (18 December, 1969): Interpretation of Radio Echo Sounding in Polar Ice Sheets. By G. de Q. Robin, S. Evans and J. T. Bailey. Pp. 437–505. 43*s*; $5.60. (London: The Royal Society, 1969.) [2312

Proceedings of the Royal Irish Academy. Vol. 68, Section A, No. 5: Reduction of the Images of the Fermi-Surface in the Phonon Spectra of Metals by Exchange Forces. By N. L. Balazs. Pp. 49–54. 2*s*. Vol. 68, Section A, No. 6: The Detection of Radon in the Atmosphere. By J. P. McLaughlin and P. J. Nolan. Pp. 55–69. 3*s* 6*d*. Vol. 68, Section B, No. 8: Fungi Associated with Irish Peat Bogs. By G. H. Dickinson and M. Dooley. Pp. 109–136 + plate 6. 5*s*. Vol. 68, Section B, No. 9: Investigations of the Plankton of the West Coast of Ireland—III. *Calanis finmarchicus* (Gunn) and *Calanis helgolandicus* (Claus) in the Plankton of Killeany Bay, Aran Islands. By B. F. Keegan. Pp. 137–147. 2*s*. Vol. 68, Section B, No. 10: Helminth Parasites from Charadriiform Birds at Galway Bay, Co. Galway. By D. Cabot. Pp. 149–159. 2*s* 6*d*. (Dublin: Royal Irish Academy, 1969.) [2412

Other Countries

Food and Agriculture Organization of the United Nations. FAO Nutrition Meetings Report Series No. 45: Specifications for the Identity and Purity of Food Additives and their Toxicological Evaluation—Some Antibiotics. (Twelfth Report of the Joint FAO/WHO Expert Committee on Food Additives, Geneva, 1–8 July, 1968.) Pp. 49, 6*s*; $1. FAO Nutritional Studies No. 22: Manual on Food and Nutrition Policy. Prepared with the assistance of B. F. Johnston and J. P. Greaves. Pp. viii + 95. 16*s*; $2. (Rome: Food and Agriculture Organization of the United Nations; London: HM Stationery Office, 1969.) [1612

WHO Technical Report Series, No. 431: Toxoplasmosis—Report of a WHO Meeting of Investigators. Pp. 32. (Geneva: World Health Organization; London: HM Stationery Office, 1969.) 3 Sw. francs; 6*s*; $1. [1612

The Walter and Eliza Hall Institute of Medical Research 1968/1969. Pp. 93. (Parkville, Victoria: The Walter and Eliza Hall Institute of Medical Research, 1969.) [1712

Annals of the South African Museum. Vol. 52, Part 10: The Behaviour of Captive Cape Rock Lobsters *Jasus lalandii* (H. Milne Edwards). By Nellie F. Paterson. Pp. 225–264. (Cape Town: South African Museum, 1969.) [1712

Yearbook of Physical Anthropology, 1967. (Yearbook Series Volume 15.) Edited by Santiago Genovés, in association with Jack H. Prost and Gabriel W. Lasker. Pp. 304. (Published by the American Association of Physical Anthropologists, in co-operation with Seccion de Antropologia, Instituto de Investigaciones Historicas, Universidad Nacional Autonoma de Mexico; and Instituto Nacional de Antropologia e Historia, Cordoba 45, Mexico, 7, DF.) (Dallas: Secretary-Treasurer, AAPA, Box 339, 1969.) [1712

India. Forest Research in India, 1963/1964, Part 1—The Forest Research Institute. Pp. 109 Rs. 8.50; 19*s* 10*d*. Forest Research in India, 1964/1965, Part 1—The Forest Research Institute. Pp. 161. Rs. 6.25; 14*s* 7*d*. Indian Forest Bulletin No. 257 (New Series): Influence of Steaming on Subsequent Drying of Green and Refractory Hardwood. By D. P. Ghosh and P. G. Gupta. Pp. 6. Rs. 1.95; 4*s* 7*d*. Indian Forest Bulletin No. 258 (New Series): Effect of Pre-Steaming on Drying Rates in Subsequent Seasoning of Green and Refractory Hardwood. By S. N. Sharma and B. I. Bali. Pp. 8. Rs. 1.60; 3*s* 9*d*. Indian Forest Records (New Series), Vol. 2, No. 9: Cultural Diagnosis of Indian Polyporaceae. 1: Genus *Polyporus*. By B. K. Bakashi, H. S. Sehgal and Balwant Singh. Pp. 205–244 + 13 plates. Rs. 10; 23*s* 4*d*. (Delhi: Manager of Publications, 1968 and 1969.) [1712

HOW TO BUY NATURE

Volumes start in January, April, July and October but subscriptions may begin with any issue.

The direct postal price per subscription for one copy of NATURE each week is:

12 MONTHS (52 issues and 4 indexes)

Great Britain and Eire .... **£14.0.0** each
United States of America .... (by air freight) .... **$48** each
Canada .... (by air freight) .... **$52** each
Elsewhere overseas by surface mail .... **£14.0.0** each
(Charge for delivery by air mail on application)
*Shorter periods *pro rata*. (Minimum three months.)

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED
4 LITTLE ESSEX STREET, LONDON WC2
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2

Subscription Department
MACMILLAN (JOURNALS) LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 5431

Advertisements only should be addressed to
T. G. SCOTT & SON, LIMITED
I CLEMENT'S INN, LONDON WC2A 2ED
Telephone 01-242 6264/01-405 4743
Telegrams: Textualist London WC2
Registered as a newspaper at the Post Office

Copyright © Macmillan (Journals) Limited, March 14, 1970

Classified Advertisements

All copy is subject to the approval of the Publishers, who retain the right to refuse or withdraw advertisements at their discretion. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes. Semi-displayed £7 4s. per single column inch. Minimum £3 12s., each additional 1/12th of an inch 12s. Full page £190, Half page across £96. Colour (orange) £25 extra. 2/6 is charged for the re-direction of replies to advertisement with a box number.

ADVERTISEMENTS SHOULD BE ADDRESSED TO: T. G. Scott and Son, Limited, 1 Clement's Inn, London, WC2A 2ED. Telephone: 01-405 4743. Telegrams: Textualist, London,W.C.2.

APPOINTMENTS VACANT

UNIVERSITY OF EDINBURGH

DEPARTMENT OF BOTANY

Applications are invited for posts of LECTURER and UNIVERSITY DEMONSTRATOR in the above Department.

LECTURER

Preference will be given to applicants having qualifications in Botany and Biochemistry. The duties involve teaching plant biochemistry and plant physiology to elementary and advanced students. The successful candidate will also be expected to engage in research. A number of workers in the Department are developing an investigation into certain aspects of the relation between protein synthesis and growth. A candidate already working in this general field would be of particular interest.

Salary Scale: £1,240 to £2,850 per annum, together with F.S.S.U. benefits.

DEMONSTRATOR

The successful candidate will be expected to assist with the teaching of advanced and elementary students, and to engage in research.

Salary Scale: £1,060 to £1,445 per annum, together with F.S.S.U. benefits.

Applications, by letter (three copies), giving full details of qualifications and experience, together with the names of two referees, should be sent to the Secretary to the University, Old College, South Bridge, Edinburgh, EH8 9YL, not later than March 20, 1970. Please quote reference 1018 Lecturer and 1019 Demonstrator.

(1006)

UNIVERSITY OF DURHAM

DEPARTMENT OF CHEMISTRY

POST-DOCTORAL RESEARCH ASSISTANTSHIP
in Organo-transition Metal Chemistry

Applications are invited for a Post-doctoral Research Assistantship tenable for a period of up to two years in collaboration with Dr. M. Kilner. The investigation will be concerned with an infrared spectral study of the interaction of small molecules, particularly CO, H_2, N_2, $RCH{=}CH_2$ and O_2, with a series of organo-transition metal complexes at high pressures and temperatures using a specially constructed high pressure cell.

Salary in the range £1,200 by £85 to £1,455 per annum with F.S.S.U. Starting date negotiable.

Applications (three copies, overseas one copy), including a curriculum vitae and the names of three referees, should be sent by April 20, 1970, to the Registrar and Secretary, Old Shire Hall, Durham, from whom further particulars may be obtained.

(1034)

MICROSCOPE SALES

WILD HEERBRUGG (U.K.) LIMITED require a **Technical Field Representative** for their Microscope Division promoting and selling Swiss precision instruments. The territory to be covered will be in the North of England.

Applicants should either be Representatives experienced in microscope selling or men with wide experience in the application of microscopes. The post is an important one in an expanding Company and a high salary will be paid to the successful applicant.

Applicants should write to:

The Managing Director,
Wild Heerbrugg (U.K.) Limited,
49-51 Church Street,
MAIDSTONE, Kent.

(1040)

IRELAND

BIOCHEMIST

required for
CORK HEALTH AUTHORITY
Salary: £2,385–£2,900.

Essential: M.Sc. in biochemistry or physiological chemistry, or equivalent, and five years' experience of biochemistry or chemical pathology. If a suitable candidate is appointed, University College, Cork, proposes to establish in the College a Part-time Lectureship in the subject. The salary envisaged for such a post is of the order of £1,000 per annum. Post-entry training in Clinical Biochemistry, if necessary, with full pay.

Application forms and further particulars from Secretary, Local Appointments Commision, 45 Upper O'Connell Street, Dublin 1.

Latest date for receiving completed application forms:
April 15, 1970

(1043)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BOTANY

CARBOHYDRATE METABOLISM IN FUNGI

Applications are invited from those with the relevant research experience in biochemistry, plant physiology or microbiology to take a major part in a programme of research in the Department of Botany into carbohydrate biochemistry of fungi with particular emphasis on the metabolism of sugar alcohols. The appointment commences on October 1, of this year and is for three years. Initial salary £1,265 per annum.

Applications, stating age, academic qualifications and experience, together with the names of two referees, should be received not later than May 2, 1970, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Please quote Ref.: RV/5753/N.

(1064)

UNIVERSITY OF STRATHCLYDE

Department of Biology

LECTURESHIP

Applications are invited from suitably qualified candidates for the above post. Candidates should have a Higher Degree and/or research experience in Parasitology.

Salary scale: Lecturer, £1,240 to £2,850 per annum with F.S.S.U.

Application forms and further particulars (quoting 16/70), may be obtained from the Registrar, University of Strathclyde, George Street, Glasgow, C.1, with whom applications should be lodged not later than March 31, 1970.

(1077)

Ministry of Overseas Development

AGRICULTURAL ECONOMIST

(graded Principal Scientific Officer)

£2674–£3671

This post is based in the Land Resources Division of the Directorate of Overseas Surveys, at Tolworth, Surrey, but could involve some service overseas.

The division undertakes integrated land resource appraisals at reconnaissance semi-detailed or detailed level, advises on planning for the development and use of these resources, and undertakes or advises on feasibility studies, under technical assistance arrangements for Overseas Governments (mainly tropical countries within the Commonwealth).

The successful candidate will be concerned with appraisal of the economic aspects of project assessment and planning, and of feasibility studies for agricultural and forestry developments (including those involving irrigation); and with the collection and processing of agro-economic data in land resource surveys.

Candidates (men only) should normally have a first or second class honours degree in Agricultural Economics, or in Agriculture with postgraduate qualification in Agricultural Economics. Experience in tropical agriculture and related development and feasibility studies, and a knowledge of modern statistical and computer techniques are important. Preference will normally be given to those between 30 and 50.

Starting salary may be above minimum of the quoted scale. There is a non-contributory pension scheme, and there are prospects of promotion to Senior Principal Scientific Officer (£4,045–£4,620) and above.

For full details and an application form, write to Civil Service Commission, 23 Savile Row, London WIX 2AA, or telephone 01–734 6010 ext. 229 (after 5.30 p.m. 01–734 6464 "Ansafone" service), quoting *S/7426/70*. Closing date 9 April 1970.

(1028)

UNIVERSITY OF LONDON

READERSHIP IN SOCIAL STATISTICS AT LONDON SCHOOL OF ECONOMICS

The Senate invite applications for the Readership in Social Statistics tenable at the London School of Economics and Political Science (salary scale £2,770 to £3,670 plus £80 London Allowance).

Applications (ten copies), must be received not later than May 1, 1970, by the Academic Registrar, University of London, Senate House, W.C.1, from whom further particulars may be obtained. (1091)

UNIVERSITY OF GLASGOW

LECTURESHIP IN STATISTICS

Applications are invited for a Lectureship in Statistics. Initial salary placement within the £1,240 to £1,470 per annum range of the Lecturers' scale. F.S.S.U.

Applications (eight copies), should be lodged not later than April 4, 1970, with the undersigned from whom further particulars may be obtained. In reply please quote Ref. No. 3005-M.

ROBT. T. HUTCHESON,
Secretary of the University Court.

(1063)

UNIVERSITY OF NEWCASTLE UPON TYNE

THE MEDICAL SCHOOL

DEPARTMENT OF DERMATOLOGY

Applications are invited from suitably qualified University graduates for appointment as Junior Research Associate for research into the aetiology and treatment of acne. The appointment will be for one year in the first instance but will be renewable up to a maximum of three years in all. A suitably qualified candidate would be encouraged to read for a higher degree. The successful candidate will be paid allowances equated to those paid in the case of M.R.C. or S.R.C. Studentships.

Applications should be sent to Professor S. Shuster, The Deparament of Dermatology, The Royal Victoria Infirmiry, Newcastle upon Tyne, NE1 4LP, as soon as possible. (1062)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

(UNIVERSITY OF READING)

BACTERIOLOGY DEPARTMENT

Microbiologist required to study the metabolism of lactic acid bacteria in pure culture and in association with other bacteria isolated from cheese. A basic study of the production of those metabolic end-products, which are related to cheese flavour, is involved. Facilities for gas-liquid chromatography and mass spectrometry are available and there are opportunities for obtaining a higher degree. Appointment will be in the grade of Scientific Officer (£1,071 to £1,827) or Senior Scientific Officer (£2,021 to £2,491) according to age and experience. F.S.S.U.

Applications, on forms obtainable from the Secretary, N.I.R.D., Shinfield, Reading, RG2 9 AT, are invited from honours graduates. Quote ref. 70/8. (1041)

UNIVERSITY OF BRISTOL

DEPARTMENT OF AGRICULTURE AND HORTICULTURE

LONG ASHTON RESEARCH STATION

FOOD CHEMIST/ BIOCHEMIST

Applications are invited for the appointment of a SCIENTIFIC OFFICER in the Domestic Food Preservation Section to undertake biochemical research on quality changes in preserved foods, with particular reference to textural changes.

The successful applicant will be required to commence on September 1, 1970, and should hold a good honours degree in Food Science, preferably having postgraduate research experience. An Honours graduate could expect to have opportunities of working towards a higher degree.

Appointment will be in the Scientific Officer grade (£1,071 to £1,827 per annum). Starting salary dependent upon age, date of degree award and postgraduate experience. Membership of the Federated Superannuation System for Universities will be compulsory.

Applications (one copy), together with the names and addresses of three referees, should be forwarded to the Registrar, University of Bristol, Senate House, Bristol BS8 1TH, by April 6, 1970, from whom further particulars of the post are available (please quote reference E.B.). (1032)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF METALLURGY

Applications are invited for the posts of

LECTURER in physical metallurgy. For one post the candidate should have a special interest in the physical metallurgy of steel; for the other post a candidate having special interest in any branch of physical metallurgy would be considered. It is hoped to make appointments from younger candidates who will have recently obtained higher degrees in the fields of research outlined. Informal inquiries should be addressed to Professor Nutting, Department of Metallurgy, Houldsworth School of Applied Science.
Reference No. 36/2/D.

SALARY SCALE: £1,240 to £2,850 (efficiency bar £2,045).

Applications (three copies), stating age, qualifications and experience and naming three referees, should reach the **Registrar, The University, Leeds LS2 9JT** (from whom further particulars may be obtained), not later than April 6, 1970. *Please quote reference number* (1047)

UNIVERSITY OF SURREY

DEPARTMENT OF MATHEMATICS

Applications are invited for the post of LECTURER IN PURE MATHEMATICS. Salary will be on the scale £1,585 to £2,045 (bar) to £2,850 per annum, with F.S.S.U. benefits.

Applications, together with the names and addresses of two referees, should be submitted by March 23, 1970, to the Academic Registrar (LFG), University of Surrey, Guildford, Surrey, from whom further particulars may be obtained.

(1035)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF BOTANY

Applications are invited for the post of **LECTURER or ASSISTANT LECTURER** in the Department of Botany. Candidates should have an interest in Taxonomy and will be expected to assist in organising the Botanic Garden and to take part in elementary teaching. Salary scales: Lecturer, £1,585 to £2,850 with a merit bar at £2,045; Assistant Lecturer, £1,240 to £1,585. The initial salary will depend on qualifications and experience.

Further particulars may be obtained from the Deputy Secretary, The University, Southampton, SO9 5NH, to whom applications (seven copies from United Kingdom applicants) should be sent as soon as possible, quoting ref.: Na. (938)

GHANA CIVIL SERVICE

Applications are invited for the following positions in the Geological Survey Department:

SENIOR CHEMIST

Duties: To take responsibility of and supervise work in chemical laboratory; training chemical technicians and other junior personnel.

Qualifications: Postgraduate degree in chemistry with at least six years experience.

Salary: N¢4,788 by N¢126 to N¢5,544 per annum.

SENIOR PETROLOGIST

Duties: To take charge of Petrological laboratories, research into relationships of Ghana rocks and non-economic minerals.

Qualifications: A first- or upper second-class Honours degree in Geology and a postgraduate degree in Petrology with four years postgraduate experience.

Salary: As for Senior Chemist.

Supplementation of salary is payable to eligible British subjects designated by Ministry of Overseas Development for the purpose.

Appointments on contract for three tours each of 15 months. Tax free gratuity at rate of 10 per cent of salary at end of each tour of duty. Also tax free resettlement gratuity of 20 per cent of aggregate salary on satisfactory completion of contract. Free passages for officer, wife and up to three children under 18 years. An education allowance for children when not resident in Ghana and attending full-time school of £100 a child for up to three children under 18 years. Generous leave on full pay. Income Tax at low local rates.

Application forms may be obtained from: The Director of Recruitment (Overseas), Ghana High Commission, 38 Queen's Gate, London, S.W.7. (1066)

UNIVERSITY COLLEGE CARDIFF

DEPARTMENT OF ANATOMY

Applications are invited from registered Medical or Science graduates, male or female, for the post of Lecturer in Anatomy and Histology. The appointment carries responsibility for teaching preclinical medical students at ordinary and honours levels. Previous teaching experience is an advantage. The appointee must engage in research work and candidates with strong investigative inclinations will be favourably considered. Duties to commence October 1, 1970. Salary will be within the scale £1,240 by £115 to £2,850. Permanent appointment of medical graduates at not less than £1,585.

Applications (two copies), together with the names of three referees, should be received by the Registrar, University College, Cardiff, from whom further particulars may be obtained not later than April 11, 1970. Quote ADV/414/N. (1065)

MINISTRY OF AGRICULTURE, FISHERIES AND FOOD

Chief Scientific Adviser (FOOD)

up to £6500

The prime accountability of this post is to advise the Minister and the Ministry generally on all questions relating to scientific and technological aspects of food and the food industry. Specifically duties will include (a) liaison with organisations conducting research and the scientific and technological side of industry; (b) representing the Ministry on committees concerned with research and consumer protection on the domestic and international fronts and (c) the co-ordination of the work of the Ministry's food science branches.

Candidates must be of high professional standing and must have a comprehensive knowledge of the general structure of the food industry and wide experience in food science and technology and in related scientific fields.

The appointment may be taken up on a short term contract if this is preferred to the normal established basis. Salary is negotiable up to a maximum of £6500.

Fuller details of this appointment may be obtained by writing to the ***Civil Service Commission, 23 Savile Row, London, W1X 2AA,*** *or by telephoning* ***01-734*** *6010, ext. 229 (01-734 6464 "Ansafone" service after 5.30 p.m.). At all times please quote* ***S/7328/U****. Closing date 31st March 1970. Candidates who have already applied should not do so again.*

(1054)

UNIVERSITY COLLEGE OF RHODESIA

Applications are invited for SENIOR LECTURESHIP OR LECTURESHIP IN DEPARTMENT OF AGRICULTURE. Appointment, available in mid-1970 may be held in one of the following disciplines:

Soil Physics: Applicants should be qualified to teach an introductory course in Physics to agricultural students as well as Soil Physics to students specialising in Soil Science. There are interesting opportunities for research in irrigation, the effect of tillage and the mechanics of soil erosion.

Crop Science: Applicants should be qualified to participate in teaching crop ecology and Physiology and Crop Pasture Production and to carry out research and to supervise that of postgraduate students.

Animal Science: Applicants should be Veterinarians or Animal Physiologists qualified to teach and carry out research in the spheres of either Bacteriology/Animal Health or Animal Physiology/Beef Cattle Production.

Salary scales: Senior Lecturers, $R5,616 by $R240 to $R7,536; Lecturer Grade I, $R4,968 by $R216 by $R5,832; Grade II, $R3,624 by $R192 to $R4,776 ($R1 = 11s. 8d. sterling). Family passages and allowance for transport of effects on appointment. Installation loan of up to half of one year's salary if required. Assistance with accommodation for three years for persons recruited from outside Rhodesia. Sabbatical and triennial visits with travel allowance. Superannuation and medical aid schemes.

Detailed applications (six copies), naming three referees by April 10, 1970, to Inter-University Council, 90/91 Tottenham Court Road, London, WIP ODT, from whom particulars are available. (1080)

AGRICULTURAL RESEARCH COUNCIL

INSTITUTE FOR RESEARCH ON ANIMAL DISEASES

COMPTON, NR. NEWBURY, BERKS

A new appointment has become available in a group studying host-pathogen relationships and other aspects of disease processes. The successful applicant would have a large measure of choice of research in relation to livestock kept under intensive methods of production. A veterinary qualification is desirable and previous experience in a relevant field such as pathology, histochemistry, immunology or microbiology, would be an advantage. Appointment in the Scientific Officer or Senior Scientific Officer grade with prospects of promotion. Superannuation under F.S.S.U.

Applications should be sent to the Secretary, quoting reference 113, together with the names of two referees, not later than April 14, 1970. (924)

UNIVERSITY OF GLASGOW

ST. MUNGO (NOTMAN) CHAIR OF PATHOLOGY

The Board of Curators invite applications for appointment to the St. Mungo (Notman) Chair of Pathology (at the Royal Infirmary), which will become vacant on August 1, 1970.

Applications (twenty copies), with the names of three persons to whom reference may be made, should be submitted not later than April 8, 1970, to the undersigned, from whom further particulars may be obtained. In reply please quote Ref. No. 1000-M.

ROBT. T. HUTCHESON,
Secretary of the University Court.
(1015)

BEECHAM RESEARCH LABORATORIES

BROCKHAM PARK, BETCHWORTH, SURREY

have the following vacancies:

IMMUNOLOGICAL TECHNICIAN

who is required to join a team of scientists working on projects connected with Immunosuppression. The work involved would include responsibility for evaluation of novel biochemicals as agents for the modification of the immune response.

Age is immaterial but the candidate, either male or female, would be expected to possess O.N.C. or A level qualifications in a Biological subject. Previous experience in animal work and/or Haematology preferred.

ANIMAL CARE ASSISTANT

A young person is required to join a team of scientists working on certain aspects of virus disease in experimental animals. No qualifications are required but I.A.T. training would be an advantage and opportunities would be given for further study. The person appointed will be expected to handle small laboratory animals, including feeding and maintenance, as well as some laboratory work. Possession of a Home Office licence would be an advantage.

Beecham Research Laboratories is part of the Beecham Group and is situated in a pleasant area of Surrey within easy reach of London. The successful applicants will qualify for the Group's generous profit-sharing and non-contributory pension and life assurance schemes. There are excellent dining facilities and a flourishing sports and social club.

For both these positions, applicants, stating briefly age, qualifications and experience, should apply in writing to the Personnel Officer, or telephone BETCHWORTH 3202, Ext. 83.

(1085)

OXFORD UNIVERSITY

BOTANY DEPARTMENT

Applications are invited for two posts of DEPARTMENTAL DEMONSTRATOR (equivalent to Assistant Lecturers elsewhere but with opportunities for College tuition) to begin in October, 1970:

(1) Appointment for up to three years in the first instance. Candidates should be interested in Genetics of Flowering Plants.

(2) Appointment for one year. Candidates should be interested in the experimental systematics of Flowering Plants.

Salary up to a maximum of £1,515 per annum.

Applications (or inquiries) should be sent with the names of two referees, to the Administrator, Botany School, South Parks Road, Oxford by March 25, 1970.

(1044)

UNIVERSITY OF HOHENHEIM

STUTTGART, W. GERMANY

CHAIR OF POPULATION GENETICS

An der Universität Hohenheim ist ein neugeschaffener ordentlicher Lehrstuhl für Populationsgenetik zu besetzen. Der Lehrstuhlinhaber soll die experimentelle und theoretische Populationsgenetik in Lehre und Forschung vertreten.

Interessenten werden gebeten, ihre Bewerbung hit Lebenslauf und Publikations-liste bis zum 31. Mai 1970 einzusenden an den Vorsitzenden der Berufungskommission, Prof. F. W. Schnell, Universität Hohenheim (LH), 7 Stuttgart-Hohenheim, Postfach 106. (1100)

Robertson Research International Limited

Further expansion in the United Kingdom and overseas has produced additional vacancies for both senior staff with up to ten years industrial experience, and for students graduating in 1970, in the following divisions:

Analytical Chemistry
Civil Engineering
Library and Information Office
Metallurgy
Micropalaeontology
Mineral Economics and Statistics
Mineral Geology
Mining Engineering
Petroleum Geology
Rock Mechanics
Sedimentary Petrography
Soil Mechanics

All permanent and pensionable appointments with incremental salary scales. Overseas allowances appropriate to different areas when operating outside United Kingdom. For further details apply to Personnel Officer, Robertson Research International Limited, Tyn-y-Coed, Llanrhos, Llandudno, Caernarvonshire, United Kingdom.

(1087)

UNIVERSITY OF STRATHCLYDE

Department of Biochemistry

RESEARCH ASSISTANT

Applications are invited for the above post in an M.R.C. Research Group studying biochemical aspects of uterine metabolism.

Applicants, with postgraduate experience, who possess either a first degree or have postgraduate experience in Biochemistry, should have interests in either: (a) nucleic acid metabolism, or (b) protein fractionation, or (c) study of interaction between chemical agents and macromolecular substances.

The appointment will be for a period of five years in the first instance. Commencing salary £1,200 to £1,300 per annum with F.S.S.U.

Applications (quoting R5/70), to Professor P. J. Heald, Department of Biochemistry, University of Strathclyde, George Street, Glasgow, C.1, from whom further particulars can be obtained. (1076)

AUSTRALIAN NATIONAL UNIVERSITY

RESEARCH SCHOOL OF PHYSICAL SCIENCES

TECHNICAL OFFICER/SENIOR TECHNICAL OFFICER

DEPARTMENT OF GEOPHYSICS AND GEOCHEMISTRY

Applicants should have experience in quantitative X-ray Fluorescence analysis and will be responsible for the operation of this facility within the Department.

Salary will be within the following ranges depending upon qualifications and experience: Technical Officer Grade I, $A3,475 to $A4,285 per annum ; Technical Officer Grade II, $A4,283 to $A4,553 per annum ; Senior Technical Officer, $A4,684 to $A6,032 per annum (limits only). In addition a salary loading at the rate of $A200 per annum is payable to Technical Officers for an approved University degree or $A100 per annum for certain recognised Technical College diplomas or membership of a professional body recognised by the University. Assistance with passages and accommodation will be provided. The successful applicant will be required to undergo a medical examination.

Written applications, quoting reference number 70040, should be forwarded to the Registrar, Australian National University, Box 4, P.O. Canberra, A.C.T. 2600, Australia, with whom applications close on April 20, 1970. (897)

POSTGRADUATE POSITIONS IN PHYSICS

QUEEN'S UNIVERSITY
KINGSTON, ONTARIO, CANADA

Research and Teaching Assistantships are available to postgraduate students, both theoretical and experimental, in the fields of solid state physics, nuclear physics, low temperature physics, and radioastronomy. Special experimental facilities include: equipment for ultra high vacuum, magnetic resonance, microwave spectroscopy, luminescence studies, and for electron structure studies of metals by positron annihilation; a 3 MV Van de Graaff positive-ion accelerator, and use of the MP Tandem at Chalk River; a 60-ft. radio-telescope and use of the 150-ft. radio telescope at Algonquin Park. Financial assistance in a combination of Scholarships and Teaching and Research Assistantships is provided in the range $3,500 to $5,000 per annum.

Postdoctorate Fellowships are available in the above fields.

Letters of application, requests for brochures and further information should be addressed to Professor A. T. Stewart, Head, Department of Physics, Queen's University. (1058)

NATURE

MACMILLAN (JOURNALS) LTD

No. 5238 SATURDAY MARCH 21 1970 Vol. 225

CONTENTS

THE NEXT DECADE IN SPACE—STRAIGHT AND NARROW 1085

Old World—Computer Policy—Nuclear Power—Drugs—Satellite—Archaeology—Physiology—Disease—European Nuclear Power—Soviet Union—Parliament in Britain 1088

New World—BLEAK RECORD OF INTERNATIONAL COLLABORATION—Intelsat—Oil Pollution—Nuclear Power—Space—Seismology 1094

News and Views—DUST-UP IN NEVADA—Planetary Nebulae—Rock Magnetism—Pheromone Syntheses—Amphibia—Evolution—Spectroscopy—Nervous System—Virology—Virus Infection—Oxygen Sensors 1099

A-LEVELS AND UNIVERSITY PERFORMANCE by D. G. Bagg 1105

REINITIATION OF RNA CHAIN SYNTHESIS *in vitro* by John P. Richardson . . 1109

FINDS AT THE SWARTKRANS AUSTRALOPITHECINE SITE by C. K. Brain 1112

OSCULATORY PACKING OF FINITE AREAS WITH CIRCLES by H. H. Kausch-Blecken Von Schmeling and N. W. Tschoegl 1119

Letters to the Editor (see overleaf) 1123

Book Reviews by J. F. Jackson, J. R. Ravetz, J. Howlett, Edward Phillips, J. F. C. Kingman, D. E. Webster, R. W. P. Drever, J. A. Salthouse, L. Magos, M. F. Dutton, J. S. Hough 1167

Obituaries—Dr A. Tindell Hopwood 1172

Correspondence—Attitudes to Conservation by Thomas H. Jukes; Information Explosion by A. Johnson; Light Carrying Electrons by Helmut Schwarz; Anonymous Bounty by Peter Scott 1173

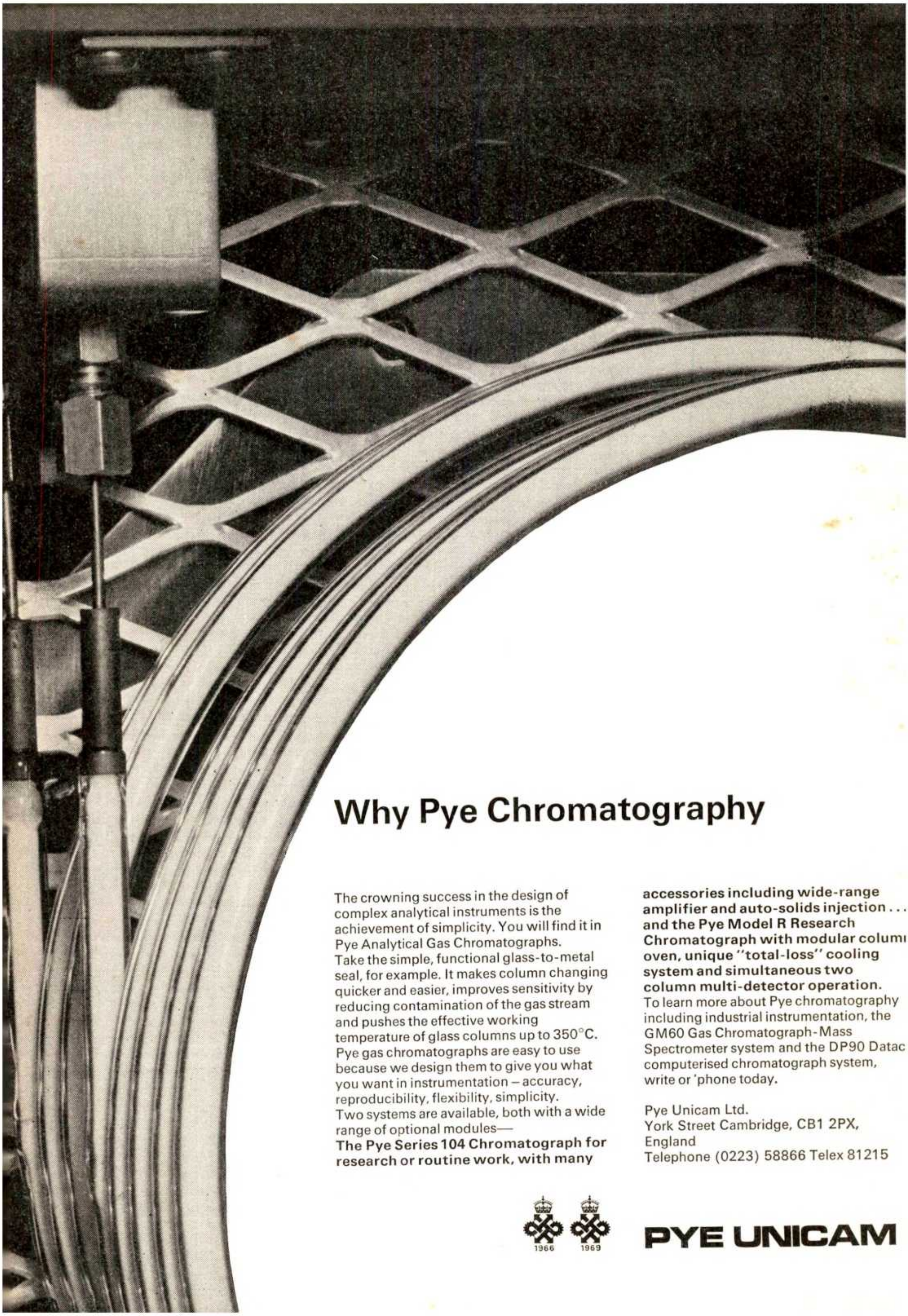
University News—Announcements—International Meetings—Sabbatical Itinerants 1174

Nature in North America: The *Nature* office in Washington DC has opened and contributors in the United States and Canada are invited to submit manuscripts for publication either to the address given below or to the present London office. The intention is that each office will be able to answer inquiries about all manuscripts and other matters. This is one of several steps by which it is hoped to provide a better service for readers and contributors.

London Office
4 Little Essex Street,
London WC2.
01-836-6633

Washington Office
711 National Press Building,
Washington DC 20004.
(202) 737-2355

(Continued overleaf)



Why Pye Chromatography

The crowning success in the design of complex analytical instruments is the achievement of simplicity. You will find it in Pye Analytical Gas Chromatographs.
Take the simple, functional glass-to-metal seal, for example. It makes column changing quicker and easier, improves sensitivity by reducing contamination of the gas stream and pushes the effective working temperature of glass columns up to 350°C.
Pye gas chromatographs are easy to use because we design them to give you what you want in instrumentation – accuracy, reproducibility, flexibility, simplicity.
Two systems are available, both with a wide range of optional modules—
The Pye Series 104 Chromatograph for research or routine work, with many accessories including wide-range amplifier and auto-solids injection . . . and the Pye Model R Research Chromatograph with modular colum oven, unique "total-loss" cooling system and simultaneous two column multi-detector operation.
To learn more about Pye chromatography including industrial instrumentation, the GM60 Gas Chromatograph-Mass Spectrometer system and the DP90 Datac computerised chromatograph system, write or 'phone today.

Pye Unicam Ltd.
York Street Cambridge, CB1 2PX,
England
Telephone (0223) 58866 Telex 81215

1966 1969

PYE UNICAM

LETTERS TO THE EDITOR

Page

PHYSICAL SCIENCES

X-ray stars—New periodic variations from Sco X-1—GRIBBIN, FELDMAN and PLAGEMANN (Cambridge) . . . 1123

Pulsars—Improved parameters for seven pulsars—MANCHESTER (US National Radio Astronomy Observatory) . . . 1124

Pulsar X-rays—Scattering by interstellar dust—BOWYER, MACK and LAMPTON (California) . 1125

Electromagnetism — Magnets in electromagnetic theory—LOVERING (Surrey) . . . 1127

Reaction kinetics—Rate equations used in thermogravimetry — MacCALLUM and TANNER (St Andrews) . . . 1127

Thermodynamics — Liquid phase inversion — LU, YU and POON (Ottawa) . . . 1128

Geochemistry — Origin of Onverwacht kerogen — SCOTT, MODZELESKI and NAGY (Arizona) . . 1129

BIOLOGICAL SCIENCES

RNA sequencing—Partial 23S rRNA from *E. coli*—FELLNER and EBEL (Strasbourg) . . . 1131

Ribosomes—Alteration of 30S subunit in *E. coli* K12 mutant causing inhibition of protein synthesis at high temperature—KANG (Chicago) . . . 1132

Alcohol dehydrogenase—Peptide differences studied in EE and SS isoenzymes—JÖRNVALL (Karolinska Institutet) . . . 1133

Collagen—Size-specific metal complexing sites in decalcified bone matrix and tendon—SPADARO, BECKER (VA Hospital, Syracuse) and BACHMAN (Syracuse) . . . 1134

Immunoglobulins — Independent genetic mechanisms controlling G subclass synthesis—RIVAT (INSERM), ROPARTZ (Centre Départmental de Transfusion Sanguine et de Génétique Humaine), BURTIN (CNRS) and CRUCHAUD (Hôpital Cantonal, Geneva) . . . 1136

Tumour immunology — Requirement for human complement in tests for antisarcoma antibody—EILBER and MORTON (US National Institutes of Health) . . . 1137

Inflammation—IgG may give rise to immunologically non-specific chemotactic factor—YOSHINAGA, MAYUMI, YAMAMOTO and HAYASHI (Kumamoto) . 1138

Lymphomas—Reticular neoplasms in SJL/J mice appear sooner after treatment with antilymphocytic serum—BURSTEIN (US National Institutes of Health) and ALLISON (MRC) . . . 1139

Antilymphocytic globulin—Effect of route of administration on skin graft survival in rats—DALTON, TOURAINE, ANDERSON and WOODRUFF (Edinburgh) . . . 1140

—Effect of adjuvant on immunosuppressive properties—ALLARDYCE, HUNTER and JAMES (Edinburgh) . . . 1141

Heart surgery—A new left ventricle as an alternative to a heart transplant—BLOOR (Royal Infirmary, Manchester) . . . 1142

Angiotensin converting enzyme—Activity and *in vivo* inhibition—NG and VANE (Royal College of Surgeons) . . . 1142

Page

Peptides—Unidentified peptides from intestine and lung have inhibitory effects on smooth muscle preparations—PIPER, SAID and VANE (Royal College of Surgeons) . . . 1144

Blood—Effect of magnetic fields on number of blood platelets in mice—BARNOTHY (Illinois) and BARNOTHY (Biomagnetic Research Foundation) . . 1146

Carotid chemoreceptors — Metabolic link between cytochrome a_3 reduction and afferent nerve discharge—MILLS and JÖBSIS (Duke) . . . 1147

Acetylcholinesterase — Immunofluorescent localization in electric eel electroplax—BENDA, TSUJI, DAUSSANT and CHANGEUX (Institut Pasteur) . 1149

Erythrocytes—Membrane stabilization properties of certain drugs—MIKIKITS, MORTARA and SPECTOR (London) . . . 1150

Anaesthetics—Effect of ether and chloroform on cation transport in human red cells — HALSEY, SMITH and WOOD (Oxford) . . . 1151

Psychopharmacology — Behavioural effects of morphine related to turnover of amines—EIDELBERG and SCHWARTZ (St Joseph's Hospital, Phoenix) . 1152

Schizophrenia—Is a genetic explanation sufficient?—JAMES (London) . . . 1153

Animal behaviour—Possible relationship between orienting and diving reflexes—GOODMAN and WEINBERGER (California) . . . 1153

Bats — Reproductive cycle of a neotropical insectivorous bat *Myotis nigricans* — WILSON and FINDLEY (New Mexico) . . . 1155

Voice recognition—Acoustic basis for the gannet—WHITE, WHITE and THORPE (Cambridge) . . 1156

Palaeontology—Faunal provinces in the Ordovician of North Atlantic areas—LINDSTRÖM (Philipps-Universität, Marburg) . . . 1158

Hybrid vigour — London plane re-created — SANTAMOUR (US Department of Agriculture) . . 1159

Forestry—Probabilities of transition from one type of tree cover to another can be calculated—WAGGONER and STEPHENS (Connecticut Agricultural Experiment Station) . . . 1160

Fungicides—Possible side effects—FIRMAN (Fiji Department of Agriculture) . . . 1161

Plant genetics—Phenotypic canalization of hemiparasitic flowering plants by hosts—ATSATT (California) . . . 1161

Algae—Possible gliding mechanism in a blue-green alga—HALFEN and CASTENHOLZ (Oregon) . . 1163

Viruses—Isolation of virus infecting strain of *M. laidlawii*—GOURLAY (ARC) . . . 1165

Microbial growth—Major thermodynamic process is large outflow of entropy from cells and energy incorporation is small—FORREST (CSIRO) . . . 1165

The titles on this page are prepared by the staff of *Nature* on the basis of the titles submitted by authors which appear in full on the pages indicated

'MICHROME'

STAINS & REAGENTS FOR BIOLOGY

Acridine orange, Aquamount, Fluormount, Lipid crimson, Matamount, May-Grünwald, Leishman, Giemsa Toluidine blue, Cresyl fast violet, Papanicolaou stains, Thioflavine S, Brilliant cresyl blue, Nile blue, Pyronin Y, Trifalgic acid, Rhodanile blue, Haplo, etc.

CATALOGUE AVAILABLE ON REQUEST

DYES FOR USE IN ALL INDUSTRIES

RESEARCH & PRODUCTION CHEMISTS: if you are seeking dyes with specific chemical and physical properties suitable for particular manufacturing and/or testing processes you are invited to contact us for supplies.

We hold stocks of an enormous variety of dyes, ready for immediate despatch, including:

Cyanine, Dicyanine, Fast Bordeaux O, Aurantia, Crystal violet, BPC-Scarlet R, BPC, Diazonium salts, Neutral violet, Lacmoid, Nile blue Janus green, Brilliant green BPC, Rosolic acid, Methyl green, p-Ethoxy-chrysoidin, Methyl red, Nitrazine yellow, Pinacryptol green, etc.

INDUSTRIAL RESEARCH ESTABLISHMENTS

are invited to make use of our professional services as consultants on the selection and use of dyes as research and production tools.

EDWARD GURR LTD

Michrome Laboratories,
42 Upper Richmond Road West, London, S.W.14
Cables: Micromlabs London SW14. Telephone: 01-876 82289

QUALITY — RELIABILITY — SERVICE
PROMPT DELIVERY

★ *A Unique Handbook now Completed*

Weighing Machines

In Three Volumes

T. J. METCALFE & E. H. GRIFFITHS
Chief Inspectors of Weights and Measures

Part III Electrical & Electronic Applications (GRIFFITHS; 248 pp., 169 illus., £3) has very wide technical interest; in the last 10 years more novel weighers have come out than in the preceding 50. The others (by METCALFE)] are **I Non-Self-Indicating Mechanisms** and **II Semi S-Ind. and S-Ind. Mechanisms;** each £3

BAUER

Precious Stones

— the long-scarce classic work — is now reprinted in two soft-covered volumes with the coloured plates, for 50s. the set (SBN 85264 195 8). Also, as a superb facsimile volume 10 × 8 in. — an opulent gift — at £8 10s. (SBN 85264 185 0)

GRIFFIN

1820

72 pp Cat. from 42 Drury La., London, WC2
has interesting Notes for Authors

Precision Instruments

Announcing—

A New Spectro-polarimeter

The Spectro-C_D ORDograph*

**A combined polarimetric and circular dichroic spectrograph*

An entirely new instrument for the measurement of Optical Rotatory Dispersion and Circular Dichroism over the wavelength range 180–800 nm of samples of optical density in the region 0–5. The data is stored photographically in an exposure time of 1 second for samples of O.D. 2; higher densities take a little longer. Both O.R.D. and C.D. data recorded simultaneously thus enabling studies of rapid reactions to be made in successive exposures. The elimination of electronics gives a low cost, trouble free, highly stable instrument with unique features.

We now have the capacity for aluminising certain types of mirrors, at short notice.

SOLE DISTRIBUTORS IN U.K. OF SCHOTT INTERFERENCE FILTERS

BELLINGHAM & STANLEY LIMITED
61, MARKFIELD RD. LONDON N15. TEL. 01-808 2675

NATURE Volume 225
MARCH 21, 1970

The Next Decade in Space

THE anticlimax after the landings on the Moon has, if anything, been accentuated by President Nixon's statement on the immediate future of the United States space programme (see *Nature*, **225**, 995; 1970). A part of the trouble, unavoidable perhaps, is that the new programme lacks a clear definition of a tangible goal which can match President Kennedy's declaration ten years ago that the United States would go to the Moon "within this decade". To be fair, it is just as well that President Nixon has chosen not to compete on terms like these. A sharp commitment to a specific goal would have been imprudent, and may even have been impossible. Although some extremists have been urging, for example, that a journey to Mars within some specified interval would be a sufficiently tangible goal to keep people working hard for the next decade, and to keep Congress sweet as well, the truth is that a journey to Mars in the next decade would serve no purpose that could not more easily and cheaply be accomplished by the proper design of automatic instruments. This time, there is not even the excuse that such a journey would help to advance the technique of sending people about the solar system. But if it is wise to have relegated the Mars journey to the list of tasks that will be attempted "eventually", it is far from easy to appreciate the philosophy on which the new programme has been put together. Is it simply a ragbag compromise between those who would be off to Mars like a shot if only somebody would provide the money and those who would shut down the space programme tomorrow if they could, spending the money instead on urgent social problems or even just the relief of taxes ?

There is a great need that the principles on which the future of space research is based should be made much more explicit than they are. A useful starting point is a simple empirical recognition that it would be a great mistake, and a great waste of resources, to dismantle the apparatus for space research which has been constructed in the United States in the past decade, chiefly the organization of the National Aeronautics and Space Administration. It is true that if it had not been for the Apollo programme, NASA would not have grown as quickly and would not now be spending more than $3,000 million a year. At the same time, however, it is clear that it would now be impossible for the United States to sustain even the most spartan space programme if NASA were, say, a tenth as expensive as it is at present. And as things are, the continuing cost of housekeeping within NASA is substantially more than that. The chances are that it would cost approximately $1,000 million a year simply to keep NASA ticking over, to maintain its laboratories, its capacity to brood about what might be done next and to preserve an efficient organization for collecting data from the Earth satellites which already exist. But with such a machine in being, is it not at least reasonable to spend twice as much again, making a total of $3,000 million a year, to allow the organization to include a modest amount of innovation in its programme ? This line of argument is as good as any other advanced in the past few days to justify the scale on which the forward plans of NASA are now determined. This sum of money, after all, is a mere 1·5 per cent of the United States budget and merely 0·3 per cent of the GNP. It is also a politically realistic sum of money—there would be great weeping in Congress in what is, after all, an election year if more of NASA's establishments had to be closed down—the shutting of the Electronics Research Center at Cambridge, Massachusetts, has shown how local prosperity depends on NASA establishments, while it would take a veritable pied piper to charm a substantially larger sum of money out of the Administration in the present climate of financial opinion. To be sure, NASA is badly served by its authorization committee in the House of Representatives, which regularly wastes the chance to make a constructive appraisal of what the agency is up to for the sake of foolish and empty gestures of goodwill such as the voting of unwanted and unspendable money (see page 1097), but it would be a splendid starting point in working out a strategy for the next few years to say that NASA should be asked to make the best it can of an annual budget which is not very different from the $3,500 million which it is likely to be getting for the coming fiscal year.

The question, then, is what should NASA do on such a pension ? This, plainly, is the kind of question that Mr Nixon sought to answer when he put together the package of new developments which he described last week. The first thing to be said is that the new programme—strategy is too strong a word—is too diverse. President Nixon said that he wants to continue with the exploration of the solar system, the application of Earth satellites to useful tasks such as telecommunications, the development of cheap methods of getting into and out of space (which to NASA means the space shuttle), the development of the techniques of extended spaceflight (which implies the experimental space station) and involvement of nations other than the United States in this ambitious programme of development. For the sake of some of the newer fun and games, NASA is plainly willing to let some urgent needs be overlooked. There is, in short, a danger that even though the panic to reach the Moon has now been exorcised, NASA will still find itself having to give less attention to the things it could accomplish well.

Scientific exploration is a case in point. President Nixon was right to say that a better knowledge of the solar system should be one of the objectives in the coming decade and there is, of course, great elegance in the scheme for sending a rocket to the outer planets by using the chance coincidence of planetary positions in the eighties to make the journey to Pluto with a less powerful rocket than would ordinarily be needed. The problem, unhappily for the new programme, is that NASA does not have a monopoly of the exploration of the solar system, and that there has never been a serious attempt to balance the very large sums of money to be spent on the grand tour, and, for that matter, on the complicated telescope to be carried on the first space station (see *Nature*, **225**, 994; 1970), against the much more modest sums of money being spent on other kinds of basic research in this field. Certainly it is irrational that these ventures should be launched at a time when the United States is being waywardly deprived of radio telescopes, to name only one way in which the cause of scientific exploration of the solar system might be advanced. It must also provoke questions that NASA could afford $145 million in 1969 for physics and astronomy, when the pressure of the Apollo programme was intense, but has only asked for $127 million in the coming year, when ordinary people would have thought that there might be a chance to fill in some of the gaps left behind in the scamper to the Moon.

There is also a danger that the plans for the better application of Earth satellites which have now been handed down as if by Moses will spend too little effort on important work. To be fair, the programme for the year ahead does include the development of the improved weather satellites, to be launched in 1972 and 1973, which will make it possible to recover a detailed temperature profile in the atmosphere of the Earth and thus to provide numerical data of a kind which may yet provide a basis for an international weather forecasting system. The value of the programme which may make this possible has been a welcome surprise, and a prudent NASA will be ready to commit extra funds to schemes like this in the years ahead. But why should "budgetary restraints" have forced the postponement by a year of the two satellites called ATSF and ATSG, originally to have been launched in 1972 and 1973 respectively? These, after all, will provide the first tests of the feasibility of air traffic control, millimetre waves and even lasers in telecommunications and, for that matter, the experimental use of satellites for educational broadcasting in India. If, as President Nixon says, one objective for the next few years must be the speedy exploitation of such uses as there may be for Earth satellites, surely these instruments should have been given a place closer to the Sun. (By comparison, Earth resources satellites are still nebulous entities, hardly deserving of the over-sharp talk still frequently lavished on them.) If President Nixon and NASA wish to be taken seriously, this is also a field in which good sense requires that useful jobs which can be done should urgently take priority over the more speculative ventures also now included in the shopping list.

But should there not be some consideration for the continuing development of the technology of space research and even of space travel? Having travelled as far as this, it would obviously be foolish to let the techniques ossify, yet, even so, it is a great mistake that President Nixon's statement should have made conspicuous the two particular notions of cheaper space travel (implying re-usable launchers) and extended space travel. To be sure, these are schemes which have been widely talked of for much more than a year, and were explicitly mentioned in the report of the Task Force which advised the President on what should happen next. It is also the case that NASA has already worked hard on both of them—so much is clear from the study on the subject carried out by the staff of the House Science and Astronautics Committee and published on February 12 this year. But are these schemes as urgent as they are being made to seem? What damage would be done if the space station were postponed a year? Most probably, only the potential contractors would notice the difference. And is it really acknowledged that NASA will have by the late seventies the need to launch the thirty or so satellites a year that will be necessary to repay within five years the difference between the cost of developing the shuttle rather than using rockets which already exist? And does not this imply that by then the budget of NASA should have risen substantially, so that the cost of making rockets is only a small part of the total? Even though this estimate of the point at which the shuttle project will break even depends on the assumption that military interests will also need to launch some thirty 25,000 pound payloads into orbit every year, which lends the space programme a sense of importance which it has been able to manage without so far, this is quite a tall order. Has the Administration really appreciated how great will be the cost of making the new space shuttle cheap?

These uncertainties are one reason why NASA is mistaken in hoping to kindle international interest in collaboration on the shuttle. Dr Paine is probably right in believing that there must be some way in which space could become a focus for international cooperation, but he seems bent on offering his potential partners the wrong end of the stick. It is unfortunately a plain fact that the journey to the Moon has not budged the industrialized nations which are the most likely collaborators in the new space programme from their pragmatic view that the best ventures are those most likely to yield some economic benefit. Telecommunications, weather analysis and air traffic control (with navigation as a kind of sideline) are the only immediate candidates. In each case, however, successful collaboration would require not merely a thorough sharing of costs but joint planning as well. The kinds of collaboration of which Dr Paine is so far rightly proud raised only a few of the thorny problems which must be created by programmes of collaboration which consist not simply of the parcelling out of work but the permanent commitment of hopes as well as of resources to some common venture. There is every reason why the United States should take the initiative in this field, but it should begin at the humdrum end of the spectrum. There is no reason why NASA should not be able to look forward to a time when it is a kind of international public service agency, living as much off fees for services provided as from the bounty of a taxation-conscious Congress.

This is probably the sense in which NASA and the Administration have most seriously misjudged the present need. By now, there is probably good reason to believe that space research and exploration has come to stay. Such benefits as there may be will be diverse and scattered. In the long run, however, this implies that no single agency within one state can hope to remain a dominating influence. Much of what is now considered NASA business will eventually be regulated by private or international organizations. But this means that NASA should be working hard to divest itself of its responsibilities. It should also be vigilant in making sure that what can be done properly is done well. The most disappointing feature of the new space programme is that it seems to be somewhat aimlessly intended to keep the machine in being without specifying what it is intended for.

Straight and Narrow

THERE is nothing openly wrong with Mr Callaghan's bill on the control of drugs. The notion that pushers should in future be dealt with more severely than possessors is sensible enough, and fourteen years in gaol is not too severe a penalty for those who make a business of addiction. The attempt to relate the terms of imprisonment which drug possessors will in future risk to the pharmacology of the materials on which they are dependent is less successful.

What precisely is the logic by means of which it has been decided that the possession of heroin and LSD should both incur the risk of seven years in gaol, that cannabis should rate a mere five and that the amphetamines and pep pills derived from them should be worth just two years? The World Health Organization, from which this classification is ultimately derived, was right to say that LSD is as great a hazard to the health of an individual as heroin or opium. The social risk, by contrast, is of a quite different order. Presumably these sentences are meant to be deterrents, ways of protecting people from themselves and ways of restricting the market for narcotic drugs—if the potential customers can be frightened off, there will be less business for the pushers. Certainly there is no reason to hope that a prison sentence will actually cure an addict (in the old-fashioned sense) of his addiction. But in circumstances like these, it would have been worth the trouble for the Home Office to have tried to relate these prison sentences not so much to the pharmacology of the material concerned as to the manner in which it is used. Why not, in other words, let judges and magistrates know explicitly that some degree of restraint in the way in which the drug of choice is shared with friends and acquaintances should be a substantial mitigation of the misdemeanour, for example? The danger, as things are, is that the arithmetic of the sentences now spelled out will only perpetuate the arbitrariness in sentencing and even prosecution which has been one of the most harmful features of the British handling of drugs in the past few years. This is one of the points to which the House of Commons should pay close attention.

Most argument in the weeks ahead is, however, very likely to be concentrated on the proposal that doctors who systematically over-prescribe dangerous drugs for their patients should be banned from prescribing these materials. This is an extremely tricky proposal, as the Home Office is no doubt well aware. Ever since the Brain Committee, it has been well understood that the so-called "English system" of dealing with drug addiction, under which the drug-dependent were given maintenance doses by their physicians, finally broke down when a handful of doctors were found to have lavished unwarranted quantities of narcotics on their patients. One difficulty, however, is that of knowing how to define what "over-prescribing" means. Is it now to be supposed that physicians will be required to vouch not merely for the physical needs of their patients for materials such as the amphetamines but for their honesty as well? And what are the implications of an arrangement under which medical practitioners are barred from some kinds of medicine? There are genuine difficulties in a system under which the government, even with the approval of a medical tribunal, has the right to curtail a doctor's licence. How can a general practitioner function properly if he is unable to treat his patients for specified conditions? And is there even a danger that the next step will be to prevent surgeons with bad records at some kinds of operations from repeating their performance? The trouble is that any attempt to restrain doctors from socially disagreeable practices must necessarily involve the kind of qualitative judgment of a doctor's work which society has usually avoided. Especially because of the retributory air which the new proposal carries, any alternatives to this proposal which the House of Commons can devise would be most welcome.

No amount of patching in the House of Commons can, however, turn the new Home Office bill into the positive measure which might have some lasting influence on the social problems of drug dependence. The most urgent need is for better ways of treating drug dependence on heroin and such hard drugs. Not merely research but facilities are needed. Everybody is aware of the haunting difficulty of this problem, but there are some hopeful experiments—the use of methadone as a substitute for heroin, for example, and the several experiments in the United States in which people who are dependent on drugs help each other to escape from their problems. There is a danger that the new array of penalties, devised with good intentions, may make it even harder than it has now become to treat drug dependence as if it were what it is —a physical condition.

Mr Callaghan is not the kind of man who takes risks in what will almost certainly be an election year. "Jim the Pot" would be an awkward label on the hustings in Cardiff. But is it wise to avoid the illogicality of a system under which commonly used but damaging drugs such as alcohol, barbiturates and even nicotine (or the carcinogens which accompany it) are permitted, but materials such as cannabis carry the risk of two years imprisonment? As a temporary expedient, such as the ban which King James I once imposed on the consumption of coal in London, it may have some value. In the long run, it will prove to be unstable.

100 Years Ago

He was about to apply his spectroscope to the observation of a solar prominence when his attention was attracted to certain shadows traversing the disc of the sun, which became bright streaks when they had passed beyond it. At first he thought these appearances were due to sparks in the tube of the telescope, but the phenomenon lasted too long for this explanation to be available. He next thought that perhaps a system of meteors might be in transit, and prepared to subject the phenomenon to careful scrutiny. The equatorial was set in motion, the sun's disc being projected on a screen. The shadows were seen persistently traversing the solar disc, but at different velocities, the larger ones travelling most swiftly. There appeared to be two streams. He noticed that when the sun was in focus the objects were indistinct, and that they appeared very distinctly when he focussed on a distant cloud. At length, while he was attentively scrutinising the phenomenon he saw one of the objects come suddenly to a standstill, and then whisk off in a different direction; and then he perceived that the phenomenon he had been examining with such anxious care was not in reality an astronomical phenomenon at all, but consisted merely of a flight of locusts. He considered, however, that not only was the existence of so enormous a swarm of locusts as the duration of the stream indicated, an interesting fact in itself, but that we might find in the occurrence the explanation of many statements which had been made respecting meteors supposed to have transited the sun, and also of some peculiarities noticed by astronomers in America during the total eclipse of last year. Mr. Stone said that it was important when appearances of this sort were noticed that the observer should examine, as Lieut. Herschel had done, whether the objects seen in transit required the same focus as the sun. This was the best way of determining whether the objects were terrestrial or not.

From a letter written to Professor Alexander Herschel by his brother and read to the Royal Astronomical Society (Nature, **1**, 543, *March* 24, 1870).

OLD WORLD

COMPUTER POLICY

Computer Society's Lack of Push

ALL aspects of the British computing scene were covered during the appearance of the British Computer Society before subcommittee D of the House of Commons Select Committee on Science and Technology last week. Eager for change, the society in its evidence talked of the poor state of computer education in Britain, the doubts surrounding government policy, and the uncertainty that clouds the future prospects of British computer firms. But enthusiasm alone is not enough, and the subcommittee under the chairmanship of Mr Airey Neave was disappointed that the society has not been more forthright in pressing its views on the government.

The deputy president, Mr A. d'Agapeyeff, pleaded the voluntary nature of participation in the society as the reason why the society's recommendations on education could not be backed by more research. In particular the society was taken to task for presenting a memorandum to the subcommittee talking of computer courses "offered by bodies reputable and disreputable" without being prepared to back up its allegations with evidence.

The British Computer Society nevertheless represents most sections of the British hardware and software industry as well as user organizations, and is the nearest the subcommittee has yet come to a disinterested party in its investigation of the British computer industry in the seventies. Although some of the society's recommendations were dismissed by Mr E. Leadbitter as a series of generalizations, the witnesses were prepared to go further when speaking as individuals. Professor S. Gill startled the subcommittee with his ideas for pruning the jungle of departments which deal with computer purchasing for the government. These include the Stationery Office, the Civil Service Department and the Ministry of Technology, which has the final say. Professor Gill thought it would be a good idea to remove the Mintech link from the chain. This would avoid the clash of interests caused by competition from a foreign computer, between the ministry, which also has the job of fostering the British computer industry, and the Civil Service Department, which is responsible for the efficient working of the civil service.

On the subject of education and training the society was keen to see changes from school level upwards. University training was called "formal and irrelevant", chiefly because the universities provide little expertise in the growing field of business computing, although the witnesses agreed that this is a difficult area for the universities to cover.

The society had a lot to say about government policy. In particular it complained that the government could be more aware of the importance of software, that departments are too cautious (citing the slow rate of installations in the Inland Revenue) and, echoing earlier witnesses from IBM (UK) and from Honeywell, that there should be less hypocrisy over the policy of favouring British computer manufacturers. The society said it should be consulted by government on matters of computer policy. But the subcommittee found the society's lack of initiative in making representations to the relevant departments, as other professional bodies do, an unimpressive comment on this suggestion.

NUCLEAR POWER

Fuel Company Announced

BRITAIN'S nuclear fuel business is to be handled by a new government controlled company, British Nuclear Fuels Ltd, according to a bill put forward by the Minister of Technology, Mr Anthony Wedgwood Benn, published last week (*Atomic Energy Authority Bill*, HMSO, 3*s*). The AEA's smaller but buoyant radio-isotopes business will also be established as a separate company, to be known as The Radiochemical Centre Ltd.

The setting up of British Nuclear Fuels is the second stage of the reorganization of the nuclear industry that was begun by Mr Benn in 1968 and represents the next step in switching from a research-oriented to a market-oriented industry. The third stage of the reorganization was outlined in the green paper, published in January this year, which envisaged the merger of the research side of the AEA with other research establishments of the Ministry of Technology to form a new British Research and Development Corporation.

Five establishments of the AEA will be transferred to British Nuclear Fuels if the new bill is passed. These are at Capenhurst in Cheshire, Calder Hall in Cumberland, Chapelcross in Dumfriesshire, Springfields in Lancashire and Windscale in Cumberland. Radiochemical Centre will be comprised of the radiochemical centre at Amersham in Buckinghamshire and a contingent from the Atomic Energy Research Establishment at Harwell. BNF will become the British component in the joint scheme negotiated by the governments of the Netherlands, Britain and West Germany for the production of enriched uranium by the gas centrifuge process, and will assume a minority shareholding in the two reactor consortia, TNPG and BNDC.

There is not expected to be much opposition to the setting up of the fuel companies. The present British market for nuclear fuels is estimated at £25 million, and is expected to rise to £100 million by 1980. The world market is likely to have exceeded £1,000 million by 1980, and the need for a strong commercially oriented body to manage this expanding business is widely recognized. Nuclear fuel is, of course, the most lucrative side of the nuclear power business. Sales of radio-isotopes are also growing, with half the radiochemical centre's £4 million trade coming from overseas.

The exact composition of the BNF shareholding is not yet known, but companies believed to be interested in participating include Imperial Chemical Industries, Rolls-Royce and Rio Tinto Zinc. Mr Benn is known to be in favour of cooperation between government and private industry in this sort of venture, a view which may, however, be challenged by the opposition spokesman on trade and technology, Sir Keith Joseph, when the bill is debated in the House of Commons. The bill was introduced in the House of Lords last week, but even the most optimistic estimates make it unlikely that either of the companies will start operating before October.

DRUGS

Hard and Soft Distinguished

STIFFER penalties for drug "pushers" and stringent provisions to prevent doctors prescribing "irresponsibly" are the main features of the Home Secretary's comprehensive new drugs bill. Controlled drugs are divided, according to their harmfulness, into three groups so that for the first time "hard" and "soft" drugs such as heroin and cannabis are treated differently (*The Misuse of Drugs Bill*, HMSO, 5*s*).

The bill assigns different penalties for misuse in each of the three controlled groups. The group considered most dangerous includes heroin, morphine, cocaine and LSD, and the penalties for producing or supplying these "Class A" drugs are up to fourteen years imprisonment and an unlimited fine. The penalty of seven years for being in possession of these substances is less than the present maximum sentence of ten years for drugs included under the Dangerous Drugs Act, 1967, a concession which reflects the present practice in the courts of imposing lighter sentences on drug users than on drug pushers.

Amphetamines, cannabis and codeine are bracketed together as "Class B" drugs. Maximum penalties for pushing these drugs are still fourteen years imprisonment and a fine, but the penalty for illegal possession is down to five years. Sentences for possession of the least harmful group of drugs ("Class C"), which includes some of the less dangerous stimulants, are up to two years imprisonment and a fine. Barbiturates are not specifically carried by the new bill but their position is under review.

A new power proposed in the bill is that of adding new drugs to the controlled list as soon as it seems that they may become dangerous. The danger would be assessed by an advisory committee and a panel of experts, but the formal procedures involved in getting a drug classified could take place quite quickly, within a few weeks if necessary. The present system of putting new drugs on the list requires international agreement and can take up to two years.

Another new provision in the bill concerns information on the supply of drugs. In regions where the misuse of drugs is causing "social problems", the Home Secretary can ask doctors or pharmacists for details of the quantity of drugs and the number of prescriptions they have supplied.

SATELLITE

SRC Advertises its Vacancy

THE Science Research Council has strange ways of announcing its achievements. Advertisements in the daily press last week (*Daily Telegraph*, March 13) for a satellite engineer to work on a new satellite in the Ariel series concerned with cosmic X-rays have revealed that at least project definition and feasibility studies are complete for the most attractive British satellite payload yet attempted. This is part of the long-standing US-UK cooperative programme (drawn up in 1959) according to which NASA provides the launch and Britain the scientific ideas and hardware. So far three satellites have been launched and one more is in the pipeline—called UK-4 until 1971 when a successful orbit will rechristen it Ariel-4.

As the programme has progressed, the partition of activity has become more equal. For Ariel-3 British industry has undertaken design and construction of the satellite body. The fourth in the series is to carry an American experiment in addition to four British (see *Nature*, **223**, 3; 1969). This hospitality is being repeated on the UK-5 stellar X-ray satellite.

The six experiments planned for UK-5 are designed to complement each other. All are concerned with stellar X-rays. A spin-stabilized design enables both broad sky surveys and precisely pointed detailed observations of individual sources to be carried out from the same vehicle. The four experiments concerned with individual sources will look along the spin axis of the satellite which, by means of fine gas-jets, can be aimed with an accuracy of one degree to any point in the sky. The two survey experiments look out of the side of the spinning satellite so that between them the whole sky will be covered. The US experiment, a survey instrument from the Goddard Spaceflight Center, has a wide fan-shaped field of view forward and aft of a Leicester University survey instrument.

To date, some 50 X-ray sources have been observed but less than ten of these have been precisely fixed. Precise location of individual sources is the predominant problem at present. Some X-ray sources are thought to be short-lived or highly variable so that it will be useful to compare the Goddard and Leicester survey findings with data from an American X-ray survey satellite (SASA—small astronomical satellite A) which is to be launched shortly.

A fine-pointing instrument designed by University College, London, is intended to obtain positional accuracy of individual sources to within 1 arc minute. Another University College experiment employs a proportional counter which is expected to obtain high sensitivity but low resolution of the spectra of individual sources. A complementary experiment mounted by Leicester University makes use of a crystal spectrometer giving low sensitivity but high resolution of spectra and also a measure of polarization.

The Imperial College experiment extends the observations of X-ray sources to energies in the Mev range. It derives from techniques using a shielded scintillation counter developed by the cosmic ray group for observing magnetosphere particles.

Because all the work has been carried out from brief rocket flights, the total amount of observing time of X-ray sources probably does not exceed 3 hours. Satellite observations should increase the sensitivity by a factor of 100, thereby substantially increasing the number of known sources. At present teams at University College and Leicester are keeping British work in X-ray astronomy to the fore.

The development by Elliott Automation, Frimley, of a refined gas-jet system for obtaining fine alignment of instruments on target in space has played its part in maintaining British success in this exacting new field. This system is in view for controlling the attitude of the UK-5 spacecraft. Elliott Automation is now part of the GEC complex and another member of the group, GEC–Marconi, is the prime contractor for the satellite expected to be launched by an American Scout rocket in 1973. Because the ride for the Goddard experiment is being offset against launch costs, the total cost of the project to Britain is only £2 million.

ARCHAEOLOGY

The Amsterdam and the Lawyers

THE manifestations of underwater archaeology have come late to this country. Now that worthwhile material is emerging it is clear that we have neither the law nor enough suitably trained people to cope, which is a curious situation for a traditionally maritime country. The predicament is highlighted by the romantic case of the Amsterdam, the fully laden Dutch East Indiaman that has been stuck in the Hastings mud since 1749, and received its most complete exposure for 221 years in the press last week.

It is beginning to be appreciated that, properly excavated and preserved, the Amsterdam offers a unique view into trading life and conditions at sea in the mid 18th century. No ship—much less a merchant ship—is known from such an early period with virtually all its contents intact and even stowed in the positions they were placed at loading. Not even Sweden's 17th century flagship Wasa can claim this because, being half empty when she sank, she heeled right over, and the final location of objects found within her on raising bore little relation to their original position.

Yet at present none of this has any protection by law. The salvage law (which goes back to the Merchant Shipping Act, 1894) tends to encourage vandalism by rewarding quick recovery of goods regardless of the methods by which they are obtained.

An attempt was made in the Commons last week through a joint-party backbench motion to introduce clauses into the government's current Merchant Shipping Bill which would provide for the protection of historic wrecks by scheduling them like historic buildings. The Merchant Shipping Bill is framed to amend the law as it relates to seamen's pay and conditions of service in line with the Pearson Report, and is not a total revision of the old Merchant Shipping Act. The government spokesman (Mr Goronwy Roberts, Minister of State, Board of Trade) made sympathetic noises but declined to extend the measure to include the protection of historic wrecks. The clauses were withdrawn but may be reintroduced when the bill

Wreck of the Amsterdam, sunk in Hastings quicksands since 1749 and here exposed to the greatest extent in 221 years through a combination of equinoctial low spring tide and mechanical excavators.

reaches the Lords shortly if only to give an opportunity for the Board of Trade to reveal if it has an official inquiry or other action in mind, as seems likely.

In theory, an historic wreck could be scheduled as a public monument under present law. But the Ancient Monuments Board, which is responsible for unoccupied and immobile historic buildings, classes a ship as "a chattel" and therefore not within its purview.

Meanwhile the gaps in the British regulations are becoming a disgrace. Almost all other European maritime nations have introduced legal protection for historic wrecks and cargoes, although few can rival Britain's nautical heritage.

PHYSIOLOGY

Study in Depth

The Royal Navy last week broke the world record for deep diving on dry land by taking two volunteers to an equivalent of 1,500 feet in a pressure chamber. The subjects endured ten hours in an atmosphere of oxygen and helium at 666 pounds/inch^2 (just over 45 atmospheres) and spent the next eleven days in a gradual mock ascent. The exercise was organized by Dr Peter Bennett of the Royal Naval Physiological Laboratory, who seems to have achieved his aim of disproving the "helium barrier" at 1,200 feet which was thought to represent the greatest pressure at which an oxygen–helium mixture could be breathed. There is no sign yet of any physiological limit for diving, and at least a further 300 feet of the sea are now opened up to explorers who take the requisite precautions.

Besides the intrinsic interest of seeing what depths the human frame can attain, the experiment has provided an opportunity for studying the tremors and dizziness often suffered when high-pressure helium mixtures are breathed, and at the same time has made possible a wide range of physiological observations which have yet to be analysed.

Because of the experimental nature of the exercise, frequent safety checks were made during the descent; the subjects paused for 24 hours at a time in atmospheres corresponding to 600, 1,000 and 1,300 feet, and made intermediate one-hour halts at 1,100, 1,200 and 1,400 feet. One of the subjects was temporarily affected by helium tremors after changes of pressure, but the other only suffered from occasional dizziness. It seems that limitations of time rather than physiology are the chief obstacle to really deep dives. Descents must be gradual to allow divers to acclimatize to each small increase in pressure, and there is also the need to decompress very slowly on the way up. Even with the eleven days that the experiment allocated to resurfacing, one of the subjects suffered slightly from "bends" so that the decompression time had to be extended by several hours.

The belief in a helium barrier came from a Franco-American simulated dive in 1968. When the subject reached 1,190 feet he found himself falling asleep unless he stayed constantly active, and the pressure was reduced after only four minutes because his EEG showed serious abnormalities. Dr Bennett says that the stimulus for his own test came from a later exercise in which 1,150 feet was held successfully for five hours. People at the naval laboratory became convinced that so marked a difference was unlikely to occur over just 40 feet and that another trial was needed.

DISEASE

Mortality Patterns

People who live south of a line joining the Wash to the Severn have a better chance of living to a ripe old age than those living in the north. But what is the significance of that information? Professor G. Melvyn Howe, of the University of Strathclyde, whose revised and enlarged edition of the *National Atlas of Disease Mortality in the United Kingdom* (Nelson, 105*s*) is published this week, believes that the mapping of diseases may provide a useful tool in understanding and pinpointing underlying factors associated with disease. The regional variations may help to exhibit associations with climatic conditions, type of soil, pollution, or other physical parameters, or they may reflect the habits or mental tensions which are more common in certain areas. In a relatively immobile population, area differences in mortality could also reflect hereditary mechanisms for some diseases.

The patterns certainly show that mortality from all causes is well above the national average in parts of the north of England and Scotland, while the south coast and most of southern England are relatively healthy. Salford earns the unenviable title of the unhealthiest town in the UK, and other disease black spots are central Scotland, the mining towns of South Wales, and the rest of Merseyside and South Lancashire.

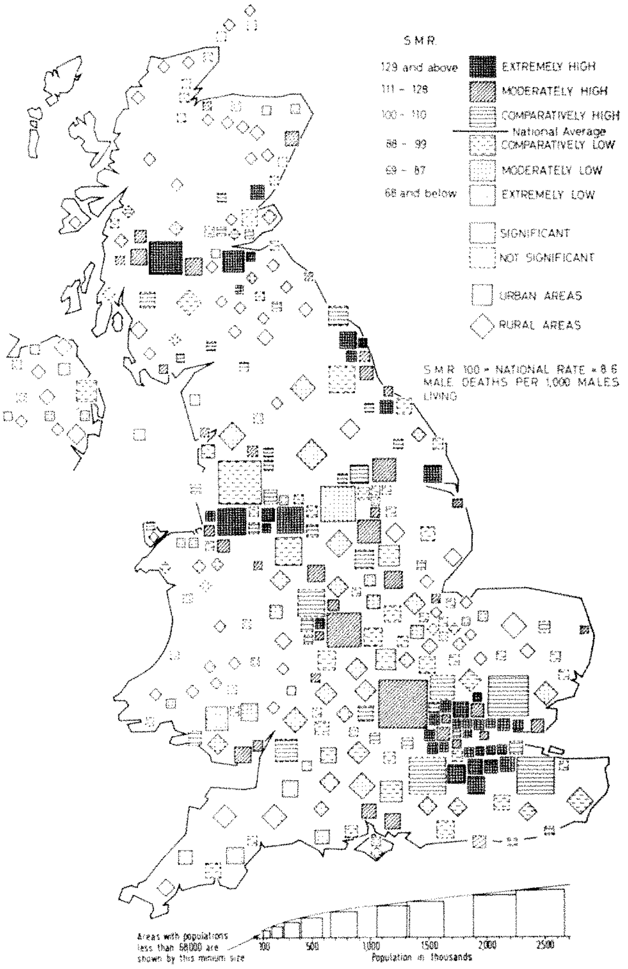


Fig. 1. Mortality rates for cancer of the trachea, lung and bronchus (males). *National Atlas of Disease Mortality in the United Kingdom*, p. 110.

London fares relatively well in the health league, although the boroughs of Southwark, Shoreditch, Stepney and St Marylebone have mortality rates well above the national average. In contrast, Bournemouth seems to be the healthiest town in the UK. These variations seem to suggest that disease mortality is highest in areas of poor housing and heavy industry.

The variations in mortality from specific diseases are even more striking. The similar distribution of high mortality from lung cancer for both males and females suggests that an environmental factor is particularly important in this disease. As might be expected, the disease is more common in areas of heavy industry, the four areas with the highest incidence being greater London, Merseyside, northeast England and Central Scotland. A different environmental factor seems to be operating in the case of diseases of the circulatory system. Although Professor Howe does not rule out the possibility of genetic factors and regional habits, his maps seem to support the theory that there is an inverse correlation between high incidence of these diseases and the hardness of the water supply. A particularly interesting finding is that the coronary mortality in Birmingham, which has soft water, is below the national average. The Birmingham water authority has neutralized the acidity of its water for several years.

Mortality from bronchitis follows the expected pattern, with the areas of highest incidence being those associated with heavy industry. London, in particular, fares worst in the central boroughs. Although the richer boroughs of London escape many of the diseases associated with poor housing and polluted air, they come off badly in mortality associated with mental stress. In Westminster, Hampstead, Chelsea and Kensington, mortality from suicide is more than twice the national average.

EUROPEAN NUCLEAR POWER

ENEA takes the Pulse

THE application of nuclear power in Europe has lately been going through a period of reappraisal rather than of innovation. This is the verdict of the European Atomic Energy Agency in its report for 1969 which was published last week. Largely because of the economic situation in Europe, the processes of decision-taking in the area of nuclear power are becoming more protracted. But according to the report, these changes in attitude are not greatly affecting forecasts of the growth of nuclear power in Europe.

The European Atomic Energy Agency is, of course, the agency of the OECD which promotes the Dragon high temperature project at Winfrith, the boiling heavy water reactor project at Halden in Norway, and the Eurochemic fuel reprocessing plant at Mol, Belgium. The report notes with pleasure that recent months have seen decisions to continue with each of these projects, all of which came up for renewal recently. As well as supporting other less ambitious cooperative projects, such as the maintenance of a computer program library at Ispra in Italy and a Neutron Data Compilation Centre at Saclay (France), the agency keeps a watching brief over the fortunes of European nuclear power programmes.

Because the demand for electricity has not risen quite as quickly as expected, there has been a slackening in the ordering of new power stations, and this is particularly so in Britain and France, which have so far made the running in the growth of European nuclear power. The report points out that neither lack of confidence in the nuclear power nor concern over uranium supplies, two popular bogies, is implicated.

But the report makes the point that the economics of plants for enriched uranium, such as the British–Dutch–German centrifuge project, are extremely sensitive to the amount of enriched uranium which is to be produced, so that forecasts of demand need to be accurate. Unfortunately the predictions seem to be less certain now than they have been. With the demand for electricity not rising at the expected rate, the estimate of 100,000 MW of nuclear capacity installed in Europe in 1980 is in doubt. This estimate followed from the anticipated need for 40,000 MW of nuclear power by 1975, which with 26,400 MW already installed by half-way through last year implied the ordering of 13,600 MW during the next year or so to meet the deadline. The protagonists of the centrifuge project, who promise to produce enriched uranium competitive with the American product, will no doubt be keeping a close eye on the way the demand develops.

SOVIET UNION

Lightning Communication

from our Soviet Correspondent

THE launching last month of the latest in the series of "Molniya-1" ("Lightning") communications satellites of the Soviet Union, coinciding as it does with the Washington conference on communications satellites, must inevitably draw attention to what may be classed as one of the less publicized achievements of the Russian space programme.

It may well be true to say that in no other country are communications satellites less of a luxury and more of a necessity. The sheer geographical extent of the USSR, sprawled as it is across so many time-zones, has always placed rapid communications at a premium. And since, in spite of the great development schemes of the 1950s, much of the vast area of Siberia is still an emerging rather than a developed region, the problems posed by a satellite communications system have been solved in rather a different manner than in, say, the USA. Indeed, the basic concept of Molniya-1 differs from the American Comsats, since Molniya-1 is not a geostationary satellite, and more than one must be employed if round-the-clock coverage is to be achieved.

Molniya-1 is a heavy satellite, with a reported transmitter power of 40 W. The accepted proportion for communications satellites is that each watt of radiated power corresponds to 2 kilograms of transmitter weight, and that the transmitter weight is about a quarter of the total weight. Thus the Molniya-1 satellites must have a weight of the order of 320 kg.

With satellites of this power, it is possible to use comparatively small ground stations. Under conditions in the Soviet Union, this is a considerable advantage, since a wide network of ground receiving centres can be erected relatively quickly and at comparatively low cost—an important factor when dealing with the rapidly developing areas of Siberia.

In the words of an official bulletin (*Pravda*, July 22, 1969) the Molniya-1 satellites are "intended to guarantee the working of the system of long-distance telephone and telegraphic communications, as well as transmitting the programmes of the central television network of the USSR to points of the 'Orbita' network (the ground stations) situated in the Far North, Siberia, the Far East, and Central Asia".

The Molniya-1 satellites are given a highly elliptical orbit to ensure the maximum transmission time over Soviet territory. The first successfully launched Molniya-1, which went into orbit on April 23, 1965, had an apogee of 40,000 km and a perigee of 500 km, giving it a period of 12 hours and an effective transmission time of 6 to 8 hours. These parameters have apparently proved satisfactory, since the latest Molniya is close to these values with an apogee of 39,175 km and a perigee of 487 km.

One of the most interesting uses of the Molniya-1 satellites has been the transmission of newspapers from Moscow and Leningrad to the Far East. Test transmissions carried out as early as February 1968 proved highly successful. The system of video transmission used has not been published in full; it has been stated, however (*Elektrosvyaz'*, **5**; 1969), that the necessary frequency multiplexing is most simply achieved on the video frequency, for this does not require a special transmitter and eliminates the danger of transient noise in the onboard retranslator of the satellite. It is arranged that the newspaper columns can be transmitted simultaneously with the regular TV transmissions, by the addition at the edge of the TV video signal of a subcarrier modulated by the video signal of the newspaper.

On the expansion of the Molniya–Orbita system, V. P. Minashin, chairman of the governing body of the Ministry of Communications of the USSR, stated in a recent interview that the USSR now has the widest network of "reception points" (ground stations) in the world "especially in sparsely populated and inaccessible regions".

Colour television transmissions were introduced on July 22 last, and experimental exchanges of colour TV programmes between Moscow and Paris proved most promising.

By the end of 1969, 30 Orbita ground stations were to be in operation, covering many of the Siberian "new towns" particularly in the newly opening petroleum and natural gas belt.

An extension to the Orbita network to cover Mongolia is planned as a joint venture between the Soviet Union and the Mongolian People's Republic. This, said Minashin, will pose particular problems in view of the "local climatic conditions and requirements of the country".

Easier, technically speaking, will be another proposed extension—the launching of an "Intersputnik" Comsat system by the Soviet Union in conjunction with the countries of Eastern Europe.

One final refinement of the Molniya-1 should be noted: besides the transmitting apparatus, it has on board a "command-measuring complex" and systems for the correction of orbit and orientation, which are used to bring the satellite from its initial orbit into the optimum orbit for maximum transmission time, and thereafter to correct any deviations which may occur—thus maximizing the effective transmission lifetime of each satellite of the series.

Parliament in Britain

Britain's Space Programme

THE Prime Minister seems to be unmoved by a new request from Mr Neil Marten that more effort and resources should be put into space activities. Mr Wilson said that he does not believe that spending large amounts of money on prestige space projects is the most efficient way of using Britain's technological resources. Mr Marten thought, however, that it is high time that Britain moved towards getting its own capability for launching satellites for commercial purposes, instead of relying on the United States. (Oral answers, March 12.)

University Files

THE Privy Council cannot prevent universities from keeping files containing information on the politilac activities of students and staff. Its powers are restricted to approving amendments to university charters and statutes, and it has no jurisdiction over the internal administration of individual universities. This information was given by Mr Fred Peart, Lord President of the Council, in reply to a question from Mr J. P. W. Mallalieu, but Mr Peart also said that he believes that the practice of keeping such files should be condemned. The political opinions of staff and students are no business of a university, he said.

Mr Short, Secretary of State for Education and Science, said later that he also is powerless to prevent universities from keeping such files. Mr Short said that this is the responsibility of the university authorities, and he has no power to intervene in their internal affairs. (Oral answers, March 11 and written answers, March 12.)

Herbicide 2,4,5-T

ALTHOUGH the US government has banned the civilian use of the herbicide 2,4,5-T, and limited its use in Vietnam to unpopulated areas, it is not necessary to ban it in this country. This statement was made by Lord Delacourt-Smith, Minister of State, Ministry of Technology. The decision of the US government was based on toxicological tests which indicated that a toxic impurity in the herbicide may cause foetal abnormalities, but the British-manufactured variety is free from this particular impurity, said Lord Delacourt-Smith.

A voluntary safety scheme is operated with the assistance of a committee which gives guidance on the use of pesticides and herbicides, and Lord Delacourt-Smith said that, provided 2,4,5-T is used in accordance with the instructions laid down by the safety scheme, no danger and no hazard should arise.

Lord Brockway, for one, was not convinced. He suggested that many scientists are doubtful whether even the clean process used in Britain eliminates all the toxic impurity, because it may be regenerated by heat and light. He also accused the government of hushing up an accident in one of the factories manufacturing the herbicide, in which some people were poisoned. But Lord Delacourt-Smith said that the accident occurred because of overheating of the reaction mixture, and in such conditions the toxic impurity would be expected to form. He did not say whether anybody was injured by the accident. (Oral answers, Lords, March 11.)

NEW WORLD

Bleak Record of International Collaboration

ONE of the chief advocates of closer international collaboration in space research, Dr Thomas Paine, the director of NASA, had a sad tale to tell the Senate Committee on Aeronautical and Space Sciences last week about his agency's efforts to collaborate with others elsewhere. The most sombre part of his tale was the record of attempts at collaboration with the Soviet Union. He said that first attempts to cooperate in planning go back to the preparations for the International Geophysical Year in 1955, but that even then "agreements for exchange were held to a minimal or token level. The Soviet side has not fully complied even with these". Efforts at cooperation by the United States in 1959 are said to have come to nothing, and Dr Paine says that he considers "disappointing" the outcome of the agreement between the Soviet Union and the United States of June 1962 which allowed for the coordinated launching of experimental weather satellites and the exchange of such data as might result, the coordinated launching of Earth satellites equipped with absolute magnetometers and the use of communications satellites for joint experiments. This agreement was followed by one in November 1965 which would have produced a collaborative review of "space biology and medicine". Dr Paine explained that although the communications channels between Washington and Moscow intended for the exchange of meteorological data were established in October 1964, Russian transmissions of satellites as distinct from conventional weather data had not been "operationally useful". In the same spirit, he said that the Soviet Union has not yet said which of the data from the second of the orbiting geophysical observatories it wishes to receive, and that the exchange so far had been restricted to ground based data in an irregular fashion. The experiments with communications satellites may not be a good test of good will, for they were intended to be built around the Echo II satellite, but Dr Paine said that the Russians did provide reasonable data about radio reception even though they declined to transmit; a part of the difficulty with this experiment seems to have been at Jodrell Bank. The plan to produce a joint review of space biology, on the other hand, seems to have been frustrated by difficulties with correspondence.

Dr Paine gave a detailed account of the attempts that have been made to extend the collaboration between the United States and the Soviet Union in space research (see panel). In the past six months, according to this list, several invitations by NASA to the Soviet Union to collaborate in American space ventures have been rebuffed or ignored, although on December 12, 1969, Academician Keldysh, the president of the Academy of Sciences of the USSR, agreed that there should be a meeting "in three or four months" to discuss the further development of collaboration between the two countries while at the same time declining an invitation to mount Russian experiments in American interplanetary rockets.

Another thorn in Dr Paine's flesh seems to be the reluctance of the Russian authorities to provide more than "generalized assurances" that Russian rockets to other planets or to the Moon are in fact sterilized so as to prevent contamination. But Dr Paine seems to be a realist about this and accepts that collaboration with the Soviet Union "is not limited so much by technical considerations as by political considerations on the Soviet side", so that there is little prospect that the Russians will accept substantial proposals even though "we do not propose to stop trying".

Elsewhere, according to Dr Paine's account last week, collaboration has been more successful, even in comparatively humdrum fields such as the development of vertical and short range take-off aircraft, in which Canadian, French, German and British teams are involved. Dr Paine singled out the proposal for the German–American solar probe, towards which the West German government will contribute the greater share of the cost—more than $100 million altogether. There has been particular interest in collaboration on the space shuttle. According to Dr Paine, the principles of these agreements are that NASA should enter "only into concrete, carefully defined projects of clear mutual interest" and that the partners in the collaborative schemes should pay for their own responsibilities. Last October, 43 foreign governments as well as ELDO were given an outline of the plan for the space shuttle. Last week, ESRO, ELDO, the European Space Conference and a number of other governments were represented at a quarterly review of the development of the proposed space station. Similar meetings will follow later this year. Clearly, however, the degree to which these countries will participate, which Dr Paine says "depends on decisions that only they can make", is limited not merely by the novelty of the exercise but by the fact that countries other than the United States and the Soviet Union spend only about $300 million a year on space.

The difficulties are not merely financial. A spokesman for NASA said that the administration had been anxious not to lay down in advance the principles on which collaboration in new ventures should be based, but considered it was for "third" countries to make proposals themselves. The governments concerned are however at a loss to know how the kinds of contribution they might be able to make could win anything but a nominal part in ventures such as the space shuttle. As things are, where bilateral agreement to cooperate involves an American launching of, say, a French satellite, the French will pay for and build the satellite and NASA will provide the rocket on the understanding that information is equally shared. Where some practical use for the satellite is foreseen, as with the launching of telecommunications satellites for Intelsat, the manager of Intelsat (Comsat) will reckon to pay not merely for the satellite (which it contracts for directly) but for the direct cost of launching and something towards research and development.

Chronology of Collaboration

December 7, 1959. US offer of help in tracking Russian manned flights—Russians reply that they would ask for help "if the need arose".

March 7, 1962. Exchange of tracking and data acquisition stations declined.

September 20, 1962. No response to Kennedy's offer in the UN General Assembly for joint exploration of the Moon.

December 8, 1964. Proposed exchange visits to deep space tracking stations declined on the grounds that such visits were impossible.

May 3, 1965. No response to NASA suggestion of telecommunications experiments with Molniya I.

August 25, 1965. Invitation to launching of Gemini VI declined.

November 16, 1965. Further enquiry about the use of Molniya-1 declined by the Soviet Union as being impossible "in the present conditions".

January 6, 1966. Academician Blagonravov declined request for information about Russian experiments on Venus probe "to emphasize experiments which could complement rather than duplicate Soviet work" on the grounds that he had no authority to give the information.

November 11, 1966. Invitation to the launching of Gemini XII declined.

March 27, 1967. American request for advance information of soil analysis from Luna 13 met only after formal publication.

March 27, 1967. Proposals for small meetings between the Russian and American academies on weather prediction, planetary research and orbiting telescopes so far ignored.

June 2, 1967. Proposal for London meeting on exchange of weather data, specified under the agreement of 1962, denied—"the required semi-annual meetings have not been held since October 1965".

December 19, 1967. Proposal by Dr F. Seitz, then president of the US National Academy of Sciences, for a joint working party on Venus experiments accepted in principle on January 24, 1968, but "there has been no further Soviet response".

May 29, 1969. Invitation to Apollo 11 declined.

August 21, 1969. Invitation to planning meeting of Viking rockets to Mars declined on the grounds of lack of time (from September 3–11).

September 18, 1969. Proposal for exchange of biomedical scientists pending for further consultation in the Soviet Union.

October 3, 1969. Offer of lunar samples not taken up.

October 10, 1969. Academician Keldysh declined invitation of proposals for Soviet experiments on NASA space rockets but said that NASA and the Soviet academy should "coordinate planetary goals and exchange results of unmanned planetary investigations".

December 18, 1969. No response to invitation to Houston lunar conference.

January 29, 1970. Invitation to discussion of United States earth resources satellites declined.

INTELSAT

Agreement Snatched Away

from our Special Correspondent

Sometimes international conferences end not with a whimper but with a bang. This final week of the conference to decide on a permanent structure for Intelsat, the worldwide satellite system, began with such ferocity that the members had to retreat into private session, taking with them all the glowing press cuttings of last weekend, which had them breaking their year-long deadlock and reaching accord.

The dog in the manger once again seems to have been Comsat, the American satellite corporation, which has run Intelsat very nicely, thank you, without undue interference from its other so-called partners in the international consortium, since 1964. Comsat had seemed to be losing ground. The United States State Department surrendered first of all Comsat's majority role in the consortium and then, last week, gave away Comsat's claim to hold the managership permanently. The State Department agreed to what is called "internationalizing the managership" and decided instead to allow Comsat to hold the job—where the real power is—for six more years only. When the conference broke up at the weekend, delegates believed that they had agreed to agree on a compromise engineered by those two powerful nations least concerned with *le défi américain*, Japan and Australia. But when the agreement emerged on Monday morning in new clothes, dressed up in treaty language, it did not look the same at all. The director general, the executive who was supposed to choose a new manager when Comsat's term ended, was in plain language, emasculated.

None among the 74 delegations was really happy; there was, early in the week, no telling how it would all end. The tiny nations were particularly downcast. They have not won very much at all from the various bits of horse trading. And what they wanted was not so very much—to have their satellite ground stations counted as investment in Intelsat (instead of having their investment measured only by the amount of traffic that they sent through the system). What they also wanted, and may still be willing to fight for, was a kind of general assembly in which they had genuine power. As it looks now, the Intelsat assembly will have to be content with having one formal session, at which the main business will be to decide when they shall meet again.

Comsat will probably be manager for at least six more years, during which the next major round of contracts for a new generation of satellites will be handed out. But during that time Comsat will probably be supervised by a potent board of governors and an alert and non-American secretary general to look over its shoulder as it chooses satellite designs and dispenses Intelsat's money. The British–European bloc, while far from jubilant, is not miserable either. Its efforts and eloquence have been instrumental in demoting Comsat, something which seemed impossible two years ago. There is also general pleasure that the cumbersome conference which opened more than two years ago is crawling to a close. If the agreement that emerges is inelegant, it will nonetheless be both international and commercial and that is no small accomplishment.

OIL POLLUTION

Another Accident at Sea

The Chevron Oil Company is for the time being the most prominent member of the rogues' gallery of corporations offending against the environment because of its operation of an oil well in the Gulf of Mexico, a burning torch since February 10 which was converted into a source of about 50 barrels of oil a day when it was extinguished on Monday last week. No less a person than the Secretary of the Interior, Mr Walter J. Huckel, has gone to see for himself what damage is being done, especially at the bird sanctuary on Breton Island. Everybody—even the oil company—seems to agree that the offending well was not being operated in accordance with the more explicit regulations for offshore oil wells which were promulgated by the Geological Survey last August, after the escape of oil from an incomplete well off Santa Barbara, California.

The groups of a dozen wells now leaking oil lie about 20 miles east of the mouth of the Mississippi. Since the fire began, the Department of the Interior has suspended operations in the concession of 51 acres which Chevron has been opening up since it first leased it in 1958. Before the fire was extinguished with dynamite, it seemed as if the oil spill that would inevitably follow would be one of the most carefully controlled in the modern history of oil pollution. In the event, however, winds of ten knots in the gulf seem to have broken up the raft of booms constructed between the wild well and Breton Island, with the result that a large slick of oil began moving towards the coast. It is estimated that some two weeks will go by before the wild well can be sealed off from a hole now being drilled in at a slant.

Mr Russell Wayland of the United States Geological Survey explained last week that the new regulations require that oil wells should ordinarily be provided with devices known as storm chokes which are meant automatically to cut off the upward flow of oil in wild wells. The new regulations for offshore oil wells require that all wells should be provided with storm chokes, but the Geological Survey can waive this rule if the pressure in the pipe is too great for the chokes to operate successfully, or if the amount of sand being carried up with the oil is so great that the chokes would be worn out quickly. Although some of the wells in the Chevron field had been granted these waivers, the well which eventually caught fire, and which was unprotected, was not one of these.

In the circumstances, it is understandable that the oil company should have made valiant efforts to prevent the unavoidable oil slick causing damage to the coastline. Mr Wayland says that his department has been giving the oil company a rough time, and that all the offshore operators have been hurrying to comply with the new regulations. One problem, Mr Wayland said, is that of enforcing the regulations which exist—his small field staff cannot be present at all offshore drilling operations to ensure that they are complied with. It remains to be seen how severe will be the penalties which are visited on the Chevron Oil Company by the Department of the Interior. As well as permitting the closing of the oil field for an indefinite period, the regulations also allow the government to recover the money which it will have lost through the escape of oil—not a vast fortune—and to prosecute the offending companies in the courts, when success will bring fines of up to $2,000 a day for each violation and up to six months in prison for the violator—possibly the president of the company, possibly the operator of the rig, nobody is quite sure. The ultimate sanction is that flagrant violation of the rules may result in the cancellation of the lease to exploit a tract of offshore oilfield, but this would be accounted a thoroughly radical move by the Department of the Interior.

The delta of the Mississippi, like many other such tracts of land and water, is much frequented by wild fowl. Altogether there are close on a dozen bird refuges, the largest of which runs to close on a hundred square miles and which is maintained by the State of Louisiana, ironically with revenue derived from oil wells on the delta. Mr James Trefethen of the Wildlife Management Institute says that the species most at risk is the red-headed duck, which is at once rare and in the habit of venturing farther out to sea than other waterfowl wintering in the region, the canvas back duck and the pintail, for example. Mr Trefothen also says that if oil does eventually reach these areas, the waterfowl may nevertheless escape serious damage because the migration to the north is about to begin. There is even talk of using aircraft to speed the birds on their way to the north, which is another way of fighting fire with fire.

NUCLEAR POWER

Slow Pace of Reactor Development

The tightened budget of the Atomic Energy Commission means that the chief innovations in the coming year are likely to consist simply of the design of a 1,000 MW fast reactor using sodium as a cooling material. This much was clear last week. Mr Milton Shaw, director of Reactor Development and Technology at the Atomic Energy Commission, asked for a modest $194·6 million—substantially less than the $223·5 million that he asked for a year ago and $8 million less than his division was actually awarded for the current fiscal year. Spurred on not merely by logic but by the example of European nations, the AEC now says that the liquid metal fast breeder reactor is the cornerstone of its forward programme. What has happened so far is that three contractors are negotiating contracts for a design study, which should be completed in a year or so, when it will be possible to push ahead with the building of a 1,000 MW demonstration plant, probably starting towards the end of 1971. Although the AEC would like to build more than one plant of this kind, it has no authority to do so. So that the sodium fast breeder reactor can go ahead quickly within the confines of the tight budget, other promising candidates such as the molten salt breeder reactor, on which Oak Ridge has set its heart and which operates with thorium rather than uranium, have been put somewhat to one side.

The experimental basis of the sodium breeder reactor has now, according to Dr Shaw, been strengthened partly by the reorganization of the Argonne National Laboratory—a point urged on the AEC by the Joint Committee on Atomic Energy, the commission's most friendly critic—the commissioning of the experimental reactor EBR II (which is operating 25 per cent above its design power at 62·5 MW) and also by the resuscitation of the Enrico Fermi reactor, which has been out of commission since the accident there in 1966 but which is now being reloaded with fuel. The AEC estimates that the cost of the liquid metal breeder reactor programme will increase from just over $100 million a year in the current fiscal year to a maximum of $175 million in 1972 and 1973, by which time the contribution of the construction companies to the cost of building reactors will be $100 million a year or thereabouts. (Mr Shaw estimated last week that industrial companies are already providing $25 million a year towards the cost of a broadly based programme of research and development.) The alternative designs being considered are based either on combinations of uranium oxide and plutonium oxide (General Electric and Babcock and Wilcox) or combinations of the carbides (Westinghouse). In testing programmes already carried out, mixed oxide fuels have been carried to more than 11 per cent burn-up, and the mixed carbide fuels are not far behind.

Like the molten salt reactor, the high temperature gas reactor will also trudge along at a comparatively modest level in the coming year—each project will get $5 million under the proposed budget. Even so, the Peach Bottom reactor is now operating successfully, another high temperature demonstration plant should be critical in just over a year from now and Mr Shaw estimates that the first commercially viable plant should come into operation in 1977 or 1978, in time for this reactor type to make a contribution to the fuel economy of nuclear power stations by the eighties.

The concentration of interest in the liquid metal fast breeder reactor reflects the anxiety in the AEC to ensure the arrival of a second generation of nuclear reactors, chiefly so as to avoid the difficulty there will otherwise be with the supply of natural and enriched uranium. One calculation, described by Mr Shaw last week, is that if the fast breeder reactor can be introduced by 1984 into commercial service, it will be possible to live within a total uranium commitment of just under a million short tons of uranium oxide (chemical formula U_3O_8), the amount of uranium ore which can be won for $10 a pound or less. By the same test, if the introduction of the fast breeder reactor has to be postponed until 1994, it will be necessary to break into uranium reserves which cost up to $15 a pound. The result will be permanently to increase the cost of uranium fuel.

This year's hearings before the Joint Committee on Atomic Energy are being held in circumstances a good deal more cheerful for the commercial operators than in 1969. Although there has now been a recovery, with something like 12,000 MW of capacity ordered in the past twelve months, the rush by the utility companies into nuclear power has not yet matched again the happy days of early 1967, when 9,000 MW were ordered in the second quarter, making a total of more than 25,000 MW for 1967 as a whole. A part of the difficulty is that the nuclear construction industry has tended frequently to fall behind its promised construction dates. Another is that public opposition to the construction of power stations, nuclear or conventional, has made it hard for utilities to push ahead as quickly as they would like with new plans. The classic example, no doubt, is the proposed pumped storage scheme at Cornwall, New York, which has been held up for eight years by local objections, most of them on the grounds of amenity. Even so, it now looks as if additional nuclear generating capacity will be commissioned at a rate which averages or even exceeds 10,000 MW a year from 1971 on.

The relationship between nuclear power stations and their immediate environment has been for the past few weeks, as it was last year in October and November, a particular concern of the Joint Committee on Atomic Energy. Last year, the committee did much to uncover some of the anomalies in the regulations for the siting of nuclear plants—it turns out, for example, that the

SPACE

Vote of Affection

The House of Representatives Committee on Science and Astronautics has made its customary declaration of faith in and affection for the National Aeronautics and Space Administration by voting that the Administration's request for a budget of $3,333 million for the coming year should be increased to $3,630 million. At this early stage of the budgetary process, it is unlikely that Dr Thomas Paine and his programme managers will be lulled into the belief that there will, after all, be an extra $297 million to spend. Not merely has the Senate committee yet to have its say, but there is now what seems to be a well established tradition that the appropriations committee takes away what the authorization committee—the Science and Astronautics Committee—adds to the Administration's budget request. In these circumstances, the vote on the budget in the authorization committee is more a guide to sentiment than a promissory note.

Briefly, it seems that members of the House committee have been convinced of the rightness of most of the innovations in the new space budget and, at the same time, regret the running down of programmes with which they have become familiar. Thus the extra sums of money voted include $145 million to prepare payloads for Apollo 18 and 19 and to make preparations for Apollo 20. The committee also suggests that $100 million should be spent on potential bottlenecks in the Saturn V programme. Similarly, the committee asks that an extra $75 million should be spent on the orbital workshop, not merely to extend the work that will be done on the three planned visits to the first of the orbiting stations, but also to begin work on the design of a second and better place to visit. The committee also asks that $80 million should be spent on the space shuttle.

In a much more modest spirit, the committee has also made known its feelings by asking for modest sums of money to be spent on research into the reduction of noise caused by aircraft and the avoidance of collisions in the air. The committee's generosity does not, however, extend to that part of the programme which used to be called the University Sustaining Program and which is now due to be wound up—that is an unpopular cause this year.

AEC is required to approve all new nuclear power stations so as to provide an assurance of safety from radiation hazards, but that it is for state power commissions to regulate other features of the enterprise and in particular to decide whether the arrangements for disposing of cooling water are satisfactory. Because states seem to vary considerably in their practice, it is no wonder that what is called thermal pollution has become an important issue. One welcome result, however, is that utility companies are increasingly providing either cooling towers or cooling ponds for preventing damage to life forms in rivers or the sea by the discharge of water that is too hot—nuclear plants are comparatively serious offenders in this respect because of their comparatively low efficiency for the conversion of mechanical into electrical energy—even though it is estimated that the extra capital costs of cooling ponds may range from between $2 and $3 a kilowatt for fossil fuel plants to between $3 and $4 a kilowatt for nuclear plants, and that cooling towers may add between $4 and $6 a kilowatt to the capital cost of a fossil fuel plant and between $6 and $8 a kilowatt for a nuclear plant. Something like a quarter of the power stations now being built have such cooling systems. There are many who will say that it is not before time, given the estimate by the Water Resources Council that, by the end of the century, power stations of all kinds using rudimentary cooling systems would use five hundred thousand million gallons of water a day, more than 40 per cent of the average run-off from the surface of the United States.

SEISMOLOGY

Let More Sun Shine

SEISMOLOGY is the latest branch of science to be patted on the back by the committees of the National Academy of Sciences which are surveying the opportunities for new research and identifying the points at which money needs most urgently to be spent. The committee which has now argued that more seismology would be a good thing (*Seismology: Responsibilities and Requirements of a Growing Science, Parts I and II*, NAS, $5.50) has had as chairman Dr J. E. Oliver of the Lamont-Doherty Geological Observatory of Columbia University. A good deal of its thunder has been stolen by the National Science Foundation, which used many of the same arguments in presenting its case for a programme of earthquake engineering to Congress three weeks ago. Inevitably, however, the way in which attention has been concentrated on one of many geophysical techniques makes the committee seem guilty of special pleading, however valid its case may be. Indeed, the appearance of this latest report may draw attention yet again to the need to put together the several assessments of research opportunities in particular fields in one balanced assessment of science as a whole. This, by all accounts, is a project on which the National Academy of Sciences has now embarked by recruiting close on 80 distinguished potential members for the interlocking set of committees to produce the review which subsumes all lesser reviews.

The committee on seismology says that the rapid growth of seismology in the United States in the past two decades has been stimulated partly by the preoccupation with underground test detection under Project Vela-Uniform and partly by collaborative schemes such as the IGY and the Upper Mantle Project. Understandably, the committee is afraid that the "reorientation" of the Vela-Uniform work and the ending of schemes such as the Upper Mantle Project may decrease the support available for worthwhile projects in seismology. Especially because of the pace at which understanding of the Earth is deepening, the committee considers that the present time is one when a fresh assessment is urgently called for.

Given the value of seismology in test detection, the committee asks that there should be new federally sponsored programmes "so that seismologists can apply their special capabilities to an attack on other pressing national problems". In particular, the committee asks that there should be research related to the hazards of earthquakes and even to their prediction and control as well as an investigation of the economic and scientific implications of the doctrines of sea floor spreading and continental drift "in cooperation with other earth scientists". The committee has the practical suggestion that the government should assume responsibility for a national centre for acquiring and distributing seismological information based on the World-Wide Network of Standardized Seismograph Stations. This is a need to which others have previously drawn attention (see *Nature*, **225**, 844; 1970), and indeed there are fears that the efficacy of the WWNSSS may be seriously impaired, now that it has just been completed, by lack of funds. The committee suggests that financial support for seismology should grow from $35 million to $50 million a year in the next few years, and few people will protest. The doubt will be whether there should be what the committee calls "a national program in seismology", presumably separate from a national programme in oceanography.

The Oliver committee says that "the present and future goals of seismology must be keyed to the overall goals of society in an age dedicated not only to the acquisition of scientific knowledge for its own sake but to the application of this knowledge for the benefit of mankind". It goes on to argue that a better knowledge of seismicity and more vigorous programmes of engineering seismology will make it possible to mitigate the effects of earthquakes. Although the committee acknowledges that forecasting the time and the size of an earthquake is still beyond the bounds of possibility, it considers that there may be some hope in investigations of accumulating strains, rock creep along faults, micro-earthquakes and general geophysical processes in the crust of the Earth. The committee also asks that somebody should study the question of how populations respond to the threat of earthquakes. Whether or not the prevention of earthquakes will ever be possible, the committee says that this must "rank as a major goal of seismology", and cites as evidence the artificial triggering of earthquakes in Denver by injecting fluid into the Earth as well as in Nevada by underground explosions (see also page 1099). In the budget of the National Science Foundation for 1971 is included $2 million for earthquake research—among the schemes on which the foundation will sponsor work is one for constructing an earthquake simulator consisting of a movable oscillating platform 20 feet square (see *Nature*, **225**, 785; 1970).

NEWS AND VIEWS

Dust-up in Nevada

It is rare for a scientific article to elicit ferocious response within weeks of publication—most experimental studies and theories are poised on a superstructure of unique apparatus or theoretical formalism not immediately accessible to the reader. The experience of Emiliani, Harrison and Swanson of the University of Miami is thus all the more singular. In *Science* (**165**, 1255; 1969) they postulated that underground nuclear test detonations in Nevada were causing increased earthquake activity at distances out to 860 km from the test site. They also suggested that such explosions might be useful in controlling earthquake activity.

Predictably they have received a sharp rap over the knuckles, and three rejoinders (*Science*, **167**, 1011; 1970) have been published signed by no fewer than nine luminaries of the seismological world. The whole seismological staff of the California Institute of Technology add their weighty names to one such letter. The experience is perhaps more of a psychological than a geophysical happening—it reflects things felt rather than things known, and it reveals the danger of attempting to copyright what is no more than coffee-room musing.

The question of destressing a region by some explosive process arose in 1963, when it was appreciated that some explosions have an earthquake type of radiation pattern. Recent large nuclear tests in Nevada have triggered near-in (less than 20 km away) earthquakes by the thousand, a phenomenon which is universally known but little understood. Do the earthquakes release pre-existing stress or only stress accumulations from the explosion itself? Nobody knows for sure and a coordinated programme of earthquake control, such as the United States hovers on the brink of supporting, must regard this as one of its priorities. Hence, probably, a certain amount of irritation that Emiliani *et al.* should claim originality for their suggestion of earthquake control. To their credit they have made a full retraction of their claim to be the first to suggest it.

The issue of increased seismicity is more thorny, and this is not the first time that feathers have flown. For the past fifty years people have hunted for periodicities in earthquakes as a step towards their prediction. Such claims for periodicities have tended to diminish with the advent of improved facilities for harmonic analysis, but some apparent periodicities remain with us and call for continued study. One prerequisite, insufficiently realized, is the need to find a scoring system so that events of different magnitudes are given appropriate weight. Straightforward counting is not enough, and may be highly dependent on the network of stations used.

Another decision that needs to be taken concerns aftershocks of large earthquakes. Do they score in the same way as main shocks? Is the frequency of aftershocks controlled only by the nature of the main shock, or could a large shock render an area particularly prone to any periodic forces such as Earth tides? These questions, posed many times in periodicity studies, apply with equal force to the arguments of Emiliani *et al.* Credit where credit is due, the authors respond to their critics by showing data called for and on the basis of this there is a statistically significant increase in activity out to 800 km in the first 32 hours after a test. But there is no indication of magnitude of event, size of relevant explosions nor an adequate analysis of the incidence of aftershocks. The implications of what Emiliani *et al.* have said are great, but very few would yet attach any label other than "not proven" to the idea. A more sophisticated approach than straight counting is needed.

PLANETARY NEBULAE

Doublets give Electron Density

by our Astronomy Correspondent

A method of determining electron densities in planetary nebulae by observing the ratios of certain line intensities has been described by H. E. Saraph and M. J. Seaton, and an account of the work was given by Professor Seaton at last week's meeting of the Royal Astronomical Society. The technique is to measure the intensities of the two lines in certain doublets, and it seems that although the intensity ratio is insensitive to temperature, the ratio is very sensitive to electron density. The case of the OII doublet—3726 Å and 3729 Å—has been studied for several years, but Saraph and Seaton have extended the electron density measurements to other doublets which have not been studied in detail before.

One advantage of their new set of doublets is that the separations are greater than for the OII lines so that spectrograph dispersions need not be so high as for the OII case. The larger separation also means that electron density measurements can sometimes be taken in galaxies where the OII doublet cannot be resolved because of Doppler broadening.

The doublets which Saraph and Seaton discuss are SII (6731 and 6717 Å), ClIII (5538 and 5518 Å), ArIV (4740 and 4711 Å), and KV (4163 and 4123 Å). With the exception of SII, the intensity ratio for each doublet seems to have a larger sensitivity to electron density than OII, for a wide range of densities at least, and in particular the sensitivities are greatest around the electron density values which are typical of many planetary nebulae.

Actual measurements reported by Saraph and Seaton show good agreement between electron densities obtained from different ions. Observations of IC 418, for example, give values of log n_e of 3·9, ≤4·4 and 4·1 from the SII, OII and ClIII doublets respectively. In objects of high excitation the situation seems to be different, and for NGC 7027 values of log n_e of 3·8, 3·7, 4·3, 5·3 and 5·1 are reported from the doublets SII, OII, ClIII, ArIV and KV respectively. The electron density seems to be larger at high excitation levels, and no unique electron density can be assigned to these objects.

ROCK MAGNETISM

Oxidation and Hysteresis

from our Geomagnetism Correspondent

THE correlation between the petrographically determined oxidation state and the polarity of natural remanent magnetization (NRM) in some igneous rocks remains a puzzle. If most reversely magnetized rocks are due to field reversal—and the evidence for field reversal is too strong to be denied—there should be no correlation between polarity and the nature of the magnetic minerals present. The strength of the NRM, on the other hand, depends on the properties of the magnetic material, some of which can be assessed petrographically. Clearly the intrinsic magnetic properties of a rock are an important link between the NRM and the optical properties, and much more needs to be known about the whole chain before oxidation-polarity correlations can be understood.

The problem in trying to correlate intrinsic magnetic properties with oxidation state is that many of the properties (the Curie point, for example) can only be determined by heating, which may alter the oxidation state. Moreover, room temperature properties, such as saturation magnetization, are difficult to interpret because it is impossible to obtain from the rocks highly refined extracts of the various magnetic minerals. But Day *et al.* (*J. Geophys. Res.*, **75**, 375; 1970) have successfully demonstrated that there is another indicative magnetic property, determination of which involves neither heating nor extraction. This is rotational hysteresis, the work done against irreversible magnetization processes when a sample of magnetic material is rotated through 360° in a magnetic field.

Day and his colleagues have measured the rotational hysteresis (*W*) as a function of magnetic field (*H*, up to 21,000 Oe) for natural rock samples and synthetic titanomagnetites, magnetite, maghaemite and haematite. The *W–H* curve from each rock was then examined in relation to the rock's oxidation state (classes 1 to 5, using the scheme of Wilson and Haggerty, *Endeavour*, **25**, 104; 1966) determined petrographically. This revealed that each oxidation class has its own characteristic *W–H* curve. All *W–H* curves possess a peak at low fields with a tail extending to the highest field available; but the width and position of the peak and the size of the tail vary with the oxidation state. By comparing the rock and synthetic sample curves, Day *et al.* were able to identify the operative magnetic minerals present in the rocks (see table).

MAGNETIC MINERALS FOUND IN ROCKS BY DAY *et al.*

Oxidation class	Magnetic mineral content
1*	Titanium-rich titanomagnetite
1†	Iron-rich titanomagnetite
2	Cation-deficient spinel phase
3	Iron-rich spinel phase
4	Magnetite and small amount of haematite
5	Haematite and small amount of magnetite

* Low Curie point.
† High Curie point.

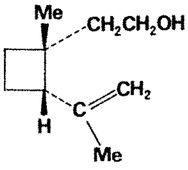
An obvious consequence of these results is that it should be possible, with a little care, to use rotational hysteresis to determine oxidation class without resort to laborious petrographic techniques. A more fundamental point to emerge, however, was that the most stable rocks fall into class 3. In other words, surprisingly, maximum stability is not associated with haematite which is known to be highly stable, and more stable than titanomagnetites. But the general point is that it is now possible to determine the magnetic mineral content of a rock without destroying it chemically or physically.

PHEROMONE SYNTHESES

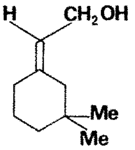
Trapping the Boll Weevil

from a Correspondent

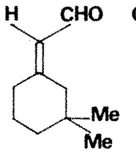
COTTON growers who read the *Journal of the American Chemical Society* will be cheered to learn that the pheromone of the pestilential boll weevil has been synthesized. Pheromones, the chemicals secreted in minute quantities by insects to influence the behaviour of other members of the same species, have great potential value in the chemical control of insect pests. The procedure would be to attract insects to traps containing insecticides by baiting the traps with pheromones.



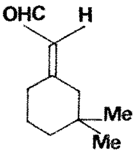
(I)



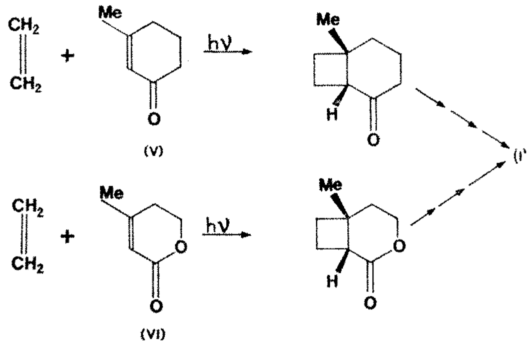
(II)



(III)



(IV)



The American cotton boll weevil does some $100 million damage annually to the US cotton crop. The male of the species secretes a pheromone which contains the four terpenes (I–IV) in faecal concentrations of 0·76, 0·57, 0·06 and 0·06 p.p.m. respectively. Stereospecific syntheses of the more complex alcohol (I) have been completed recently by research teams at the Zoecon Corporation in California and in the US Department of Agriculture's Boll Weevil Research Laboratory in Mississippi (R. Zurflüh, L. L. Dunham, J. L. Spain and J. B. Siddall, *J. Amer. Chem. Soc.*, **92**, 425; 1970). Both syntheses construct the four-membered ring by the photochemical addition of ethylene to a cyclic olefin; the former group choosing 3-methylcyclohexenone (V) and the latter group using a dehydration product of mevalonolactone, 3-methylpent-2-enolactone (VI). This photoaddition guarantees the correct *cis*-stereochemistry for the ultimate product (I) which is obtained in 10 per cent overall yield after further chemical transformations to give the correct side chains.

Without this component, mixtures of the other three synthetic compounds fail to attract boll weevils whereas the attracting power of the combination of all four chemicals in the correct proportions closely matches that of the natural pheromone. This research may not only provide a solution to the control of the boll weevil, but also assist in restricted distribution of insecticides.

AMPHIBIA

Ancestors of the Reptiles

from our Vertebrate Palaeontology Correspondent

The vexed question of the amphibian ancestry of the reptiles has been aired again. According to R. L. Carroll, the carboniferous tetrapod, *Gephyrostegus*, although too recent to be the ancestor in question, seems to have the characteristics required for such an evolutionary position (*Phil. Trans. Roy. Soc.*, B, **257**, 267; 1970).

The most important criterion distinguishing living reptiles from amphibians is their mode of development. Reptiles (and birds) usually lay an egg which provides all the material needs of the developing embryo so that it may hatch fully formed. This amniote egg has not developed in amphibia and a protracted larval stage is common, although many species have curtailed or even eliminated this vulnerable stage in their development by other means. When dealing with the fossil remains of early tetrapods it is thus very difficult to establish criteria for deciding whether a given "reptiliomorph" tetrapod is to be classified as a reptile or an amphibian.

Many years ago, the fossil tetrapods, thought to be the most primitive reptiles, were placed in three groups by D. M. S. Watson: the first represented by *Seymouria*, the second by *Diadectes* and the third by *Captorhinus*. All three "type" genera were from early Permian deposits from Texas and surrounding states. For many years the status of *Seymouria* was disputed: was it a reptile or an amphibian? On the one hand, it was a sturdy terrestrial form with many characters regarded as reptilian; and on the other hand, the structure of the middle ear region, critical to the interpretation of early tetrapods, favoured amphibian affinity. The matter was finally settled when Špinar in Prague demonstrated that closely related forms from the European Lower Permian developed external gills during their development and thus had a larval stage.

Thus *Seymouria* was an amphibian, but it was still accorded an honoured place as a relict reptilian ancestor. It was recognized, however, that while it was probably closely related to *Diadectes*, a massive form with an enormous otic notch to hold the eardrum, it was not particularly closely related to *Captorhinus* and its allies, small lizard-like forms with no otic notch, and with an eardrum that must have been situated behind the skull. Unfortunately for the status of *Seymouria* it is *Captorhinus*, or rather its less specialized predecessors, which are probably the most closely related to the ancestry of all subsequent reptiles.

These primitive captorhinomorphs occur very much earlier in the fossil record than either *Captorhinus* or *Seymouria*. The earliest known comes from a horizon in Nova Scotia which is early Pennsylvanian and probably equivalent to the Middle Coal Measures of Britain. This and another somewhat later and better preserved form (*Paleothyris*) have been described by Carroll, of the Redpath Museum, McGill University, as part of a general attack on the problem of reptile origins. He has also undertaken a detailed anatomical study of several little known and difficult fossils from Carboniferous horizons in Europe more recent than the British Coal Measures. Most important of these is the genus from Nýřany in Bohemia, but there are other specimens of disputed affinity which he considers the descendants of early offshoots of the amphibian–reptilian evolutionary stock.

Last year (*Biol. Rev.*, **44**, 393; 1969) Carroll characterized *Gephyrostegus* as an ideal relict of the reptilian ancestor, although, like *Seymouria*, too late in time to occupy a true ancestral position. However, it lacks some of the aberrant specializations of the ear region which *Seymouria* has. Carroll attempted to demonstrate by a close comparison of the whole skeletons of *Gephyrostegus* and *Paleothyris* the stages in the evolution of a reptilian skeleton from an amphibian skeleton.

His latest contribution has been to describe the anatomy of *Gephyrostegus* and other forms from Nýřany as well as an allied form from a roughly contemporary horizon in the United States. This work is likely to establish *Gephyrostegus* as demonstrating the anatomy of the reptile ancestor, but a lingering doubt remains. While *Gephyrostegus* lacks the specializations of *Seymouria* its skull is still basically similar and in critical features of the back of the skull roof and occiput thus resembles *Diadectes* rather than the certainly reptilian captorhinomorphs. Meanwhile, *Diadectes* has come to be considered by many as a sterile end form of an evolutionary line quite separate from true reptiles.

EVOLUTION

Ammonites indicate Reversal

from a Correspondent

Can the course of evolution turn back on itself? Or does some sort of momentum keep an organism proceeding in a particular evolutionary direction, even if this results in the production of aberrant and non-adaptive types, forming an evolutionary cul-de-sac ending in extinction? Among evidence often cited for the latter point of view is the production of aberrant forms by the familiar spiral-shelled ammonites, of which fossil remains are found from the early Devonian through to the Cretaceous. In these aberrant forms, called heteromorphs, either the whole shell, or part of it, is unrolled. This is interpreted as a sign of overspecialization or racial senility, preceding inevitable extinction.

A drawback of this point of view is that it effectively credits evolution with an intrinsic rhythm, comparable with that of an individual life, with each type having a youthful phase of explosive development, then a long period of advancing specialization, and a final phase of senile overspecialization and morphological degeneration. This is, of course, in direct contradiction to Darwin's selection theory, which cannot explain the appearance of any organisms which are selectively negative. On the other hand, it does tally with the "law" of the irreversibility of evolution which Dollo put forward in 1893.

The use of uncoiling ammonoids to support the idea of an endogenous rhythm in evolution has now been challenged by Jost Wiedmann, of Tübingen University (*Biological Reviews*, **44**, 563; 1969), after careful study of the heteromorph ammonoids of the Triassic, Cretaceous and Jurassic eras. In particular, he looked to see, first, whether these heteromorphs really appeared explosively at the end of evolutionary lines; second, if their development was irreversible; and third, whether they really were short lived, degenerate or overspecialized, and had lost the ability to adapt or change, thus being "condemned" to extinction.

He found that the Triassic heteromorphs were a single monophyletic group, with a long stratigraphical range through the whole of the later Triassic, suggesting that here the uncoiling was an adaptive change, possibly related to living on the sea bed, and with no indication that it was due to an unselected and somehow internally elicited mutation. The Jurassic heteromorphs, too, seem to have been a monophyletic group persisting for a long time, and some of these do show signs of re-coiling, as do several groups of Cretaceous heteromorphs. Indeed, here some of the uncoiled forms turned out to be older than their coiled supposed ancestors, and so their phylogenetic development must now be read in the opposite direction—that is, from uncoiled to re-coiled.

Wiedmann thus finds no evidence to support the assertion that "a re-coiling of forms which have become secondarily rod-like does not occur". In fact, if one regards the ammonites coiling early in their evolution in the Devonian era as being primary, the re-coiling of the heteromorphs he has found here is actually tertiary, with some of the re-coiled forms even showing a later quaternary uncoiling. This means, of course, that the final extinction of the ammonites at the end of the Cretaceous needs some explanation other than endogenous racial senility. Wiedmann points out that many other marine groups died out at the same time, so that changes in sea level provide a more satisfactory answer than either an immanent evolutionary ageing, or factors like cosmic rays or supernovae. Second, it clearly calls for a reconsideration of what he calls the "most undisputed of the phylogenetic laws", namely Dollo's "law of irreversibility". This was originally stated as "an organism is unable to return, even partially, to a previous stage already realized in the ranks of its ancestors" and the too strict application of this rule has already been challenged, especially by geneticists, who have pointed out that reverse mutations should not be ruled out *a priori*. Now Wiedmann concludes that in palaeontology the over-hasty formulation of such phylogenetic "laws" and "principles", based on insufficient information, has produced "a stifling set of prejudices" rather than "guide lines crystallized from long experience and observation". This all serves to stress very neatly the often overlooked point that in science, the so-called "laws" are, in Karl Pearson's phrase, "descriptive and not prescriptive", so that where facts clash with the law, and the exception proves (that is, tests) the rule, the facts should aways prevail.

SPECTROSCOPY

ESR for Biologists

from a Correspondent

RESEARCHERS in the biological sciences are showing increasing enthusiasm to add electron spin resonance (ESR) to their range of spectroscopic techniques. This became evident at a symposium on ESR in biological systems held at Liverpool Polytechnic on March 11 and 12.

Although the applicability of ESR to biological systems is somewhat limited by the scarcity of paramagnetic centres in biological molecules, this has the advantage of making ESR a very specific method for probing a particular part of those molecules containing a paramagnetic species. Dr J. C. Swann (Chester Beatty Research Institute, London) illustrated this when describing how the mechanism of action of the enzyme xanthine oxidase has been largely elucidated by ESR studies of the molybdenum and iron atoms and an FAD radical in its active site. The molybdenum atom has proved the most useful probe, and experiments with deuterated substrates and in ^2H_2O solution have shown that the mechanism involves direct hydrogen transfer from the reducing substrate to a position near the molybdenum atom where it is exchangeable with the solvent. The oxygen radical (O_2^-) has been detected as an intermediate in the reduction of molecular oxygen by the reduced enzyme, using ESR in conjunction with the rapid-freezing technique developed by Bray. The identity of this radical has been confirmed in experiments with oxygen-17.

The utility of the isotope substitution method was also demonstrated by Dr J. F. Gibson (Imperial College, London), who talked about the ESR of iron–sulphur proteins, which include ferredoxins, oxidative enzymes of mitochondria and xanthine oxidase. They are characterized by an iron signal with unusually low average g value (less than 2) and anomalous temperature sensitivity. The temperature sensitivity has been simulated in an inorganic iron–sulphur system, but there are no inorganic analogues with such low g values. Isotope substitution using either iron-5 or, in place of sulphur, selenium-77, reveals that each iron interacts with the other and with two selenium nuclei. Thus the characteristic signal seems to come from a two iron–two sulphur entity.

The more familiar iron compounds of biological interest are the haem iron proteins, and Dr E. F. Slade (University of Keele) discussed determinations of zero field splitting tensors in myoglobin using 2 and 4 millimetre wave ESR instruments with superconducting, liquid helium temperature magnets. A transition between the lower two Kramers doublets is not seen, presumably because of depopulation of the upper level of the ground state doublet at liquid helium temperature, but estimates of the splitting have been obtained indirectly. Results for haemin chloride agree with direct spectroscopic determination, but other results have not confirmed estimates from Mössbauer spectroscopy.

Many molecules of biological interest which have no paramagnetic centre can be modified by attaching a small synthetic probe containing a stable nitroxide radical. This spin labelling technique developed by McConnell was described by Dr D. Chapman (University of Sheffield). The method is based on the anisotropy of signals from the nitroxide radical which thus acts as a "reporter" of molecular motions (for example, whether the spin label is in a region of free or restricted movement). Applications have ranged from studying conformation changes in proteins to obtaining evidence for the bilayer structure of phospholipid membranes. Interaction of lipid membranes with drugs and anaesthetics has also proved tractable to the spin labelling approach. Chapman emphasized, however, that possible perturbation of the biomolecular structure by the spin label must be considered, and that the method should be considered as complementing rather than outmoding more classical techniques, such as polarization of fluorescence.

NERVOUS SYSTEM

Central Cholinergic Mechanisms

from a Correspondent

Much has been heard about the significance of central neurones, with monoamines as transmitters, for an understanding of neurological and psychiatric disorders and the action of centrally acting drugs. But cholinergic neurones, using acetylcholine as their transmitter, are no less important, being involved, for example, in ascending systems of neurones which may have an important role in attention, awareness and consciousness. Investigations, however, have been held up for lack of a sufficiently sensitive and specific chemical method for estimating acetylcholine. But at the international symposium on the effects of drugs on cholinergic mechanisms in the central nervous system, held at Skokloster, Sweden, from February 23 to 25, Dr D. J. Jenden (Los Angeles) thought that it should soon be possible to estimate picomolar quantities in tissue samples and fluids using a combination of gas chromatography and mass spectrometry.

Much of the meeting was taken up with an examination of the storage and release of acetylcholine and how they are affected by drugs, using superfused cortex, cortical slices and isolated nerve terminal synaptosomes. The synaptosome has turned out to be a most versatile organelle. Dr H. F. Bradford (London) explained how to make "reconstituted slices" from synaptosomes which have most of the metabolic properties of brain slices, including a large increase in oxygen uptake when electrically stimulated. He also showed that of the six or so uncombined amino-acids present in synaptosomes in considerable amounts, only those known to have transmitter-like effects on central neurones are released on stimulation. Dr M. P. Blaustein (St Louis) observed that in metabolizing synaptosomes the uptake of calcium ions is stimulated by potassium ions in a manner very similar to that of intact neurones. He considers that the metabolizing synaptosome has a normal resting membrane potential.

When the topic was the cholinergic receptor and its structural and functional relationship to acetylcholinesterase, Dr E. Heilbronn (Sundbyberg and Uppsala) described the morphological changes in brain slices which result from treatment with phospholipases. The pre-synaptic portions of the terminal membranes are selectively destroyed, leaving clumps of intact vesicle and synaptic clefts; such preparations should be a valuable new starting material for the isolation of the post-synaptic membrane, particularly if allied with the new high resolution separation techniques using zonal rotors described by Dr R. H. Mahler (Bloomington) and Dr V. P. Whittaker (Cambridge and Staten Island).

VIROLOGY

Where to get Monkey Viruses

Many virologists still do not seem to be aware of the services available to them at the simian virus reference centre of the Southwest Foundation for Research and Education in San Antonio, Texas. Dr S. Kalter, director of the foundation's division of microbiology and infectious diseases, writes: The increased use of non-human primates in biomedical research prompted the need to develop a centre to which researchers could turn with their virological problems. As a result, a simian virus reference centre was set up in 1966 at the Southwest Foundation, with support from the World Health Organization; in 1968 the US National Institutes of Health added its support. The centre is called the Collaborating Laboratory on Comparative Medicine: Simian Viruses (*WHO Chronicle*, **23**, 112; 1969).

The centre aims to develop a working repository for simian viruses, and to provide a source of reagents such as strains of certified reference seed virus and specific antisera. At present there are reagents available for all accepted prototype simian viruses. We try to obtain new simian viruses as they are described, in order to prepare stocks and develop suitable antisera.

Consultation services are available to deal with questions about the existence of antibody to viruses of human and simian origin in various primates. There are also diagnostic services, including facilities for the identification and characterization of viruses for primatologists who are unable to identify their own isolates (this includes screening for human viruses). An information service is also available, and exchanges of organisms between primate centres and other health organizations can be arranged. The Texas centre also has facilities for the training of students in the procedures of primate virology. The address of the centre is: Division of Microbiology and Infectious Diseases, Southwest Foundation for Research and Education, PO Box 28147, San Antonio, Texas 78228.

VIRUS INFECTION

Good News on Poly-I : C

from a Correspondent

During the past two to three years the synthetic double stranded RNA, polyriboinosinic acid–polyribocytidylic acid (poly-I : C), which induces interferon, has been shown to have a significant antiviral effect in animals and tissue culture. In spite of previous indications of unpleasant side effects in animals, recent clinical trials have had hopeful results. Some good news was delivered by Dr S. Baron (National Institute of Allergy and Infectious Diseases, Bethesda) and his colleagues at the international symposium on medical and applied virology in Fort Lauderdale on December 14–17, and at the Gustav Stern symposium on perspectives in virology at New York on February 2–3.

Baron *et al.* first examined the effect of poly-I : C on experimental rhinovirus infection in humans. When the dose estimated to confer protection, and still be safe, was given to volunteers, working up from one tenth of the dose, no evidence of toxicity was found. The subjects for trials against experimental virus infection were eighteen healthy volunteer prisoners, selected for a low concentration of serum neutralizing antibody to rhinovirus-13, so that they should be susceptible to infection. On the day before virus challenge, eight men were given 0·1 mg/kg body weight of poly-I : C intranasally; the rest received placebos. Further doses of poly-I : C or placebo were given for 5 days after infection, and the men were examined independently for symptoms. Five out of ten of those given placebos developed colds, compared with one in eight of those who received poly-I : C. And fewer viruses were recovered from the nasal washings of treated men. Probably more significant than the development of colds was the overall reduction in symptoms in the treated group, as measured by symptom score analysis.

When influenza virus was the challenge there was again a reduction in disease and symptoms—illness occurred in four out of nine subjects treated with poly-I : C and in seven out of ten given placebos.

Baron *et al.* also investigated the relationship between interferon titre and doses of poly-I : C in patients with either encephalitis or cancer, to look for ways of preserving a high titre of interferon for the longer periods necessary in prophylactic treatment. After one dose of poly-I : C there was a decrease in interferon after 24 hours, reaching a minimum after about 3 days. Further doses given during this period of decline had no effect. But in some cancer patients, treated intravenously, a dose given 7 days after the initial dose induced some interferon. Intravenous injections caused fever, reversible suppression of erythrocyte formation, and in one patient an allergic reaction.

These results are all encouraging, although only a few subjects were involved, both for the protection afforded by poly-I : C and for the apparent lack of side effects from intranasal treatment. On the question of toxicity, it should be remembered that experimental virus challenge doses are usually much higher than those occurring in natural infection, and so much less interferon inducer should be necessary for prophylaxis, with a reduction in possible toxicity. Certainly this is an interesting beginning to the therapeutic use of a synthetic interferon inducer in humans.

OXYGEN SENSORS

Galvanic Cell makes Good

from a Correspondent

SOME useful new developments in the measurement of partial pressures of oxygen were described when members of the Analytical Group of the Society of Analytical Chemistry met in London on March 12 to discuss oxygen sensors in biological and other systems. There has been growing interest in the direct measurement of the partial pressure of oxygen, particularly for medical purposes and in closed environments such as space capsules and mines. The polarographic oxygen electrode of Clark has been most commonly used to measure oxygen partial pressure in fluids.

Dr I. Bergman (Safety in Mines Research Establishment, Sheffield) pointed out that the galvanic oxygen electrode, which unlike the Clark electrode requires no external applied voltage to bring about the reduction of oxygen at the cathode, is essentially a polarographic device when operated in the gas diffusion controlled region. Research at the Safety in Mines Research Establishment has led to the development of several polarographic oxygen sensors in which a silver cathode is evaporated on to a polytetrafluoroethylene (PTFE) membrane. The anode is Ag/AgCl. In this way very efficient reduction of the incoming oxygen is achieved as well as a very fast response time. Bergman demonstrated an oxygen sensor which could be used to control the partial pressure of oxygen in an oxygen tent used in hospital during the care of premature babies.

Dr D. Parker and Mr A. Key (University College Hospital, London) reported the development of three silver-lead galvanic cells for measuring oxygen tension in blood. The first was a "flow-through" sensor which measured continuously the oxygen tension of blood flowing from a heart-lung machine to a patient undergoing heart surgery. The sensor electrodes were isolated from the blood by a PTFE membrane. Temperature compensation was incorporated in the device so that the cell output was linear in the range 20°–40° C for a pO_c from 0·710 mm Hg. The second sensor was a small probe used to determine glucose concentration in serum and urine. The glucose sample was added to an air-saturated glucose oxidase enzyme and the rate of consumption of oxygen measured by the oxygen sensor. The rate of consumption was proportional to the concentration of glucose initially present.

Key reported the development of an *in vivo* silver-lead galvanic oxygen sensor incorporated into the tip of a PTFE catheter of 0·8 mm diameter. A preformed PTFE membrane fits over the catheter tip. The catheter oxygen sensor is guided into the heart where the measurement of oxygen can be used to detect shunts across the chambers of the heart. The device could become a valuable diagnostic tool for the early detection of diseases of the heart.

There is a great deal of interest in galvanic cells as *in vivo* energy sources in which oxygen from the body fluids is converted directly to electrical power. In this way it is hoped to provide *in vivo* the energy to power cardiac pacemakers. At present, batteries implanted in the human body have a useful life of 18–24 months and thus their replacement necessitates expensive surgery about every two years. Dr A. C. C. Tseung (City University, London) reported the use of a platinum black–aluminium galvanic cell as an *in vivo* energy source. The cell electrolyte was an ionic ion-exchange membrane and the cell is encapsulated in silicone rubber. Such a cell planted subcutaneously near the abdominal wall of a rat gave a power output during intermittent use of 100 μW at 1·1 V for a total of 500 h. This was well above the objective of 30 μW at 1·1 V.

If the problem of the deposition of contaminants from the body on the cell membrane leading to a decrease in the membrane permeability for oxygen can be overcome, the galvanic cell promises seriously to rival present *in vivo* energy sources.

An Introduction to X-ray Crystallography

M. M. WOOLFSON

A text book for senior undergraduates and graduate students beginning a serious study of X-ray crystallography. An introductory chapter summarises the geometry of the crystalline state. Following chapters discuss scattering of X-rays, diffraction by crystals and factors affecting X-ray intensity. There is a chapter on the Fourier transform, describing mathematical techniques needed to make deductions about the arrangement of the scattering atoms from the scattered radiation, and a survey of the types of camera in common use. Finally, Professor Woolfson shows how these theories and techniques are applied to the determination of space groups and crystal structures.

380 pages; 211 figs. £4 5s *net*

The Computation of Chemical Equilibrium

F. van ZEGGEREN & S. H. STOREY

Methods for calculating the composition of a mixture of reacting compounds which has been allowed to come to equilibrium under new conditions, such as a given temperature and pressure, have been developed since the introduction of digital computers made realistic computations of this type feasible. This book provides a detailed critical survey of these methods, developed and published over the last 15-20 years. It is intended to assist those with a problem of this type to solve to select an appropriate method, and as a graduate level text in this area of theoretical thermodynamics.

175 pages £2 10s *net*

A History of Bubonic Plague in the British Isles

J. F. D. SHREWSBURY

The story of the mortality, distress and fear caused by the Bubonic Plague, from the Great Pestilence of 1348 to the Plague of London in 1665. Professor Shrewsbury, a bacteriologist, describes how the disease was transmitted to man from its natural host the Black Rat. He also discusses the effects of plague on population growth and social and economic life, the harsh and useless regulations made in vain efforts to control it, and the collapse of law and order during its great outbursts.

660 pages; 4 plates; 70 figs. £8 *net*

Clerks and Craftsmen in China and the West

JOSEPH NEEDHAM, FRS

Dr Needham discusses astronomy and meteorology, metallurgy, engineering, nautical technology, alchemy, chemistry, pharmacy and medicine in this collection of essays, which are by-products of the research for his great work, 'Science and Civilisation in China'. All these topics are related to his major theme and constant preoccupation; the enormous and hitherto undervalued contribution of Chinese culture to world science and technology.

470 pages; 40 plates; 59 figs. £7 10s *net*

CAMBRIDGE UNIVERSITY PRESS

Water, Water Everywhere...

. . . nor any drop to drink?".

It has not reached that stage, yet. But there is growing concern over the pollution of drinking water in the closely populated areas of the world, and, far more serious, the pollution of the oceans and the sea-food which forms the diet of many millions of people.

One of the most serious effects of ocean pollution is the ruin and destruction of plankton — the food on which most of the creatures of the sea depend.

As part of the search for greater knowledge of the life processes of plankton, a renowned marine biologist has successfully measured their metabolic activity. This investigation called for an instrument with incredible sensitivity — the LKB FLOW MICROCALORIMETER. In fact, the LKB Flow Microcalorimeter sensitivity was such that successful measurements were made not only of concentrated solutions of plankton, but even of such diffuse solutions as normal sea-water.

A special feature of both the LKB Flow and Batch Microcalorimeters is the ease with which they can be set up and operated. Even the newcomer to calorimetry will find them easy to use, after only a very brief training session.

LKB IN THE SERVICE OF SCIENCE

LKB INSTRUMENTS LTD. ● LKB HOUSE ● 232 ADDINGTON ROAD ● S. CROYDON, SURREY. CR2 8YD ● TEL: 01-657-0286

SALES AND SERVICE THROUGHOUT THE WORLD: STOCKHOLM, WASHINGTON, THE HAGUE, COPENHAGEN, ROME, VIENNA

10700-UK19

A-levels and University Performance

by

D. G. BAGG

Department of Chemical Engineering,
University of Manchester Institute
of Science and Technology

The principal factors which influence performance at the university and control 85 or 90 per cent of the variance of marks in the Part II examination are unrelated to A-level results.

MANY studies, mostly carried out in the United States, have sought to define the relationship between achievement at school and subsequent performance at university. In his survey of nearly three hundred such studies, Lavin points out[1] that "research is overwhelmingly of the static type", meaning that it investigates only "the prediction of performance at one point in time". Lavin strongly recommends "longitudinal" studies to cover the whole of the university course. This article describes such a study, giving my interpretation of results without many details of the statistical techniques used to obtain them. Briefly the principal technique is the analysis of the correlation matrices to obtain sets of multiple regression equations for each university examination and to show the different influences which affect the examination mark.

The influence of GCE A-levels on performance in university examinations can be said to be exerted in two distinct forms—the "direct" or "unique" influence peculiar to any particular A-level subject, and the "indirect" influence due to factors common to two or more A-level subjects. The indirect influence also includes factors common to the A-levels and such examinations as are included in the analysis which have occurred before that being considered. There are factors unrelated to the A-level subjects which are represented by the constant figures in the regression equations, and these factors can be seen to enlarge with progress through the course as A-levels are left further behind. This type of interpretation is given to the multiple regression equations presented in this article.

The Present Work

I have been concerned mostly with the performance of students in the Department of Chemical Engineering at the University of Manchester Institute of Science and Technology, where nearly a thousand student records are available. Similar techniques have also been applied to samples of records from the Departments of Civil and Mechanical Engineering in the Faculty of Science, and to samples of honours BA graduates from the schools of geography and history.

In all my work I have scaled the alphabetical grades of A-level pass numerically by conversion to "normalized T scores" which are based on frequency distribution and have a mean value of approximately fifty and a standard deviation of about ten. Knowledge of the actual scales is essential for the use of the regression equations, and those for the chemical engineering work are:

Grade of pass	*A*	*B*	*C*	*D*	*E*
Chemistry	62	52	46	40	32
Physics	66	56	48	44	35
Mathematics	66	56	49	42	34

Similar scales were prepared for other A-level subjects as the samples became available. The meaning of the regression equations given later can be illustrated easily by assuming a mean value of 50·0 for each A-level subject, multiplying this by the loading factor, and summing with the constant. It can then be seen that the proportion of the total mark which is contributed by the A-levels is large in the first examination but becomes less with progress through the university course, and is lower for the arts courses than for science and technology.

Chemical Engineering

I have analysed the records of 621 students who have graduated with honours in chemical engineering and of 116 who have graduated with ordinary degrees after taking the same course and examinations, the only difference being that at some point in the examinations they had not reached the standard demanded for honours graduation. The samples covered most of the students from the United Kingdom who graduated in the thirteen summers of 1957–69 inclusive. Overseas students presented quite a different picture in correlation[2], but the maximum size of sample, which is only $N = 65$, is really too small for the results of analysis to be fully valid. For those who wish to check the regression equations, the data for these two samples are given in Tables 1 to 4.

Table 1. RAW DATA FOR 621 HONOURS BSC GRADUATES IN CHEMICAL ENGINEERING

	A-level T scores			University percentage mark			
	Chemistry	Physics	Mathematics	Mid-session	Sessional	Part I finals	Part II finals
Mean	50·570	51·254	49·710	47·649	56·077	56·655	59·163
S.D.	8·518	8·844	9·362	11·866	9·482	9·386	8·085

Table 2. CORRELATION COEFFICIENTS FOR 621 HONOURS BSC GRADUATES IN CHEMICAL ENGINEERING

	Chemistry	Physics	Mathematics	Mid-session	Sessional	Part I
Physics	0·372	1				
Mathematics	0·199	0·282	1			
Mid-session	0·318	0·324	0·418	1		
Sessional	0·353	0·369	0·359	0·639	1	
Part I finals	0·261	0·309	0·302	0·507	0·627	1
Part II finals	0·206	0·293	0·238	0·465	0·532	0·669

I shall now examine performance session by session. For the first year mid-sessional examination the regression equation for the honours graduates is: mid-sessional mark $= 2{\cdot}185 + 0{\cdot}267 \times C + 0{\cdot}211 \times P + 0{\cdot}425 \times M$, where C, P and M denote the T scores of the appropriate A-level subjects, chemistry, physics and mathematics.

Evidently, mathematics is the A-level subject most closely related to this examination, one of the three papers actually being in mathematics. A second paper is in chemical engineering and the third is organic and physical chemistry, which apparently is moderately well correlated with the A-level paper. The lowish value for physics at this time is probably due to the small amount of chemical engineering which has so far been covered, this subject being sometimes termed "applied physics". The small size of the constant, 2·185, shows that altogether the three A-level grades of pass have a major controlling influence on performance at this stage.

For ordinary graduates a somewhat different equation is obtained

$$\text{mid-sessional mark} = -2{\cdot}424 + 0{\cdot}490 \times C + 0{\cdot}025 \times P + 0{\cdot}361 \times M$$

The first thing to note is that the constant, although of much the same absolute value as for the honours graduates, is negative, showing that there is a tendency for the

Table 3. RAW DATA FOR 116 ORDINARY BSC GRADUATES IN CHEMICAL ENGINEERING

	A-level T scores			University percentage mark			
	Chemistry	Physics	Mathematics	Mid-session	Sessional	Part I finals	Part II finals
Mean	47·276	47·750	47·138	38·885	42·571	42·378	51·397
S.D.	8·735	9·150	10·019	10·284	7·447	8·656	10·267

Table 4. CORRELATION COEFFICIENTS FOR 116 ORDINARY BSC GRADUATES IN CHEMICAL ENGINEERING

	Chemistry	Physics	Mathematics	Mid-session	Sessional	Part I
Physics	0·486	1				
Mathematics	0·111	0·079	1			
Mid-session	0·465	0·252	0·399	1		
Sessional	0·301	0·174	0·255	0·475	1	
Part I finals	−0·136	−0·155	0·039	0·101	−0·149	1
Part II finals	0·033	−0·089	0·031	0·239	0·163	0·551

A-level grades to overestimate performance at this examination. The discrepancy between the loading factors for chemistry and physics suggests to me the predominance of aptness in chemistry but inaptitude in physics. Perhaps these students take longer to "get into their stride" with the course, and their numerical facility, presumably, was not quite so good as that of the honours students.

The regression equation for the first year sessional examination and for subsequent examination marks can be expressed in two forms. The first includes the influence of the mid-sessional examination mark, denoted by MS, and the second contains this influence algebraically converted into that of the A-levels, by means of the equation given earlier. The first form then shows the influence, unique plus indirect, of the A-levels on this examination, and the second form includes their influence on the previous examination, showing the overall total influence of the A-levels on the first year sessionals.

For the honours graduates these two equations are

$$\text{sessional mark} = 18{\cdot}133 + 0{\cdot}135 \times C + 0{\cdot}141 \times P + 0{\cdot}079 \times M + 0{\cdot}419 \times \text{MS} \tag{1}$$

$$\text{sessional mark} = 19{\cdot}049 + 0{\cdot}247 \times C + 0{\cdot}229 \times P + 0{\cdot}258 \times M \tag{2}$$

The first point to note is that the constant is much larger than for the mid-sessional examinations, showing that factors independent of previous examination results are beginning noticeably to affect performance, and by far the largest loading factor is for the immediately preceding examination; this point is noticeable throughout the equations in this article.

In equation (1) the influence of mathematics is very small, evidently exerted indirectly through the mid-sessional examination, as shown by its increase from 0·079 to 0·258 in equation (2). The overall influence of each A-level subject is nearly equal.

For the ordinary graduates the corresponding equations are

$$\text{sessional mark} = 23{\cdot}717 + 0{\cdot}085 \times C + 0{\cdot}017 \times P + 0{\cdot}065 \times M + 0{\cdot}281 \times \text{MS} \tag{1}$$

$$\text{sessional mark} = 23{\cdot}036 + 0{\cdot}223 \times C + 0{\cdot}024 \times P + 0{\cdot}167 \times M \tag{2}$$

The constant is appreciably larger than for the honours graduates, indicating that the influence of external factors or factors other than A-levels is a greater proportion of the total. Equation (1) shows that the direct A-level influence, particularly of physics, is lower, and in equation (2) the difference between the factors for chemistry and physics is again very noticeable, as for the mid-sessional mark. Apparently, students who will eventually become only ordinary graduates are continuing to show an inaptitude for physics. The reduced factor for the mathematics predictor certainly suggests less numerical facility than for the honours graduates, and my comments about the mid-sessional discrepancies also apply here.

For the honours graduates the equations for the part I finals mark are

$$\text{part I mark} = 17{\cdot}741 + 0{\cdot}009 \times C - 0{\cdot}069 \times P + 0{\cdot}043 \times M + 0{\cdot}123 \times \text{MS} + 0{\cdot}480 \times \text{S} \tag{1}$$

$$\text{part I mark} = 27{\cdot}159 + 0{\cdot}160 \times C + 0{\cdot}205 \times P + 0{\cdot}219 \times M \tag{2}$$

Equation (1) indicates that the direct influence of each of the three A-level subjects is very considerably reduced at this stage, the loading factors being so small that "errors of chance" might conceivably predominate. Most of their influence is now indirect, in factors common to more than one of the variables. This is shown by the algebraic substitution in equation (2), where the influence of the mid-sessionals and the sessionals (S) is analysed into an increase in the constant as well as in the total influence of each A-level subject. The value of the constant, now about 27, shows an increase in the influence of factors unrelated to the A-levels, the constant for the sessional examinations being only about 19.

For the ordinary graduates, these equations are

$$\text{part I mark} = 56{\cdot}119 - 0{\cdot}153 \times C - 0{\cdot}112 \times P - 0{\cdot}000 \times M + 0{\cdot}262 \times \text{MS} - 0{\cdot}267 \times \text{S} \tag{1}$$

$$\text{part I mark} = 49{\cdot}326 - 0{\cdot}084 \times C - 0{\cdot}112 \times P + 0{\cdot}050 \times M \tag{2}$$

In both these equations, the constant is larger than the mean part I finals mark, which is 42·278, showing that the overall performance of the ordinary graduates in this examination is quite inconsistent with previous performance and that the factors controlling this performance must be largely unrelated to the original A-level results. In equation (1) the loading factor for mathematics is actually −0·0002.

For the honours graduates, these equations for the part II finals examination are

$$\text{part II mark} = 23{\cdot}309 - 0{\cdot}031 \times C + 0{\cdot}060 \times P - 0{\cdot}024 \times M + 0{\cdot}082 \times \text{MS} + 0{\cdot}104 \times \text{S} + 0{\cdot}455 \times \text{pt. I} \tag{1}$$

$$\text{part II mark} = 37{\cdot}826 + 0{\cdot}090 \times C + 0{\cdot}195 \times P + 0{\cdot}138 \times M \tag{2}$$

Evidently, by this time, the direct influence of the A-level predictors is small enough probably to be mostly "errors of chance", being shown in equation (1) as positive and negative factors of minor value. Equation (2) shows that the overall influence of the three A-level predictors on the performance of the honours graduates is not very large, the constant comprising the external factors being about 64 per cent of the mean mark of 59·168. A-level physics has the largest influence of the A-levels on part II honours finals, and chemistry has the least.

For the ordinary graduates we now have

$$\text{part II mark} = 12{\cdot}158 + 0{\cdot}046 \times C - 0{\cdot}090 \times P - 0{\cdot}094 \times M + 0{\cdot}188 \times \text{MS} + 0{\cdot}299 \times \text{S} + 0{\cdot}673 \times \text{pt. I} \tag{1}$$

$$\text{part II mark} = 51{\cdot}962 + 0{\cdot}114 \times C - 0{\cdot}155 \times P + 0{\cdot}032 \times M \tag{2}$$

Equation (2) shows that, with a constant of almost exactly the mean part II mark of 51·397, the overall influence of the three A-level subjects on the performance of the ordinary graduates in the final examinations is practically zero, and the positive and negative loading factors must be accounted for as errors of chance resulting from the erratic and inconsistent performance of these students, which is evident from their departmental records. The factors controlling their performance in finals must be almost, if not quite, unrelated to A-levels and strongly related to the "external" factors which controlled their performance in part I, for the loading factor for that examination mark in equation (1) is large – 0·673.

Civil Engineering

I have used the records of all 174 graduates in this subject for the four summers of 1965–68. I found it practical to take only two A-level subjects, physics and mathematics, for the third varied, being chemistry, applied mathematics, or further mathematics. It was not practical to separate the honours from the ordinary graduates, there being relatively few of the latter.

The equation relating the physics and mathematics A-level grades of pass to the first year mid-sessional examination performance of civil engineering students is:

$$\text{mid-sessional mark} = 14{\cdot}330 + 0{\cdot}386 \times P + 0{\cdot}262 \times M.$$

The mean mark of 48·218 is moderately controlled by the A-level results, physics having a somewhat larger influence than mathematics. The constant of 14·330 indicates a larger proportion of factors external to these two A-levels in this examination than for the chemical engineering honours graduates, for whom this constant was only 2·185.

The equations for the first year sessional examination taken by civil engineering students are

$$\text{sessional mark} = 13{\cdot}360 + 0{\cdot}107 \times P + 0{\cdot}183 \times M + 0{\cdot}558 \times \text{MS} \quad (1)$$

$$\text{sessional mark} = 21{\cdot}349 + 0{\cdot}322 \times P + 0{\cdot}330 \times M \quad (2)$$

In equation (1) the direct influence of each A-level subject is less than that on the mid-sessional mark, although equation (2) shows that the overall influence of mathematics has increased, with a slight reduction in that of physics. The increase in the constant of equation (2) over that in the equation for the mid-sessional examination is almost exactly equal to the increase in the mean mark to 55·408, this increase therefore being an influx of factors external to the A-levels, the overall influence of which has scarcely changed with time.

For the second year examination, the equations for civil engineering students are

$$\text{second year mark} = 15{\cdot}146 - 0{\cdot}009 \times P + 0{\cdot}015 \times M + 0{\cdot}151 \times \text{MS} + 0{\cdot}620 \times \text{S} \quad (1)$$

$$\text{second year mark} = 30{\cdot}543 + 0{\cdot}249 \times P + 0{\cdot}259 \times M \quad (2)$$

From equation (1) it can be deduced that the direct influence of the A-levels is approaching zero. Equation (2) shows that the overall influence of the A-levels is somewhat reduced at this time and that the "external" factors become more predominant, amounting to a little more than half of the mean mark, which is 57·058.

For the third year final examination in civil engineering, the equations are

$$\text{final mark} = 20{\cdot}618 + 0{\cdot}100 \times P - 0{\cdot}003 \times M + 0{\cdot}053 \times \text{MS} + 0{\cdot}052 \times \text{S} + 0{\cdot}431 \times \text{2nd yr} \quad (1)$$

$$\text{final mark} = 35{\cdot}642 + 0{\cdot}244 \times P + 0{\cdot}139 \times M \quad (2)$$

The direct influence of A-level mathematics on the civil engineering finals mark is practically zero, and that of physics is only small, contributing, say, five marks to the total. The overall influence of physics, however, is just about equal to that in the second year examination, with that of mathematics reduced by nearly 50 per cent. With a mean mark of 58·552, the A-levels contribute in total almost 40 per cent to civil engineering finals, the influence of physics being nearly twice that of mathematics.

Mechanical Engineering

I used the records of 113 students of mechanical engineering who graduated in the four summers of 1965–68. Again, it was practical to use only A-level physics and mathematics in the correlation. For the first year mid-sessional examination the regression equation is

$$\text{mid-sessional mark} = 24{\cdot}835 + 0{\cdot}236 \times P + 0{\cdot}221 \times M$$

The constant is larger than for the other samples I have discussed, being just about half of the mean mark of 48·664, which indicates fairly strong influence of factors other than the A-levels quite early in the course.

For the second examination in mechanical engineering (the first year sessional examination) the equations are

$$\text{sessional mark} = 10{\cdot}712 + 0{\cdot}136 \times P + 0{\cdot}129 \times M + 0{\cdot}661 \times \text{MS} \quad (1)$$

$$\text{sessional mark} = 27{\cdot}138 + 0{\cdot}293 \times P + 0{\cdot}274 \times M \quad (2)$$

The direct influence of the A-levels is reduced a little, although their indirect influence through the mid-sessional examination makes their total influence a little greater than before. The increase in the constant of equation (2) over that of the mid-sessional equation is less than half of the increase in the mean mark to 56·726, so that the influence of factors unrelated to A-levels is, if anything, less than in the mid-sessional examinations.

For the second year examination in mechanical engineering the equations are

$$\text{second year mark} = 18{\cdot}056 - 0{\cdot}009 \times P + 0{\cdot}023 \times M + 0{\cdot}239 \times \text{MS} + 0{\cdot}442 \times \text{S} \quad (1)$$

$$\text{second year mark} = 35{\cdot}999 + 0{\cdot}177 \times P + 0{\cdot}197 \times M \quad (2)$$

The direct influence of physics is practically zero, and that of mathematics is very small. The overall influence of the A-levels is substantially less than for the first year, and the increased constant indicates a growth in the factors unrelated to the A-levels.

For the third year final examination in mechanical engineering the equations are

$$\text{finals mark} = 30{\cdot}856 + 0{\cdot}027 \times P - 0{\cdot}055 \times M + 0{\cdot}246 \times \text{MS} + 0{\cdot}071 \times \text{S} + 0{\cdot}204 \times \text{2nd yr} \quad (1)$$

$$\text{finals mark} = 46{\cdot}229 + 0{\cdot}142 \times P + 0{\cdot}058 \times M \quad (2)$$

The direct influence of the A-levels, with one positive and the other negative together with the small size of the sample (113), is only of the order of errors of chance, and their indirect influence is small, amounting to about 17 per cent of the mean mark of 56·655. The total influence of A-level physics on the final examination in mechanical engineering is apparently two and a half times that of A-level mathematics.

Geography and History

I used the records of 116 honours BA graduates in geography. A-level predictors were restricted to geography and history because of variations in the third subject, but I included GCE O-level grades of pass in both geography and history, and these are included in the equations, using similar scales of normalized T scores. To distinguish the O-level from the A-level items, I have used the initials *OG*, *OH*, *AG* and *AH* in the equations.

The equation for the prediction of the first year sessional examination mark in geography is

$$\text{sessional mark} = 50{\cdot}208 - 0{\cdot}009 \times OG - 0{\cdot}014 \times OH + 0{\cdot}029 \times AG + 0{\cdot}063 \times AH$$

The overall influence of O-level and A-level subjects taken together is almost zero, these four predictors adding only 3·4 marks to the constant for the mean mark of 53·616. If O-level predictors are omitted, this equation becomes

$$\text{sessional mark} = 49{\cdot}619 + 0{\cdot}025 \times AG + 0{\cdot}055 \times AH$$

Although the influence of the A-levels is very small, that of history is twice that of geography.

For part I finals in geography (second year), the equations are

$$\text{part I} = 36{\cdot}926 - 0{\cdot}114 \times OG + 0{\cdot}252 \times OH + 0{\cdot}064 \times AG - 0{\cdot}131 \times AH + 0{\cdot}233 \times S \quad (1)$$

$$\text{part I} = 48{\cdot}620 - 0{\cdot}116 \times OG + 0{\cdot}249 \times OH + 0{\cdot}071 \times AG - 0{\cdot}116 \times AH \quad (2)$$

Although the overall effect of the GCE predictors is only about four marks at the mean point, the apparently strong influence of O-level history in this examination is surprising and difficult to explain without a detailed study of the two sets of examination questions, unless it is an "error of chance". Omitting the O-level predictors from equation (2) gives

$$\text{part I mark} = 49{\cdot}548 + 0{\cdot}103 \times AG - 0{\cdot}040 \times AH \quad (3)$$

In this case the total influence of the A-levels is only about three marks.

The equations for the part II final examinations in geography are

$$\text{part II mark} = 24{\cdot}970 + 0{\cdot}055 \times OG - 0{\cdot}141 \times OH + 0{\cdot}014 \times AG + 0{\cdot}082 \times AH + 0{\cdot}173 \times S + 0{\cdot}457 \times \text{pt. I} \quad (1)$$

$$\text{part II mark} = 55{\cdot}865 - 0{\cdot}000 \times OG - 0{\cdot}029 \times OH + 0{\cdot}052 \times AG + 0{\cdot}039 \times AH \quad (2)$$

Since the mean part II mark is 58·959, the overall influence of the O-level and A-levels is the addition of only about three marks to the constant at the mean point, and the finals mark is controlled chiefly by factors unrelated to the GCE examinations. Omitting the O-level predictors from the part II equation gives

$$\text{part II mark} = 55{\cdot}282 + 0{\cdot}046 \times AG + 0{\cdot}027 \times AH \quad (3)$$

The total influence of the two A-levels is then nearly four marks, of which history contributes a little more than one-third.

A small sample, from two sessions, of eighty-one honours BA graduates in history has been analysed, with data somewhat more limited than for the previous samples. The records of two A-level subjects, history and English, were taken, but a third was again too variable for use. Analysis of the first year examination results was found to be impractical, and the equation for the part I final examination is

$$\text{part I mark} = 48{\cdot}457 + 0{\cdot}065 \times H + 0{\cdot}037 \times E$$

The direct influence of the two A-level subjects is enough to add only about five marks to the constant which includes any indirect A-level influence through the first year examinations.

With the part II final examination, the influence of A-level history amounts to about 12 per cent of the mean mark, the equations being

$$\text{part II mark} = 15{\cdot}040 + 0{\cdot}093 \times H - 0{\cdot}027 \times E + 0{\cdot}724 \times \text{pt. I} \quad (1)$$

$$\text{part II mark} = 50{\cdot}108 + 0{\cdot}140 \times H - 0{\cdot}0005 \times E \quad (2)$$

Performance in part I finals clearly has a large influence on the part II finals result in history, perhaps larger than in some other courses. This figure may be affected by the absence of figures for the first year examinations, which I was not able to obtain.

Influence of A-level Diminishes

My first conclusion from this work is that, although the influence of the A-levels on the first year mid-sessional examinations is quite appreciable, this influence diminishes with progress through the 3 years of the university course until it is of no real importance in the part II final examinations. The first examination at the university is usually held about 7 months after the A-level examinations, and the student is presumably still using some of the knowledge which he gained at school, the university syllabus not at that point being very far ahead of the A-level limits. By the end of the course, there is probably little real connexion between the subject matter of the finals examination and that of the A-levels.

My principal conclusion is that A-level grades of pass form an unreliable and possibly hazardous assessment, or prediction of the future academic performance, of a candidate. Such an examination mark is scarcely a measure of the subject by which it is labelled, but is more a measure of the various specific abilities used by the candidate for the application of his knowledge to the questions. In addition, this measure will include an element measuring the efficiency of the school teaching, in its coverage of syllabus and communication with the student. I believe that the admissions tutor may fall into a trap, particularly in the case of a candidate from a school which indulges in "cramming", for the A-level grade may be a close approximation to a measure of the "ceiling of attainment" or "maximum cognitive capacity" of that student in that subject. Such is the only explanation I have for the cases of students who have entered the university with, say, three grade *A* passes, but whose performance has deteriorated to ultimate failure in part II finals. It is sometimes said that the reason for such performance is simply that the student "did not work", but to me this is not a reason but a statement of what happened, and a reason for the lack of ability to work is required. Maybe such a student has reached his limits in that type of subject after A-level and should transfer his studies to a school better suited to his abilities. The existence of such a "ceiling of attainment" is quite widely recognized, if only subconsciously, as is illustrated by the remark "Oh ! I'm not mathematical" which I have often heard.

The "specific abilities" to which I have referred are elements such as verbal fluency, numerical facility, spatial ability, logical reasoning, vocabulary, and so on, but the measurement of such abilities by A-level grades of pass has not been established in detail, nor has their identification in the various university examinations. We do not know whether for the assessment of a prospective student the more important factor is the quantity of his knowledge of a subject before admission, or whether it would be better to assess his ability towards the future application of such knowledge of that subject as will be acquired after admission, his previous knowledge of that subject then being only of minor importance. Such a distinction might perhaps be described as a balance between memory ability on the one hand and cognitive capacity on the other, bearing in mind that "memory" is not always synonymous with the "comprehension" of the matter memorized.

---

[1] Lavin, D. E., *The Prediction of Academic Performance* (Russell Sage Foundation, 1965).

[2] Bagg, D. G., *Brit. J. Educ. Psychol.*, **38**, part 2, 197 (1968).

Reinitiation of RNA Chain Synthesis *in vitro*

by
JOHN P. RICHARDSON[*]
Institut de Biologie Moléculaire,
Université de Genève,
Geneva

During transcription of bacteriophage T4 or T5 DNA in 0·2 M KCl, RNA polymerase is able to terminate the synthesis of RNA chains, dissociate from the DNA template and reinitiate new RNA chains on new DNA templates.

THE complete process of RNA transcription in the cell involves initiation, polymerization, termination and release of specific RNA molecules. Some of these steps are also characteristic of the reaction catalysed by RNA polymerase *in vitro*; when T4 or λ phage DNA is the template, the purified enzyme from *Escherichia coli* is capable of specific initiation[1-4] and rapid polymerization of RNA chains[5]. There is, however, some question whether this reaction also includes termination and release of the RNA.

During synthesis at low salt concentration ($I < 0·1$), it is evident that there is some defect either in the termination or in the release of the RNA: synthesis usually ceases completely after only 60 min[6] and each enzyme initiates only one RNA chain that is rarely released from its synthetic complex with enzyme and DNA[7-9]. At higher salt concentrations, however, RNA synthesis can continue for several hours[10,11], suggesting that salt may overcome the defect. I demonstrate here that during synthesis of T4 RNA in 0·2 M KCl, each RNA polymerase molecule can initiate more than one RNA chain; further, the enzyme molecule can be released from its DNA template and reinitiate a new RNA chain on a new DNA template. Thus, in these conditions of synthesis, the RNA polymerase catalysed reaction is functionally complete, and continued synthesis is the outcome of repetitions of the transcription cycle.

It has been shown recently by Roberts[12] that *E. coli* possesses a protein factor called ρ that causes specific termination and release of λ RNA molecules in an *in vitro* reaction. From the existing evidence, however, it seems that the termination and release that occur in 0·2 M KCl are different from those mediated by ρ. It is thus of interest to learn more about the termination that occurs in high salt concentration in the absence of ρ, for it may also have some significance in the control of RNA transcription.

Kinetics and Stoichiometry of Initiation

RNA chain initiation was assayed by measuring the incorporation of γ-^{32}P-ribonucleoside triphosphates into RNA[8]. In 0·05 M KCl (Fig. 1*a*), most T4 RNA chains are initiated in the first few minutes of synthesis, and after 20 min there is no further initiation even though there is still appreciable RNA synthesis at that time. In 0·2 M KCl (Fig. 1*b*), however, not only does total RNA synthesis continue for a much longer time, but so does the initiation of new chains. The yield of synthesis after 160 min, which is three-fold greater at the higher salt concentration, is nearly matched by the 2·6-fold increase in the number of chains initiated. Similar findings have been reported recently by Millette[13] and by Maitra and Barash[14].

From the data in Fig. 1, it is possible to estimate the number-average length of the RNA chains initiated, and thus determine whether the salt concentration also influences the size of the RNA synthesized. After 60 min, when there is no further increase in the average length of the RNA in either case, the RNA made at 0·2 M KCl is, on

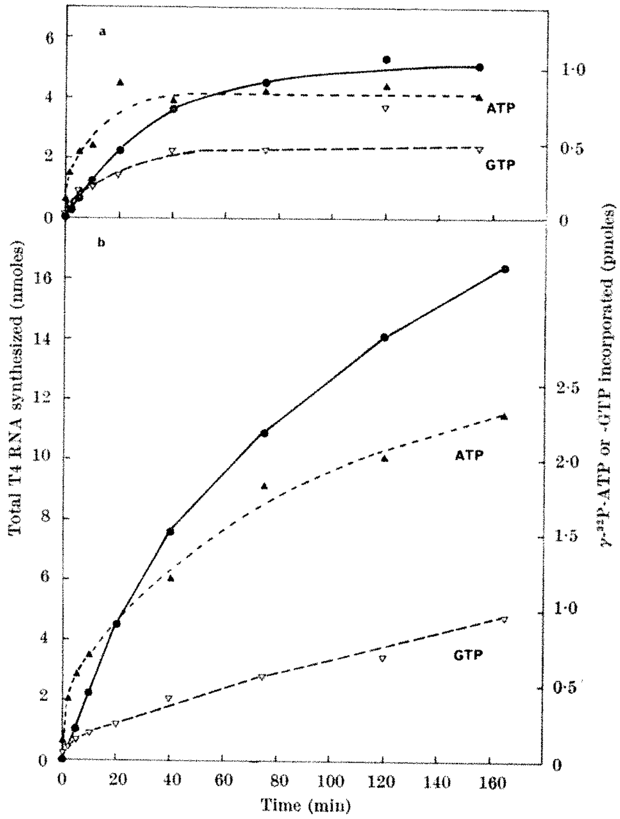


Fig. 1. Effect of salt concentration on RNA chain initiation. Reaction mixtures of 0·1 ml. contained 0·04 M Tris-HCl (*p*H 7·9) buffer; 0·012 M $MgCl_2$; 0·2 mM ATP, GTP, UTP and CTP; 1·1 mM 2-mercaptoethanol; 0·1 mM EDTA; 0·2 mg/ml. bovine serum albumin; 4·7 μg T4 DNA (prepared by the method of Thomas and Abelson[28]); 1·0 μg hydroxylapatite fraction *E. coli* MRE 600 RNA polymerase (prepared by the method of Richardson[15]); and either (*a*) 0·05 or (*b*) 0·2 M KCl. Separate mixtures contained either γ-^{32}P-ATP (0·89 × 10^3 c.p.m./pmole); or γ-^{32}P-GTP (0·83 × 10^3 c.p.m./pmole) all contained ^{14}C-UTP (1·9 × 10^3 c.p.m./nmole). The γ-^{32}P-nucleoside triphosphates were prepared by the method of Glynn and Chappell[29] and ^{14}C-UTP was purchased from Schwarz BioResearch. Reaction mixtures were incubated at 37° C and RNA synthesis was terminated at the specified times by adding rapidly at 0° C: 0·35 ml. H_2O; 0·05 ml. 25 mM ATP or GTP (depending on which is labelled with ^{32}P in the reaction mixture); 0·1 ml. 1 per cent bovine serum albumin; and 0·5 ml. 5 per cent trichloroacetic acid. After 5 min at 0° C, the precipitate was collected by centrifugation (10 min at 1,500*g*) and redissolved in 0·5 ml. 0·1 M $Na_4P_2O_7$ plus 0·05 ml. 25 mM ATP or GTP. After reprecipitation of the RNA, this washing procedure was repeated once more and the precipitate formed, after adding trichloroacetic acid for the third time, was collected on a Whatman 'GF/C' filter (2·5 cm diameter) and washed on the filter with 10 ml. 2 per cent trichloroacetic acid containing 1 per cent $Na_4P_2O_7$–10 H_2O and 0·5 per cent NaH_2PO_4 and with 1 ml. ethanol. After drying, the filter was placed in a plastic vial containing 5 ml. PPO-POPOP-toluene scintillation fluid and radioactivity was counted in a Packard 'Tri-Carb' liquid scintillation counter. Incorporation of all labelled substrates was completely dependent on both enzyme and DNA. The levels of unincorporated ^{32}P were equivalent to 0·17 and 0·16 pmoles, respectively, of ATP and GTP, and the data given in the figure have been corrected for these background values. The total incorporation of γ-^{32}P-UTP and γ-^{32}P-CTP into RNA, measured separately in other experiments, was less than 5 per cent of the total initiation in all cases. Total nucleotide incorporation was calculated from the value of ^{14}C-UTP incorporated assuming that the mole fraction of UMP in the T4 RNA synthesized was 0·29 (ref. 30). ●, Total nucleotide incorporation; ▲, incorporation of γ-^{32}P-ATP; ▽, incorporation of γ-^{32}P-GTP.

---

[*] Present address: Department of Genetics, University of Washington, Seattle, Washington 98105.

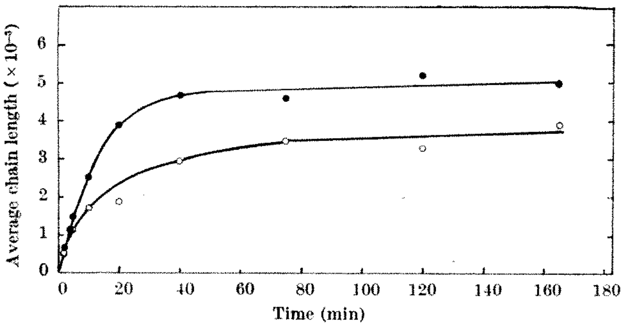


Fig. 2. Average chain lengths of T4 RNA as a function of the time of synthesis. These values are calculated from the data in Fig. 1; each point is the ratio of the total nucleotide incorporation divided by the sum of the γ-^{32}P-ATP and γ-^{32}P-GTP incorporated. ○, In 0·05 M KCl; ●, in 0·2 M KCl.

the average, 1·3 times larger than that made at 0·05 M KCl (Fig. 2). Thus salt concentration does affect the average size of the RNA synthesized, but not to the same extent as it affects the number of chains initiated. Furthermore, the fact that the average length of RNA synthesized in 0·2 M KCl is constant after 60 min indicates that further RNA synthesis after that time is a direct consequence of continued initiation.

To show that continued initiation in 0·2 M KCl reflects reinitiation rather than a slow activation of enzyme molecules that do not function in 0·05 M KCl, it is necessary to consider the molar yields of the reaction. In the experiment shown in Fig. 1*b*, a total of 3·3 pmoles of γ-^{32}P-ATP and γ-^{32}P-GTP had been incorporated after 165 min in 0·1 ml. of a reaction containing 1 µg of enzyme protein (determined using 0·65 as the specific absorbance of purified RNA polymerase at 280 nm, ref. 15). After 5 h of synthesis, this value increased further to 4·4 pmoles. Because the particle weight of the active unit of RNA polymerase is believed to be in the range 400,000 to 500,000 daltons[15–18], this yield indicates that at least 1·7 chains have been initiated per enzyme molecule after 5 h synthesis in 0·2 M KCl.

When T5 DNA was used as template instead of T4 DNA, the yield was even greater. After 4 h synthesis at 30° C in 0·2 M KCl, 0·6 µg of RNA polymerase catalysed the incorporation of 3·2 and 0·3 pmoles of γ-^{32}P-ATP and γ-^{32}P-GTP, respectively, which is equivalent to at least 2·3 T5 RNA chains per enzyme molecule. Because the RNA polymerase preparations used may have contained some inactive protein, the yields are only minimal estimates. The number of chains initiated per active enzyme molecule is probably even higher.

Evidence for Reinitiation on a New DNA Template

During synthesis at low salt concentrations, nearly all enzyme molecules become irreversibly attached to the DNA template within the first few minutes of incubation[19]. It is possible, however, that in conditions in which reinitiation occurs, enzyme molecules dissociate from the template before initiating new RNA chains. If the enzyme molecules dissociate, they should then be free to transcribe a second DNA species added at a later time. The following experiment suggests that many of the RNA polymerase molecules are released from the DNA template molecules during transcription in 0·2 M KCl.

When RNA polymerase is added to a mixture of T4 and T5 DNA, it transcribes T5 DNA with a slight preference that is nearly the same in both 0·05 and 0·2 M KCl (Table 1). But if the enzyme is preincubated with one DNA template for 10 min in a complete reaction mixture before adding the other, the salt concentration strongly influences the fractions of RNA specific to the two DNAs. In 0·05 M KCl, very little RNA is transcribed from the second DNA, whereas in 0·2 M KCl the fractions are much closer to the values found in the experiment without preincubation. The total amount of RNA synthesized is also greater in 0·2 M KCl, so it is concluded that many more enzyme molecules are free at the higher salt concentration to initiate RNA chains on a DNA template added at a later time.

Table 1. TRANSCRIPTION OF T4 AND T5 DNA MIXTURES

Exp.	DNA present during preincubation	DNA added after 10 min	KCl concentration (M)	^{3}H-UMP incorporated per 5 µl. (c.p.m.)	Fraction of RNA made between 10 and 20 min (per cent) T4	T5
1	T4 + T5	—	0·05	542	45	55
	T4 + T5	—	0·20	1,100	40	60
2	T5	T4	0·05	354	5	95
	T5	T4	0·20	775	19	81
3	T4	T5	0·05	486	88	12
	T4	T5	0·20	968	70	30

Each reaction mixture of 0·1 ml. contained initially 0·04 M Tris-HCl buffer (*p*H 7·9); 12 mM $MgCl_2$; 2·8 mM 2-mercaptoethanol; 0·05 mM EDTA; 0·66 mM each of ATP, GTP and CTP; 0·2 mM UTP; 2·5 µg of DNA (either T4 or T5); and 1·3 µg of RNA polymerase. After incubation for 10 min at 37° C, 2·5 µg of a second DNA (in 10 µl.) and 2 µCi of ^{3}H-UTP (1·2 Ci/mmole in 5 µl.) were added and the incubation was continued for a further 10 min at 37° C. RNA synthesis was stopped by adding 0·4 ml. of 0·5 per cent sodium dodecyl sulphate containing 0·12 M sodium acetate buffer (*p*H 5·2). After incubation for 3 min at 37° C, a 5 µl. sample was removed to assay the total incorporation of ^{3}H-UTP into acid insoluble material, and the proteins in the remaining solution were extracted by the hot-phenol method of Scherrer and Darnell[31]. Unincorporated nucleoside triphosphates were removed after phenol extraction by dialysis for 15 h against 0·3 M NaCl containing 0·03 M trisodium citrate. The fractions of RNA specific to T4 and T5 DNAs were estimated by the RNA-DNA hybrid assay procedure described by Gillespie and Spiegelman[32]. Membrane filters (Sartorius 'MF 50') with 2 µg of alkali-denatured DNA or with no DNA were annealed with RNA (approximately 3,500 c.p.m.) in 1·1 ml. of 0·3 M NaCl containing 0·03 M trisodium citrate for 20 h at 65° C. Each RNA preparation was annealed in duplicate to T4 DNA, T5 DNA and a blank filter. The amount of RNA remaining on each DNA filter was corrected first by subtracting the amount on the blank (< 0·4 per cent of c.p.m. added), then by dividing by the efficiency of hybridization to that filter (determined with preparations of pure T4 and T5 RNA: 0·27 for T4 DNA and 0·35 for T5 DNA). The fractions (as per cent) are the ratios of the corrected value for hybridization to each filter over the total of the corrected values. In all cases the total of the corrected values was within 10 per cent of the total amount of RNA added.

Although this result is expected if enzyme molecules dissociate from the DNA before initiating new RNA chains, a similar effect might have been seen if RNA chain initiation were much slower in 0·2 M KCl. The initial rate of RNA synthesis and the rate of RNA chain growth are both about 1·3 times higher in 0·2 M KCl than in 0·05 M KCl[5], however, so that there is probably very little difference in the rates of initiation. It is therefore much more likely that the increase in transcription in 0·2 M KCl

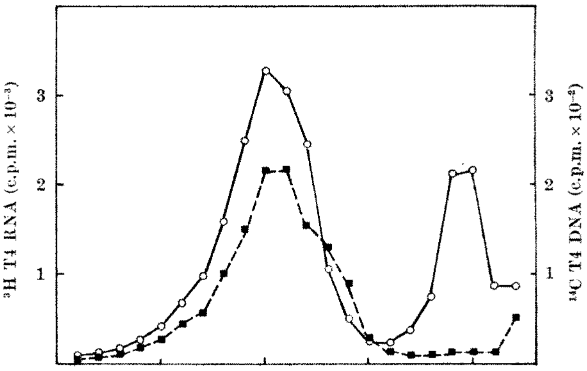


Fig. 3. Stability of the RNA polymerase–nascent RNA–T4 DNA complex in 0·3 M KCl. The reaction mixture was the same as that containing 0·2 M KCl described in Fig. 1, but with the following modifications: ^{3}H-UTP was the labelled nucleoside triphosphate (12 Ci/mole) and initially the mixture contained 6·2 µg T4 ^{14}C-DNA (416 c.p.m./µg) and 3·8 µg RNA polymerase in a total volume of 0·1 ml. After incubating for 3 min at 37° C, the reaction mixture was diluted with 1·4 ml. of an ice-cold solution of 0·3 M KCl containing 0·01 M Tris-HCl (*p*H 7·9) buffer, 0·01 M $MgCl_2$, 0·5 mM 2-mercaptoethanol, 0·05 mM EDTA and 0·2 mg/ml. bovine serum albumin ("gradient buffer") and was layered on a 35 ml. 10 to 30 per cent linear glycerol gradient in the same buffer. After centrifugation for 4 h at 27,000 r.p.m. in a Spinco '*SW* 27' rotor, the gradient was fractionated by pumping the solution from the bottom of the tube at a rate of 3·1 ml./min. Fractions of 1·6 ml. were collected in tubes containing one drop of 0·5 per cent bovine serum albumin and precipitated with 0·6 ml. 16 per cent trichloroacetic acid. The precipitates were collected on Whatman 'GF/C' filters and radioactivity was counted as described in Fig. 1. The recoveries of applied material were 89 per cent for ^{3}H-RNA and 71 per cent for the T4 ^{14}C-DNA. ○, ^{3}H-RNA; ■, T4 ^{14}C-DNA.

of a DNA added later is a reflexion of a greater rate of release of enzyme molecules previously attached to the first DNA template.

Continued Synthesis with Enzyme–Nascent RNA–DNA Complex

When an RNA polymerase molecule has initiated synthesis of an RNA chain on T4 DNA, it forms a stable complex with the template and nascent RNA chain[9]. This complex sediments much faster than free RNA polymerase, and it is sufficiently stable to be isolated by zone centrifugation on a glycerol gradient containing 0·3 M KCl (Fig. 3). RNA polymerase can also bind to T4 DNA in the absence of ribonucleoside triphosphate substrates, but this binary complex is much less stable and dissociates completely in 0·3 M KCl (ref. 20 and personal communication from G. Cassani). Consequently, the ternary complex isolated in 0·3 M KCl should be free of enzyme molecules that have not initiated RNA chains.

The enzyme in the complex with nascent RNA and T4 DNA isolated in this way does retain some RNA synthetic activity, but by itself it is apparently unable to initiate new RNA chains even in 0·2 M KCl. The kinetics of synthesis are the same in 0·06 M KCl and in 0·2 M KCl: in both cases, synthesis stops very rapidly (Fig. 4). Further, the enzyme in the complex is unable to transcribe added T5 DNA (Table 2, experiment 1). The possibility that the DNA in the complex is altered is ruled out by the fact that free RNA polymerase added to this same mixture transcribes both T4 and T5 DNA with the usual preference ratio (Table 2, experiment 2). It seems therefore that the enzyme is defective.

The possible nature of the defect is suggested by the fact that *E. coli* RNA polymerase requires a protein factor known as σ for the initiation of RNA chains from T4 or T5 DNA (ref. 21 and personal communication from G. Hager). Normally, σ is associated with the enzyme, but there is some indication that it may be released after the enzyme initiates an RNA chain[17,22], in which case it could be separated from the enzyme on the glycerol gradient containing 0·3 M KCl. Because of this possibility, the effect of added σ was tested, and the results show that it does stimulate RNA synthesis, particularly in 0·2 M KCl, and that it does allow the enzyme to transcribe T5 DNA (Table 2, experiment 3). Thus the RNA polymerase in the isolated complex is able to initiate new RNA chains, but only in the presence of σ-factor. Further, the fact that the enzyme in the complex is deficient in active σ-factor is consistent with the interpretation that σ is released after RNA chain initiation.

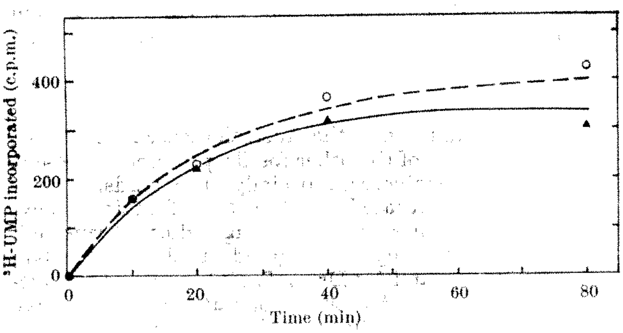


Fig. 4. Kinetics of RNA synthesis with complex. The nascent RNA–RNA polymerase–T4 DNA complex was prepared by the procedure described in Fig. 3, except that the reaction mixture of 0·3 ml. contained 15·6 μg T4 ^{32}P-DNA (950 c.p.m./μg) and 11·4 μg RNA polymerase. After incubating for 3 min at 37° C, 1·1 ml. of cold "gradient buffer" was added, and the diluted sample was centrifuged and fractionated exactly as described in Fig. 3. The position of the complex was determined by assaying 200 μl. samples of each fraction for ^{32}P radioactivity, and the peak fractions were pooled. RNA synthesis was assayed in mixtures of 0·25 ml. containing 0·04 M Tris-HCl (pH 7·9) buffer; 12 mM $MgCl_2$; 2·8 mM 2-mercaptoethanol; 0·2 mM each ATP, GTP and CTP; 0·04 mM ^{3}H-UTP (100 Ci/mole); 0·2 mg/ml. bovine serum albumin; 50 μl. of gradient fraction (containing 0·027 μg T4 DNA complexed with RNA polymerase and nascent RNA) and either 0·06 or 0·2 M KCl. Incubation was at 37° C and the amount of ^{3}H-UTP incorporated into acid insoluble material was assayed by the method described in Fig. 1, except that no unlabelled UTP was added during the washes. The background level of unincorporated UTP in these experiments was 20–35 c.p.m. ○, Incorporation in 0·06 M KCl; ▲, incorporation in 0·2 M KCl.

Table 2. TRANSCRIPTION OF T4 AND T5 DNA IN MIXTURES CONTAINING RNA POLYMERASE–NASCENT RNA–T4 DNA COMPLEX AND T5 DNA

Exp.	Addition	KCl concentration (M)	^{3}H-UMP incorporated per 200 μl. (c.p.m.)	Relative composition of RNA (per cent)	
				T4	T5
1	None	0·06	205	90	10
		0·20	208	93	7
		0·06	2,740	41	59
2	RNA polymerase	0·20	3,460	44	56
3	Pure σ-factor	0·06	426	60	40
		0·20	669	50	50

The basic composition of each reaction mixture was the same as that described in Fig. 4. Each mixture of 1·25 ml. contained 0·25 ml. of gradients fraction preparation of complex plus 0·14 μg of T5 DNA and the components listed in the table. For RNA polymerase, 0·5 μg in 5 μl. was added, and for σ-factor (prepared by a modification of the method of Burgess *et al.*[21] and kindly supplied by Mr G. Hager), 0·12 μg in 5 μl. was added. After incubation for 40 min at 37° C, a 200 μl. sample was removed to determine the total incorporation into acid insoluble material and the remaining solution was treated with detergent. After extraction, the RNA was analysed as described in Table 1. It should be noted that the amount of T5 RNA synthesized could not result from the presence of RNA polymerase in the added σ protein. Control assays indicated that less than 5 per cent of the σ protein preparation was RNA polymerase, which should account for no more than 6 per cent of the RNA made in experiment 3.

Experiment 3 of Table 2 also shows that enzyme from the complex can transcribe T5 DNA even in 0·06 M KCl when σ is added. Because there is normally no release of RNA polymerase during synthesis in 0·06 M KCl, this result is surprising, and it suggests either that the complex may contain some enzyme molecules that have not initiated an RNA chain or that there may have been some dissociation of the complex after its isolation. Another possibility, however, is that the added σ-factor may have promoted the release of enzyme molecules, particularly because the amount of σ added may have been in several-fold molar excess over the amount of RNA polymerase present.

Conditions for Release and Reinitiation

These results indicate that during transcription of T4 or T5 DNA *in vitro* in 0·2 M KCl, RNA polymerase is able to terminate the synthesis of RNA chains and to initiate new ones *de novo*. In order for an enzyme molecule to initiate a new RNA molecule, it is presumed that the one synthesized previously has to be released at least from the site of polymerization. Thus the observation presented previously[16] that 0·2 M KCl also stimulates the release of RNA from the enzyme–DNA complex is consistent with this interpretation. Further, because the enzyme also seems to be released from the DNA template during synthesis in 0·2 M KCl, the release of RNA may be linked to the complete dissociation of the complex between nascent RNA, RNA polymerase and DNA.

Reinitiation might not be possible at lower salt concentrations because the RNA cannot be released from the enzyme. Krakow[23], Fuchs *et al.*[11] and Bremer[24] have presented evidence that in restrictive conditions of synthesis RNA polymerase is randomly inactivated by the nascent RNA. In this case the synthesis of many RNA molecules would be terminated prematurely, which might be one explanation for the inability of the enzyme to release the RNA. It is also possible, however, that even if the synthesis of the RNA molecules is not terminated prematurely, release might not occur at low salt concentrations because the binding affinity between enzyme and RNA is too high. In general, the binding affinity of nucleic acids to RNA polymerase decreases as the ionic strength is raised[25]. Consequently, a weaker binding of the nascent RNA to enzyme in 0·2 M KCl may, in one case, reduce the probability that the RNA inactivates the enzyme and, in the other case, facilitate the release of terminated RNA chains.

If termination and release are separate steps, the release step might be much slower. Thus other components that stimulate RNA synthesis by increasing the level of chain initiation—such as polyamines[26] or the combination of ribosomes with the factor C of Revel *et al.*[27]—may act by facilitating the release of RNA chains.

It is possible that ρ or a similar factor present as a contaminant in the enzyme preparation could be responsible for the termination that is observed in these experiments. There is, however, no evidence of a polypeptide contaminant in the enzyme preparation used with electrophoretic properties similar to the polypeptide component of pure ρ factor. Further, because the RNA products are much larger than those found when ρ is present[5,12], it is not likely that this factor itself is a contaminant. Although another factor could still be responsible, termination could actually be an intrinsic property of the reaction catalysed by RNA polymerase. It therefore will be important to determine whether the RNA molecules synthesized in 0·2 M KCl are terminated at specific sites on the template.

I thank Professor A. Tissières for his encouragement and advice; Professor B. Hall and Dr G. Gussin for discussions; Mr G. Hager for the gift of pure sigma protein; Dr G. Cassani for labelled T4 DNA; and Mrs L. Richardson for technical assistance. This work was supported by the Fonds National Suisse de la Recherche Scientifique. I am a postdoctoral fellow of the American Cancer Society.

Received February 4, 1970.

[1] Milanesi, G., Brody, E. N., and Geiduschek, E. P., *Nature*, **221**, 1014 (1969).
[2] Naono, S., and Gros, F., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 363 (1966).
[3] Roberts, J. W., *Nature*, **223**, 480 (1969).
[4] Cohen, S. N., Maitra, U., and Hurwitz, J., *J. Mol. Biol.*, **26**, 19 (1967).
[5] Richardson, J. P., *J. Mol. Biol.* (in the press).
[6] Stevens, A., and Henry, J., *J. Biol. Chem.*, **239**, 196 (1964).
[7] Bremer, H., Konrad, M. W., Gaines, K., and Stent, G. S., *J. Mol. Biol.*, **13**, 540 (1965).
[8] Maitra, U., and Hurwitz, J., *Proc. US Nat. Acad. Sci.*, **54**, 815 (1965).
[9] Bremer, H., and Konrad, M. W., *Proc. US Nat. Acad. Sci.*, **51**, 801 (1964).
[10] So, A. G., Davie, E. W., Epstein, R., and Tissières, A., *Proc. US Nat. Acad. Sci.*, **58**, 1739 (1967).
[11] Fuchs, E., Millette, R. L., Zillig, W., and Walter, G., *Europ. J. Biochem.*, **3**, 183 (1967).
[12] Roberts, J. W., *Nature*, **224**, 1168 (1969).
[13] Millette, R. L., *Fed. Proc.*, **28**, 659 (1969).
[14] Maitra, U., and Barash, F., *Proc. US Nat. Acad. Sci.*, **64**, 779 (1969).
[15] Richardson, J. P., *Proc. US Nat. Acad. Sci.*, **55**, 1616 (1966).
[16] Richardson, J. P., *Prog. Nucleic Acid Res. and Mol. Biol.*, **9**, 75 (1969).
[17] Travers, A. A., and Burgess, R. R., *Nature*, **222**, 537 (1969).
[18] Berg, D., Barrett, K., Hinkle, D., McGrath, J., and Chamberlin, M., *Fed. Proc.*, **28**, 659 (1969).
[19] Jones, O. W., and Berg, P., *J. Mol. Biol.*, **22**, 199 (1966).
[20] di Mauro, E., Snyder, L., Marino, P., Lamberti, A., Coppo, A., and Tocchini-Valentini, G. P., *Nature*, **222**, 533 (1969).
[21] Burgess, R. R., Travers, A. A., Dunn, J. J., and Bautz, E. K. F., *Nature*, **221**, 43 (1969).
[22] Krakow, J. S., Daley, K., and Karstadt, M., *Proc. US Nat. Acad. Sci.*, **62**, 432 (1969).
[23] Krakow, J. S., *J. Biol. Chem.*, **241**, 1830 (1966).
[24] Bremer, H., *Mol. Gen. Genet.*, **99**, 362 (1967).
[25] Richardson, J. P., *J. Mol. Biol.*, **21**, 83 (1966).
[26] Petersen, E. E., Kröger, H., and Hagen, U., *Biochim. Biophys. Acta*, **161**, 325 (1968).
[27] Revel, M., Herzberg, M., Becarevic, A., and Gros, F., *J. Mol. Biol.*, **33**, 231 (1968).
[28] Thomas, jun., C. A., and Abelson, J., in *Procedures in Nucleic Acid Research* (edit. by Cantoni, G. L., and Davies, D. R.), 323 (Harper and Row, New York, 1966).
[29] Glynn, I. M., and Chappell, J. B., *Biochem. J.*, **90**, 147 (1964).
[30] Bautz, E. K. F., *Cold Spring Harbor Symp. Quant. Biol.*, **28**, 205 (1963).
[31] Scherrer, K., and Darnell, J., *Biochem. Biophys. Res. Commun.*, **7**, 456 (1962).
[32] Gillespie, D., and Spiegelman, S., *J. Mol. Biol.*, **12**, 829 (1965).

New Finds at the Swartkrans Australopithecine Site

by

C. K. BRAIN

Transvaal Museum, Pretoria

New discoveries at Swartkrans, in South Africa, an important cave site for the study of the australopithecine stage of human evolution, include a new series of australopithecine remains and an associated stone culture. Recent investigations have clarified the original form of the cave and a study of the accumulations of bones in the cave suggests that the australopithecines who lived there may have been the prey of leopards. In this article, Dr Brain introduces the new discoveries: further reports will follow.

SWARTKRANS, one of the five South African australopithecine cave sites, has produced remains of more than sixty individuals of the large hominid, *Paranthropus robustus*, and is the only cave site where fossils of *Homo* have been found in direct association with those of australopithecines.

The new investigations described here have clarified a number of the important Swartkrans issues. A new series of hominid fossils has been discovered (including two vertebrae which will be described in detail by Dr J. T. Robinson[1]), and it has been possible to associate indisputable stone culture with the hominid remains. The tools have been critically examined by Dr Mary Leakey, who has compared them with an equivalent series from Olduvai Gorge in Tanzania and has found striking similarities with the assemblage from Bed II of that site[2]. In the absence of absolute dating for the Swartkrans fossils, cultural comparisons of this sort assume particular significance. Unexpected new information has come to light on the nature of the second hominid at Swartkrans[3], while a study of the bone accumulation as a whole has led me to conclude that the australopithecine remains, together with many of the other fossils, probably represent food remains of carnivores, especially of leopards.

Swartkrans is situated in the Sterkfontein valley, approximately 8 miles north of Krugersdorp, Transvaal. It was first scientifically investigated in 1948 by the late Dr Robert Broom and by Dr J. T. Robinson, who transferred the Transvaal Museum's operations to the cave from Sterkfontein, on the opposite side of the valley. Excavation of *in situ* breccia continued throughout 1949 and resulted in the recovery of about 3,000 fossils, including numerous *Paranthropus* specimens and remains of a second hominid, named *Telanthropus capensis*[4], but subsequently reclassified as *Homo erectus*[5].

The palaeontological work of 1949 revealed a substantial seam of pure travertine along the north wall of the excavation, and this was exploited commercially by a lime-miner in 1950 and 1951, who left the site in a chaotic state. Robinson excavated the site again in 1952, but the cave was then abandoned until the new investigations, reported here, were started in April 1965.

New Investigations

The new fieldwork at Swartkrans has had two important achievements: first, order has been restored after the chaotic mining episode and excavation of undisturbed breccia is now possible; second, a large and representative sample of 14,000 bone fragments has been recovered.

Our first task was to sort through a substantial dump on the hillside below the cave. Unexpectedly, we found very numerous blocks of fossiliferous breccia, obviously not derived from the 1950–51 mining operations. In fact, more than 200 tons of breccia were recovered from the dump and stockpiled for subsequent attention. Enquiries among old residents of the area revealed[6] that Swartkrans had, in fact, been mined for lime on a previous occasion between 1930 and 1935. The miners had later refilled their excavations with rubble, thereby confusing the situation and leading Robinson[7] to believe that, unlike Sterkfontein, Swartkrans had not been modified by mining before its palaeontological exploration.

After working through the hillside dump, rubble was removed from the excavation and hand-sorted. This work is now almost complete and, after a grid over the excavation area has been erected, selected blocks of breccia from different stratigraphic levels will be removed for detailed comparative study of their fossil contents. It is fortunate that a substantial bank of *in situ* breccia survived the first mining operations and remains today as an inclined surface along the breadth of the cave's south wall. A profile is thus preserved of the whole thickness of the original breccia deposit.

Form of Original Cave

Clearing of the site has clarified the extent of the deposit and form of the original cave, and there will have to be some modification of the original interpretation[8]. In this interpretation, the cave was reconstructed as having comprised two filled chambers, an outer and an inner, with pink unstratified breccia in the former and a brown, stratified deposit in the latter. The two deposits were separated by an unconformable contact, the brown breccia proving to be younger than the pink. After our new investigations, separation into inner and outer chambers remains valid, but study of the east end of the deposit strongly suggests that the outer-cave breccia is not the oldest in the Swartkrans cave and that a still older breccia occupies the eastern extension of the site. This is separated from the normal australopithecine-bearing deposit by a steeply inclined unconformity. Each of the three unconformable contacts between the Swartkrans breccias seems to have resulted from progressive slumping into an underground, lower cavern system. Neither the oldest of the breccias, nor the underlying cavern, has yet been investigated.

In 1958 (ref. 8), the entrance to the outer cave was interpreted as an incline, opening obliquely into the hillside. The walls of the cave can now be seen, however, on all sides except the south-east, where extensive decalcification has occurred. On the evidence of the new exposures, it seems very likely that the original entrance was a precipitous, shaft-like one, perhaps no more than 5 feet in cross-section, linking the cavern with the surface above. Such shaft-like entrances to caverns are a common feature on the undulating dolomite hillsides in the area today. Soil, rock and other debris are funnelled down a shaft of this sort during rain storms, to build up a talus cone in the cavern below the discharge point of the shaft. The discharge point itself is marked by a concentration of rocks and coarse particles in the sediment, while the finer fractions tend to be carried into the further recesses of the cave. It is likely that a vertical shaft discharged in this area, from a hillside depression perhaps 50 feet above it (Fig. 1).

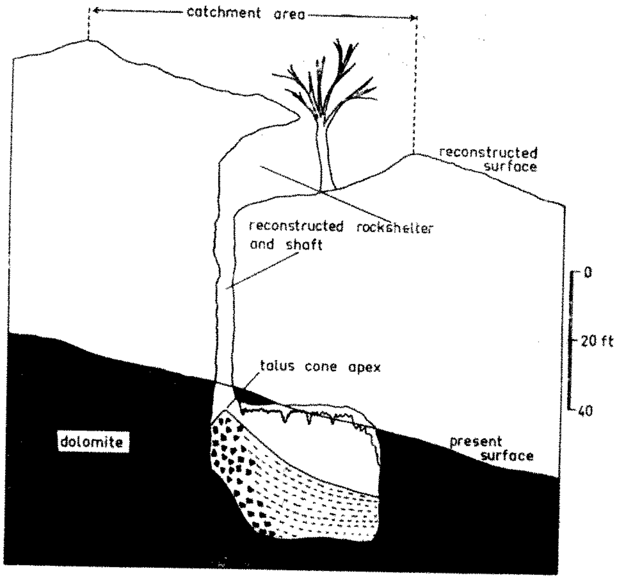


Fig. 1. Diagrammatic N–S section through the Swartkrans hillside. The upper reconstructed part has been removed by erosion since the accumulation of the fossiliferous deposit.

The Swartkrans cave system has developed at the intersection of two fault planes, clearly visible on an aerial photograph. They intersect at an angle of about 87 degrees and it is probable that a large rock-shelter may have existed on the surface in this area, with the shaft descending from it to the underground cavern. If this interpretation of cave form is correct, it is important to realize that the cavern containing the fossiliferous breccia would have been inaccessible to most animals, other than bats and owls. The deposit would, nevertheless, reflect events in the catchment area on the surface during the accumulation period. Relics left by animals or men in the upper rock shelter would ultimately find their way down to the subterranean fossilization site. Stratigraphy in a talus cone is difficult to follow because it is steeply inclined close to the apex, but becomes progressively flatter in those parts of the cave further from the shaft (see Fig. 1). Today, erosion has removed the overlying hillside with its shaft and rock shelter. The original form of these missing parts of the cave system is thus a matter for speculation, but it may be reconstructed on the known geological conformation of the immediate area.

New Hominid Finds

The current fieldwork at Swartkrans has produced a little more than 11,000 bone fragments among which are seventeen recognizable hominid fossils. The hominids, which I shall describe briefly, are all presumed to represent

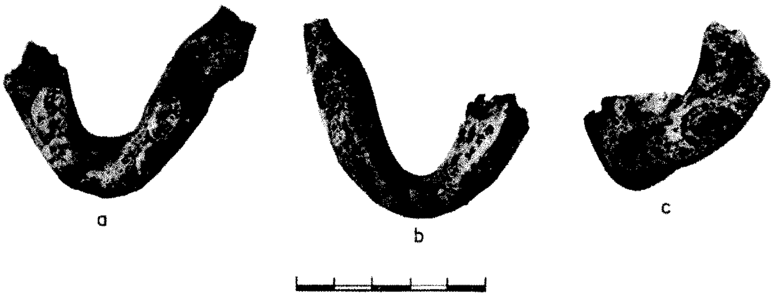


Fig. 2. An infant *Paranthropus* mandible (*SK* 3978) in superior (*a*), inferior (*b*) and lateral view (*c*).

Paranthropus robustus. It has been observed[6] that teeth in two of the mandible fragments (*SK* 1587 and *SK* 1588) were unusually small, with dimensions falling outside the observed range for *Paranthropus* from the site. At the time, it was suggested that these two mandibles may have represented a hominid other than *Paranthropus*, but this now seems unlikely—instead, the hominids probably extend the range of tooth dimensions for the Swartkrans *Paranthropus* sample. Measurements were taken on all teeth which had not suffered damage or undue wear. In the following descriptions mesio-distal length is quoted first, followed by bucco-lingual breadth, in millimetres.

Cranial and Vertebral Remains

SK 3978	Perfectly preserved and undistorted infant mandible lacking rami (see Fig. 2). Left dm$_1$ (10·2 × 7·8 mm) slightly worn, dm$_2$ (13·0 × 10·5 mm) unworn; right dm$_1$ (9·9 × 8·2 mm), dm$_2$ (12·9 × 10·7 mm). Sockets are present for deciduous incisors and canines while the unerupted left M$_1$ is visible in its crypt. Found August 8, 1967.
SK 1585	A natural endocranial cast from a hominid, presumably *Paranthropus* (see Fig. 3). The skull was blasted out by lime miners in the early 1930s and was broken into several pieces before being dumped on the hillside. Two of the surrounding parts of the cranial vault have been recovered, but the face has not yet been found. It is clear that the skull originally came to rest in the cave on its right side and was filled with fine-grained sediment to the level of the top of the foramen magnum. The filling was subsequently hardened with calcium carbonate and is almost completely undistorted. The state of the sutures suggests that the individual was subadult. The endocast has recently been prepared and studied by Dr R. Holloway, who estimates the capacity of the skull to have been 530 cc and whose detailed report will be published elsewhere. Found January 17, 1966.
SK 3981	Two vertebrae, thought to belong to *Paranthropus*; (*a*) a last thoracic, (*b*) a last lumbar. These specimens have been described in detail by Dr J. T. Robinson[1]. Found March 1968.
SK 1587	(*a*) Portion of the left corpus of a mandible with roots of P$_3$, complete P$_4$ (10·6 × 11·6 mm), M$_1$ (14·7 × 12·9 mm); (*b*) isolated right M$_2$ (15·2 × 12·9 mm) complete, from the same block and almost certainly from the same individual. Found July 19, 1966.
SK 1588	Part of a left corpus of a mandible with roots of P$_3$, complete P$_4$ (11·0 × 11·6 mm), M$_1$ (14·0 × 12·3 mm) and root of M$_2$. The specimen is of particular interest because the dimensions of M$_1$ are well below the hitherto observed range for *Paranthropus* from Swartkrans. Found October 6, 1966.
SK 1590	From an adult skull: two palatal fragments (*a*) and (*b*), with an incomplete right I^2, C, P^3 (9·25 × 13·3 mm), P^4 (10·2 × 14·5 mm) and M^1 (13·2 × 14·9 mm), all worn; crushed and distorted mandible (*c*) with parts of premolar and molar teeth. Associated were the head of a femur (*d*) and parts of an innominate bone in poor condition. Found April 13, 1965.
SK 1586	Almost complete adult mandible, severely shattered during fossilization, with right I$_1$, I$_2$, M$_1$ (broken), M$_2$ (15·0 × 14·9 mm) and M$_3$ (16·7 × 15·0 mm); also left I$_1$, M$_1$, M$_2$ (15·0 × 13·8 mm). One of the most robust mandibles from Swartkrans. Found September 1, 1965.
SK 1648	Anterior part of an adult mandible, severely distorted with worn right M$_1$ and M$_2$ (15·7 × 14·5 mm), left M$_1$ (incomplete) and roots of other teeth. Found November 4, 1966.

Isolated Teeth

SK 1593	Right P$_3$ (9·8 × 12·2 mm), well worn, with almost complete root system. Found March 24, 1966.
SK 3974	Right M$_1$ (14·9 × 13·4 mm), perfect crown, slightly worn, without roots. Found July 14, 1967.
SK 1594	Right M$_1$, moderately worn, most of the buccal half including the root. Found September 12, 1966.
SK 3976	Left M$_2$ (17·3 × 16·0 mm), complete and slightly worn. Found May 5, 1967.
SK 14001	Left P^3 (10·5 × 12·8 mm), complete crown but partly damaged root system. Found July 14, 1967.
SK 1589	Right P^4 (11·0 × 14·4 mm), complete crown of an unerupted tooth. Found October 14, 1966.
SK 3977	Right M^3 (15·5 × 17·9 mm), slightly worn. Found May 5, 1967.
SK 3975	Left M^3 (14·6 × 16·7 mm), slightly worn. Found July 28, 1967.
SK 14000	Upper molar broken through middle, showing pulp cavity. Found November 11, 1967.

The following eight hominid fossils were recently found in blocks of breccia brought back to the Transvaal Museum during the Broom/Robinson operations at Swartkrans. They came to light when the breccia was processed in acetic acid. All are assigned to *Paranthropus robustus*.

SK 1596	Isolated upper canine, heavily worn, with almost complete root system.
SK 1591	Isolated left M^1 (13·0 × 14·4 mm), fairly worn with part of the root system.
SK 1524	Isolated left M^3; most of the mesial portion of an unerupted and incompletely developed crown.
SK 1514	Mandibular fragment in very poor condition with roots of two molars and crown of one.
SK 1595	Fragment of a maxilla with slightly worn left dm^2 (11·5 × 12·4 mm) and pieces of erupting anterior teeth.
SK 1512	Palatal fragment with roots of several cheek teeth and part of a premolar.
SK 1592	Palatal fragment with broken right P^4, M^1, M^2 and part of M^3; also the root of one of the anterior teeth.
SK 14003	Part of a crushed skull with left M^1 (incomplete), part of right M^1, M^2 and complete M^3 (15·0 × 16·2 mm).

Stone Culture

The presence of a stone culture at Swartkrans was first suspected in 1956 when several quartzite tools, encased in breccia, were found there[8]. Recent examination of breccia blocks, blasted from the outer cave by the lime miners, has led to further recovery of occasional pieces of rock, foreign to the immediate vicinity of the cave and, in some cases, artificially worked. To date, 195 foreign stones have been found, fifty-one of which show signs of artificial fracture. The whole collection has been studied in detail by Miss S. M. Johnston, who has very kindly made her results available to me (see Table 1).

Table 1

Rock type	No. of pieces	Per cent of total foreign stones	Average wt (g)	No. of waterworn pieces	No. of worked pieces
Quartzite	148	75·9	507	87	33
Vein quartz	36	18·5	226	3	8
Diabase	6	3·1	450	1	5
Other	5	2·5	166	0	5
	195	100·0	337 (overall average)	91	51

The most numerous rock type present, quartzite, has its origin on the Witwatersrand escarpment a few miles to the south of Swartkrans; vein quartz occurs sporadically in the dolomite formation, while the closest outcrop of diabase is about 1 mile south-east of the cave, on the opposite side of the valley. Although fifty-one of the stones showed some signs of artificial fracture, only thirty of them could be termed artefacts and these have been studied and described in detail by Dr Mary Leakey[2]. All the tools, with the exception of the two diabase bifaces and one quartzite chopper, have edges so sharp that they were

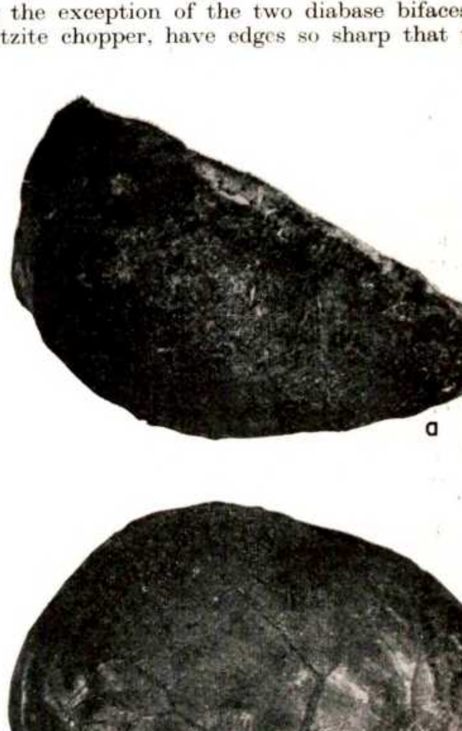


Fig. 3. A natural endocranial cast (*SK* 1585) from a Swartkrans hominid in superior (*a*) and right lateral view (*b*).

clearly made close to the cave entrance shortly before their inclusion in the deposit. The diabase tools both have weathered cortices, but it is known that diabase weathers while it is enclosed in a damp breccia deposit. The one quartzite chopper had been waterworn after manufacture, but before fossilization. It was originally assumed that all the foreign stones must have been brought to Swartkrans by human (or proto-human) agency; it now seems likely, however, that an old river gravel may have existed on the Swartkrans hillside when the cave deposit was accumulating and that some of the pebbles found their way naturally into the cavern, together with the soil and debris which were washed down into it. Tools that were made at the cave entrance were presumably fashioned on pebbles which happened to be in the immediate vicinity.

In view of the possibility that the Swartkrans foreign stones may have originated in a river gravel, a detailed study of contemporary gravels in the area has been made. The cave is situated 700 feet from the present course of the Blaaubank River, but this stream does not normally flow except after heavy rain. Sparse river gravels occur along the sides of the valley and, from three separate localities within 1·5 miles of the cave, Miss Johnston was able to collect a sample of 152 foreign stones for comparison with the Swartkrans specimens. The results of this comparison indicate that the Swartkrans foreign stones are very similar to those associated with the stream course in the vicinity of the cave. Waterworn quartzite pebbles proved to be slightly more abundant in the Swartkrans sample than in the gravel, but the percentage abundance of vein quartz pieces did not differ significantly between the two. Weights of the pieces in the two samples were found to be almost exactly comparable. The stones in the gravel sample also did not differ significantly in shape from those in the Swartkrans collection.

On the basis of this study, it seems that the foreign stones at Swartkrans were almost certainly derived from an old gravel, associated with the stream course. Artificial transportation need not be invoked because, if the gravel lay within the catchment area of the cave entrance, it would have been washed into the underground cavern together with other surface debris. The stone tools are nevertheless of special significance, because they are indicative of sporadic hominid activity near the cave entrance during the Swartkrans accumulation period. Two different hominids are known at the site, *Homo erectus* and *Paranthropus robustus*, but it is not certain which was responsible for the stone tools. Three of the stones in the collection show evidence of heat-spalding, suggesting that they had been in a fire shortly before inclusion in the deposit. On this slender, but interesting, evidence it would be premature to suggest that the Swartkrans hominids had mastered the deliberate use of fire. Grass fires, started by lightning, are a recurrent feature of the Transvaal highveld and, when sweeping through a leaf-strewn rock shelter, can generate a good deal of heat. Nothing resembling a bone tool has yet been found at Swartkrans, in spite of examination of every fragment in the collection.

Interpretation of the Bone Accumulation

In the interpretation of any bone accumulation preserved in a cave deposit, the original form of the cave is of crucial significance because it is this that determines the kinds of animals likely to have made use of the site. As already described, Swartkrans seems to have consisted of a hillside rock-shelter, probably supporting several large trees, but enclosing a vertical shaft which descended to an underground cavern, in which the fossiliferous deposit accumulated (Fig. 1). The latter cavern was simply a receptacle into which surface-derived debris was funnelled, while the nature of the rock-shelter on the surface was presumably such that it is likely to have been used by a wide variety of animals, as well as primitive man. The sample of 14,000 fossil bone fragments currently being analysed comes from a thickness of deposit which probably took several thousand years to accumulate. At Swartkrans we are not dealing with an impressive concentration of bones, but rather with a more or less even scatter throughout the calcified soil which originally filled the cave. It is probably not necessary to account for the collection of more than about ten bones per year and these could have found their way into the cave in a variety of ways. Some of the possibilities will be considered.

It is, for instance, known that many animals, when injured or sick, will retreat into caves to die. If unmolested, complete skeletons or scattered undamaged bones can be expected to result. Nothing approaching a complete or undamaged skeleton has been found in the Swartkrans breccia and natural deaths are not thought to have been important. The same is true of artificial burials which are unlikely to have occurred during the australopithecine period.

A recent study of important factors in Southern African bone accumulations (my work, in preparation) has shown that porcupines are of the greatest significance as collectors of bones in caves. They are known to horde large numbers of bones in their lairs, but all their collections so far studied have been characterized by a high percentage of gnawed bones on which the marks of the rodents' incisors are unmistakable. The final figure for the percentage of porcupine-gnawed bones in the Swartkrans sample has yet to be determined, but it will probably be less than 5. It is therefore concluded that, although porcupines almost certainly contributed to the Swartkrans bone collection, their influence was small.

Second to porcupines, primitive men rated high in importance as collectors of bone fragments in caves during the South African Stone Age. A detailed comparative study of human bony food remains from various Southern African caves has led me to conclude that these accumulations are characterized by the extreme fragmentation of the bones (my work, in preparation). A high percentage abundance of long-bone flakes and unrecognizable fragments is indicative of human activity, in which the bones are broken with stone tools for the extraction of marrow. As already mentioned, fifty-one stone artefacts have been recovered from the Swartkrans breccia, many of them chopping tools suited to the breaking of bones for the extraction of marrow. Bone flakes and fragments do occur in the accumulation but they are not abundant, suggesting that human feeding activity occurred sporadically within the catchment area of the Swartkrans cave but that this was not of major importance in the building up of the bone collection.

Carnivore Activity

The overall impression given by the Swartkrans bones is that they represent carnivore food remains; they come predominantly from antelope, baboons, australopithecines, hyraxes, and so forth, and are usually fragmentary, often showing damage apparently caused by carnivore teeth. Antelope are characteristically represented by a wide variety of damaged skeletal parts, but remains of many other animals are essentially cranial in origin, with very little else. The Swartkrans breccia has yielded an interesting series of carnivores, some of which were almost certainly involved in the collection of bones at the site. Forms identified to date are as follows (taxonomy according to Ewer[9–11]): hyaenids: *Crocuta crocuta venustula* (spotted hyaena), *Hyaena brunnea dispar* (brown hyaena), *Lycyaena silbergi nitidula*, *Leecyaena forfex*; sabre-toothed cats: *Therailurus* sp., *Megantereon* sp., *Nimravidae indet.*; felids: *Panthera pardus incurva* (leopard), *Panthera* sp. (lion); canids: *Canis mesomelas* (jackal), *Vulpes* sp. (fox); viverrids: *Cynictis penicillata* (mongoose).

Both the *Crocuta* and the *Hyaena* are regarded as early representatives of the extant spotted and brown hyaenas, while *Lycyaena* and *Leecyaena* represent extinct genera, the former showing characteristics which have been interpreted as "reflecting a secondary adaptation to a more predacious, less scavenging habit—a change presumably brought about under the selective pressure of competition with more efficient scavengers"[11]. *Leecyaena* is characterized by anterior cheek teeth resembling those of an advanced *Hyaena* and could possibly be ancestral to *H. bellax*, described from Kromdraai.

The three different sabre-toothed cats are represented by rather fragmentary material. Ewer has pointed out that, as a result of highly efficient carnassial shear, these carnivores were adapted to the slicing of meat and had premolars so reduced that they "must have been unable to deal with more than the very smallest bones"[11]. The conclusion that sabre-toothed cats left the skeletons of their prey almost intact has important implications, in that a niche was thus created for various species of hyaena, not necessarily capable of crushing the more resistant bones. Hyaenas like *Lycyaena* and *Leecyaena* may have owed their livelihood to the remains left by the abundant sabre-tooths of the day. As Ewer[11] has remarked: "The period of hyaena abundance in the Lower Pleistocene is thus also a period of abundant sabre-tooths and the disappearance of the latter during the Middle Pleistocene corresponds with the shrinkage of the hyaenid fauna to those few species which have succeeded in surviving today, in association with modern Felinae". Regrettably little is known about the behaviour of South African sabre-tooths and it is uncertain whether they were dominant to the associated hyaenas or whether they were forced to retreat with their prey into the seclusion of caves or trees. If so, it is likely that they would have contributed bones to the Swartkrans accumulation and that some of these may have been modified by the less competent bone-crushing hyaenas, such as *Lycyaena* and *Leecyaena* before fossilization. (It is unlikely that the bones found today as fossils were ever thoroughly worked over by spotted or brown hyaenas; if they had, few would have remained to become fossilized.) Sabre-tooth involvement at Swartkrans can neither be demonstrated nor disproved, but it is very likely that the bone accumulation was added to by these cats and that the remains were modified by primitive hyaenas before being fossilized.

The Role of Leopards

Leopards are well represented among the Swartkrans fossils, where remains of at least thirteen individuals have been found. As *Panthera pardus incurva*[10], they differ from the living leopard on the subspecific level only, with an indication that the fossil form may have been a little smaller than its contemporary counterpart. It has already been noted that the presence of sabre-toothed cats could have been important to the survival of primitive hyaenas; in the same way, the direct association of leopards and spotted hyaenas was probably a crucial factor in the building up of the Swartkrans bone accumulation.

Where leopards share their hunting area with spotted hyaenas, it is well known that, if they are to retain their prey, they are obliged to feed in places inaccessible to the scavengers. I recently studied fifteen leopard kills in the Kruger National Park. Spotted hyaenas were attracted to all these kills, but in twelve cases the leopards took their prey into trees sufficiently promptly to avoid interference. In three they were not quick enough and, as a result, lost their prey to the hyaenas.

In a woodland habitat, leopards have little difficulty in finding suitable trees for storage of their prey, but the situation in open country is rather different. Swartkrans is situated today on the open highveld, an area of undulating grassland, almost devoid of trees except along the watercourses. Study of the composition of the antelope fauna from the Swartkrans deposit suggests that, during the infilling of the cave, open grassland conditions prevailed as they do today, the fossil fauna being dominated by gregarious plains-living species such as springbuck, gazelles and alcelaphines of various sizes. Leopards hunting on the open highveld have to make use of suitable cliffs, caves and the few available trees for the protection of their prey. An interesting and highly significant correlation is found to exist between the occurrence of large trees and caves in the dolomitic areas of the Transvaal highveld. In this gently undulating countryside, caves are typically of the shaft or sinkhole variety, with depressions surrounding their entrances. Whereas the hillsides themselves are devoid of trees, the cave entrances characteristically support several large stinkwood (*Celtis kraussiana*) or wild fig (*Ficus* spp.) trees, which flourish there as a result of protection afforded to them from frost and fire. Leopards are thus inevitably attracted to the dolomitic caves as places of safe retreat, while the associated trees are invaluable for the storage of their prey. Food remains of leopards are consequently introduced into the catchment areas of the cave entrances and some will ultimately find their way down to the subterranean fossilization sites. Assuming that the Swartkrans deposit accumulated during a period of perhaps 20,000 years, it would be surprising if the cave and its associated trees had not been used on innumerable occasions by leopards as a feeding place and lair. The cave was inhabited throughout its life by owls whose regurgitated pellets have built up a concentration of microfaunal remains in the deposit, to give a remarkably complete picture of the animals hunted by the owls in the vicinity of the cave. In the same way, the larger faunal remains at Swartkrans are very likely to contain evidence of the range of animals hunted by leopards in this particular area.

Studies on living leopards have made it clear that the nature of their food remains is considerably influenced by the presence or absence of hyaenas (my work, in preparation). In the Kruger Park study area, where the leopards are constantly harried by hyaenas, they were found to do considerable damage to the skeletons of their prey (largely impala). They would typically return to the carcass in the tree during a period of 3–4 days, by which time most of the body had been consumed. The head and lower leg segments would invariably be left, usually hanging on strips of skin, but the rest of the skeleton generally disappeared. As part of this investigation, leopards were also studied in South West Africa, in an area where no hyaenas or other significant scavengers occur. Here the pattern of leopard food remains was completely different. The leopards made no attempt to drag their prey into inaccessible places, except where the kill had been made close to human habitation. They typically ate where they killed and did comparatively little damage to the prey skeletons, eating only a small quantity of meat before moving on to make another kill a few days later. It is consequently impossible to define the characteristics of "typical" leopard food remains. Their nature will depend on the availability of food and the pressure applied to the leopards by dominant scavengers such as spotted hyaenas.

The greater part of the Swartkrans bone accumulation shows characteristics consistent with those of leopard food remains, and suggests a situation where the leopards had been regularly harried by hyaenas. There are, however, several features of the bone collection which require special explanation. These involve the disproportions in the skeletal parts preserved. In the case of bovid skeletons, it has already been established that certain parts are more resistant to destruction than others and it is possible to predict which parts are likely to survive any particular treatment[12]. At Swartkrans, for instance, bovid distal humeri are almost five times as common as proximal ones. Such a disproportion is to be expected and means simply that the carnivore involved was able to chew away the relatively fragile proximal end of the humerus, but had trouble with the resistant distal one. The difficulty is encountered when we compare the recognizable remains of primate skeletons (such as of baboons and australopithecines) with those of bovids. The latter are typically represented by a wide variety of damaged skeletal parts, while the former are largely cranial in origin with very little else. In fact, the Swartkrans *Paranthropus* sample consists of 190 separate pieces, representing a minimum of at least sixty individuals. Of these fossils, only eleven are post-cranial bones.

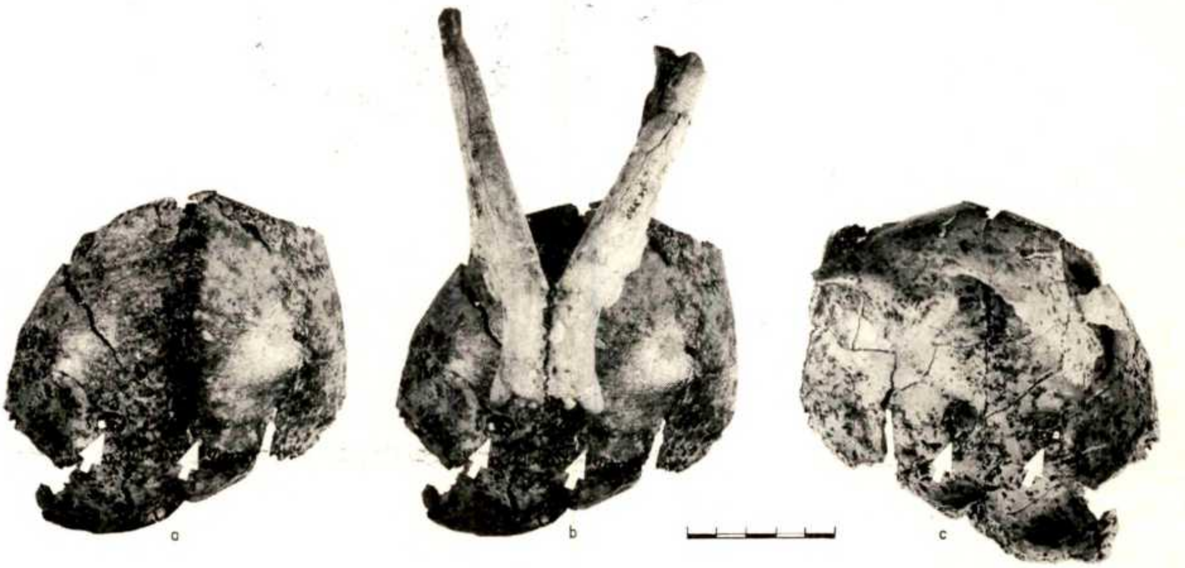


Fig. 4. Part of a hominid skull (*SK* 54) from Swartkrans showing holes thought to have been made by canine teeth. (*a*) Posterior view of left and right parietals and part of the occipital (arrows indicate position of holes on either side of lambda); (*b*) same view as in (*a*) to show how spacing of holes is matched by that of canine tips in the type mandible of the Swartkrans leopard; (*c*) internal view showing flakes of bone lifted when the parietals were pierced.

The probable reasons for this remarkable state of affairs have been suggested by a series of feeding experiments with cheetahs that I have recently carried out. A natural group of five wild cheetahs was caught on Valencia Ranch in South West Africa and maintained in a large semi-natural enclosure there, through the cooperation of Mr A. F. Port. Within a short period the cheetahs tolerated human observers and fed readily on the animals provided. Previous observations had indicated that cheetah damage to bovid skeletons, from animals in the 50–150 pound range, was minimal. It was restricted to fragile parts such as ribs, vertebral processes and scapulae. These observations were confirmed in the feeding experiments and, when the cheetahs had fed on a springbuck for instance, almost the whole skeleton remained. When a baboon of equivalent weight was eaten, however, a very different result was obtained. The cheetahs were able to consume the whole of the vertebral column, the hands and feet, as well as to do appreciable damage to the ends of the limb bones. The reason for this difference clearly lies in the fact that the construction of a baboon skeleton is considerably less robust than that of a bovid of equivalent live weight. The baboon vertebral column is fragile enough to be consumed, while the hands and feet are found to be fleshy and palatable.

It is not suggested that cheetahs were involved in the Swartkrans situation: they are simply amenable experimental animals. Nevertheless, conclusions drawn from them on the comparative robusticity of prey skeletons will be equally applicable to leopards, which are known to be capable of doing far more extensive damage to the skeletons of their prey. A leopard is potentially capable of eating virtually a whole baboon, with the exception of the skull, which is invariably left. Conclusions drawn about baboons will be equally applicable to hominids, built on the generalized primate pattern, provided that their larger size is taken into account. One of the reasons for the fact that so few primate limb bones have been recognized in the Swartkrans accumulation may lie in the fact that the diagnostic ends of such bones are typically chewed off by leopards, with the result that damaged and undiagnostic shaft pieces remain. Many of the indeterminate bone fragments at Swartkrans could well be of australopithecine origin.

One of the australopithecine skull pieces from Swartkrans appears to bear direct evidence of leopard activity. The specimen, *SK* 54, consists of much of the calvaria of a subadult hominid thought to have been a *Paranthropus*. It was found at the site in 1949 and subsequently prepared by Dr Robinson in Wisconsin, using the acetic acid technique. As shown in Fig. 4, the fossil consists of left and right parietal bones, part of the occipital and much of the frontal. The frontal has been distorted downwards, approximately across the width of the coronal suture, while the whole specimen has been slightly flattened by pressure in the deposit. From a comparison of the size of the parietal bones with those of other Swartkrans specimens, it is estimated that the endocranial capacity of this skull was approximately 500 cm^3. The two holes in the back of the skull, one in each parietal bone, close to lambda (see Fig. 4), are particularly interesting. Each is slightly elongated in a lateral direction and is approximately 6 mm in its shortest diameter. It is clear that the holes were made by two pointed objects, whose tips diverged slightly. On the internal surface of the parietals, flakes of bone have been lifted in a characteristic manner, suggesting that the bone was fresh and pliable when the injury was inflicted. If the two holes were made simultaneously, the points of the two sharp objects responsible must have been approximately 33 mm apart.

In a recent survey of the evidence for intrahuman killing in the Pleistocene, Roper[13] mentions this particular specimen and quotes Ardrey's argument[14] that the holes indicate that the australopithecine was struck twice on the back of the head with a pointed object. I believe that it is more likely that the two holes were made simultaneously by the canine teeth of a carnivore spaced about 33 mm apart. The question remains as to which species may have been involved and in what position the australopithecine's head could have been held when the damage was inflicted.

The Swartkrans fossils include remains of carnivores ranging in size from lion to mongoose. In many cases they represent forms closely related to living species so that, although the fossil material is fragmentary, modern skulls may be used to give an indication of typical canine spacings. Making use of the collection in the Transvaal Museum, distances between upper and lower canine tips have been measured on skulls of seven extant species (Table 2).

Results are plotted in Fig. 5, where the estimated ranges for some of the extinct species are also shown. On the basis of rather inadequate material, it is concluded that the known Swartkrans sabre-toothed cats had canine spacings intermediate between those of lions and spotted hyaenas. The type skull of *Leecyaena forfex* (*SK* 314) is comparable in size with the skull of a *Crocuta* and had upper canines spaced about 46 mm apart. Specimens of *Lycyaena* from Swartkrans appear to have had spacings intermediate between those of the living hyaenas and leopards.

It will be seen that the figure of 33 mm, representing the distance between the holes in the fossil skull, falls well within the leopard range and coincides with the mean figure for lower canine spacings in the female skulls measured. It must be remembered, however, that the fossil leopards seem to have been a fraction smaller than their living counterparts. It can naturally not be proved that a leopard (or any other specific carnivore) was responsible for the damage to the fossil skull, but, considering the various lines of evidence from Swartkrans, it is very probable that leopard canines did, in fact, produce the holes. Similar holes in the skulls of baboons eaten by leopards in the Suswa caves of Kenya have been reported by Simons[15]. A leopard typically kills its prey with a firm grip across the throat but, when subsequently dragging a dead animal to a protected feeding place, will often grip the head in its jaws. Fig. 6 shows how this may have been done in the case of the Swartkrans *Paranthropus* child. Under the weight of the child's body, the leopard's lower canines may have penetrated the rather thin parietal bones, while the upper canines were firmly embedded in the face.

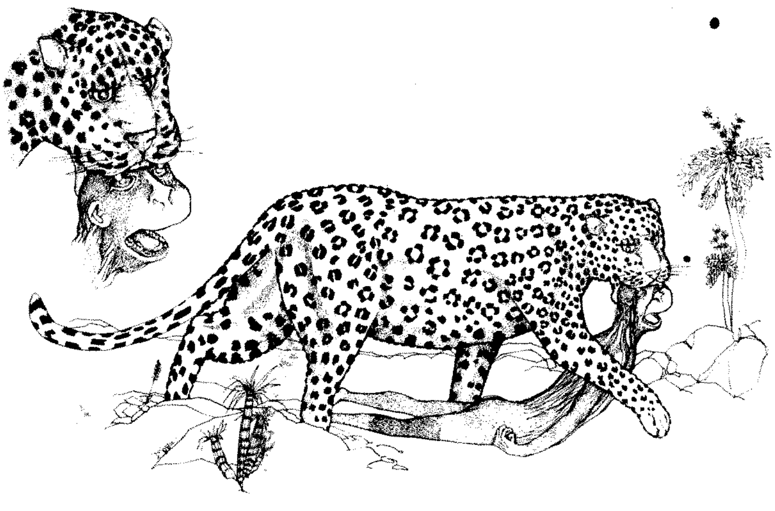


Fig. 6. Reconstruction showing how the observed damage to the skull of a Swartkrans *Paranthropus* child could have been caused by a leopard. The leopard's lower canines are thought to have penetrated the parietals of the dead child while it was being dragged to a feeding place.

Table 2. DISTANCES BETWEEN UPPER AND LOWER CANINE TIPS ON SKULLS OF SEVEN CARNIVORES

Carnivore	*n*	Upper canine spacing, mm Range	Upper canine spacing, mm Mean	Lower canine spacing, mm Range	Lower canine spacing, mm Mean
Lion, *Panthera leo*	17	60–81	67	53–69	59
Brown hyaena, *H. brunnea*	17	44–57	52	43–57	52
Spotted hyaena, *Crocuta crocuta*	8	49–57	54	42–54	46
Male leopard, *Panthera pardus*	10	33–47	39	31–41	36
Female leopard, *Panthera pardus*	14	33–42	38	30–38	33
Jackal, *Canis mesomelas*	20	21–28	25	21–26	23
Fox, *Vulpes chama*	11	14–19	16	11–16	13
Mongoose, *Cynictis penicillata*	25	10–13	11	9–13	10

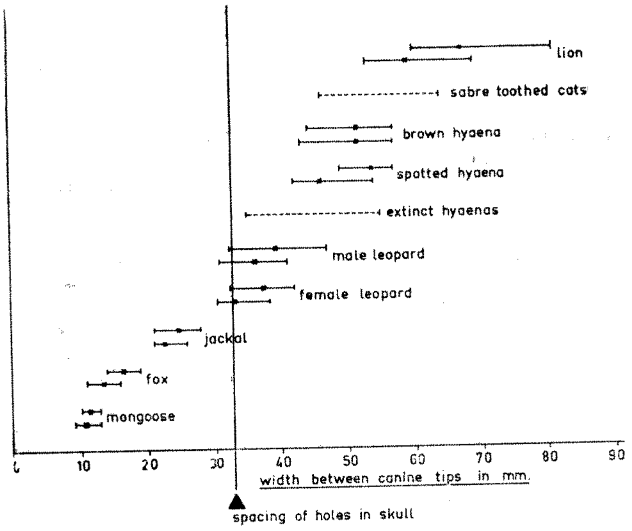


Fig. 5. Canine spacings in some South African carnivores. Observed ranges and means are plotted for upper and lower teeth while estimated ranges for Swartkrans sabre-tooths and extinct hyaenas are included.

The evidence presented here suggests that *Paranthropus* individuals were preyed upon by leopards in the same way as were baboons, hyraxes and antelope. Leopard predation on humans still occurs today; such predation would presumably have been much more prevalent at a stage of human evolution when australopithecines were neither physically formidable nor protected by the weapons of an advanced technology.

The renewed work at Swartkrans would not have been possible without the support of the Wenner-Gren Foundation for Anthropological Research; in particular I wish to thank Mrs L. Osmundsen for her interest in the project. I also thank the trustees of the Transvaal Museum and the South African CSIR for their support; the University of the Witwatersrand, present owners of Swartkrans, for help and permission to continue research there; Professor P. V. Tobias and Professor F. Clark Howell for helpful discussions; the South African National Parks Board for research facilities in the National Parks; Mr and Mrs A. F. Port for the opportunity to study living carnivores on Valencia Ranch, South West Africa; and my wife, Mr A. C. Kemp and Mrs E. A. Voigt for their help with the research.

Received January 16, 1970.

[1] Robinson, J. T., *Nature* (in the press).
[2] Leakey, M., *Nature* (in the press).
[3] Clarke, R. J., Howell, F. C., and Brain, C. K., *Nature* (in the press).

[4] Broom, R., and Robinson, J. T., *Nature*, **164**, 322 (1949).
[5] Robinson, J. T., *S. Afric. J. Sci.*, **57**, 3 (1961).
[6] Brain, C. K., *S. Afric. J. Sci.*, **63**, 378 (1967).
[7] Robinson, J. T., *Ann. Transv. Mus.*, **22**, 1 (1952).
[8] Brain, C. K., *Transv. Mus. Memoir*, No. 11 (1958).
[9] Ewer, R. F., *Proc. Zool. Soc. Lond.*, **124**, 815 (1953); **124**, 839 (1953); **125**, 587 (1954).
[10] Ewer, R. F., *Proc. Zool. Soc. Lond.*, **126**, 83 (1956).
[11] Ewer, R. F., in *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.), 109 (University of Chicago Press, 1967).
[12] Brain, C. K., *Scient. Pap. Namib Desert Res. Stn.*, **32**, 1 (1967); **39**, 13 (1969).
[13] Roper, M. K., *Current Anthrop.*, **10**, 427 (1969).
[14] Ardrey, R., *African Genesis*, 300 (Collins, London, 1961).
[15] Simons, J. W., *Bull. Cave Exploration Group E. Afric.*, **1**, 51 (1966).

Osculatory Packing of Finite Areas with Circles

by
H. H. KAUSCH-BLECKEN VON SCHMELING*
N. W. TSCHOEGL
California Institute of Technology,
Pasadena, California 91109

Osculatory packing of a finite area by circles requires that any uncovered area or interstice be filled always with the largest possible non-overlapping circle. The strength of this requirement makes it possible to derive a general analytic expression that approximates closely to the distributions of radii calculated for various sets of boundary conditions.

THE problem of covering a finite area with a given set of circles has received frequent attention. In particular the maximum packing fraction, that is, the ratio of the covered area to the total area, has been determined for a variety of boundary conditions[1-8]. The densest packing of non-overlapping uniform circles is the hexagonal lattice packing[1,2] with a packing fraction of $\varphi = \pi/\sqrt{12} = 0{\cdot}9069$.

The biggest packing fraction with bimodal circle size distributions is obtained if the larger circles, of radius R_1, form a hexagonal lattice, the interstices of which are filled by a large number of smaller circles with radius R_2. If the smaller circles within each interstice are also packed hexagonally, then in the limit that $R_2 \ll R_1$, the packing fraction of all circles will be given by $\varphi_2 = 1-(1-0{\cdot}9069)^2$. The limiting packing fraction for k different circle sizes, with each size packed hexagonally, is

$$\varphi_k = 1-(1-0{\cdot}9069)^k \tag{1}$$

Clearly φ_k approaches unity for large values of k. It follows immediately from the condition $R_k \ll R_{k-1}$ and from the size of the interstice ($0{\cdot}323\ R_{k-1}^2$ in a hexagonal lattice) that the number of smaller circles necessary to cover an interstice is very large. Evidently the condition $R_1 \gg R_2 \gg \ldots R_k$ minimizes the number of different circle sizes required to cover a given area to a given extent. We present here an approximate solution to the problem of minimizing the number of circles without regard to the number of different circle sizes.

The maximal covering of a finite area by a minimum number of non-overlapping circles will be called optimal covering. Optimal covering requires a distribution of circle sizes which, when truncated at any arbitrary lower limit, will always yield a larger packing fraction than any other distribution with the same number of circles, truncated at the same limit. Following Melzak[7], we shall call a distribution osculatory if any available area is always covered by the largest possible circle. If the original area to be covered is either a triangle or a tricuspid area, as shown in Fig. 1, then the first circle to be placed must touch the boundaries of the area at exactly three points. Further, all circles placed subsequently in the remaining spaces must touch the boundaries or neighbouring circles at exactly three points. In the limit of an infinitely small lower bound of the distribution, optimal and osculatory packing lead to the same distribution of circle sizes. Our investigation of the osculatory packing of circles is a first attempt to learn about optimal distributions of particle sizes with various boundary conditions and modes of packing.

Osculatory Packing of Finite Areas

The relationship between the radii of four osculating circles has been described by Soddy[9] in a poem that appeared in *Nature*. In a more prosaic formulation, and solved with respect to R_4, this relation is

$$R_4 = \frac{R_1R_2R_3}{R_1R_2+R_2R_3+R_1R_3+2\sqrt{R_1R_2R_3(R_1+R_2+R_3)}} \tag{2}$$

As shown in Fig. 1, three new interstices are created by placing the circle with radius R_4 into the tricuspid interstice formed by the three circles with radii R_1 to R_3. The radius of the touching circle placed into any of the new tricuspid interstices can be calculated from equation (2) by making the proper substitutions of radii. The radii of all subsequent smaller circles can be calculated similarly. (From Lemma 7 of ref. 8, the square rooting can be avoided after the first stage.) We call the circles with radii R_1 to R_3 the parent generation, and the circle with radius R_4 the zeroth generation. Because the number of circles per generation increases by a factor of 3 in all further generations, we use K subscripts to denote a radius of the Kth generation and allow each subscript to assume the values 1, 2 or 3. The radii of the circles of the first generation are thus denoted by r_i ($i = 1,2,3$), those of the second generation by r_{ij} ($i, j = 1,2,3$), and so on. The distribution of circle radii within any one generation is determined

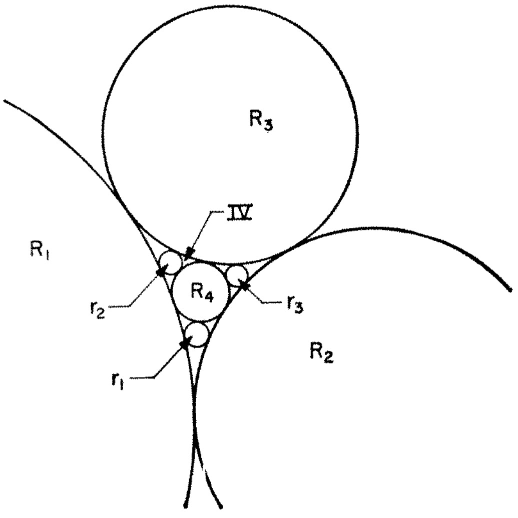


Fig. 1. Tricuspid interstices.

---

* Present address: Battelle-Institut E.V., 6 Frankfurt am Main 90, Germany.

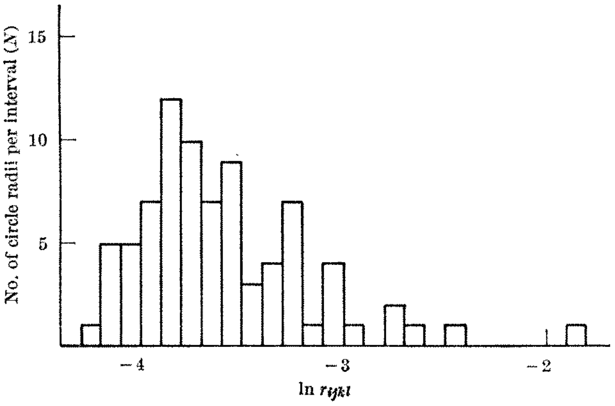


Fig. 2. Distribution of fourth order circle radii obtained by optimal filling of ideal tricuspid interstice.

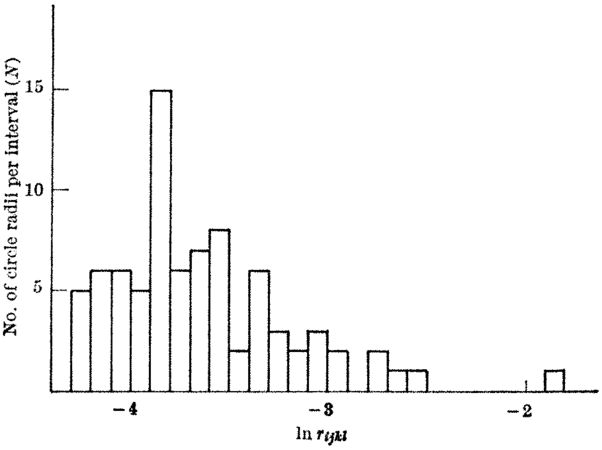


Fig. 3. Distribution of fourth order circle radii obtained by optimal filling of subcell of equilateral triangle.

largely by the order of the generation. Using equation (2), the first generation radii r_i can be computed from R_j, R_k and R_4. Then the second generation radii r_{ij} can be obtained, and so forth. We shall always arrange the parent radii so that $R_1 \geq R_2 \geq R_3$.

Fig. 2 shows the distribution of the radii of the fourth generation of circles filling the tricuspid area (see Fig. 1) formed by three parent circles the radii of which decrease by the constant factor 0·3460 so that $R_1 : R_2 : R_3 = 1 : 0{\cdot}3460 : 0{\cdot}1197$. The constant factor 0·3460 results from the requirement that $R_1/R_2 = R_2/R_3 = R_3/R_4$. To define a convenient reference point for the ratios of radii, R_4 was arbitrarily taken as unity. The distribution following from this particular choice of boundary conditions has features which facilitate the derivation of the analytic expression to be obtained later. Fig. 3 shows a similar plot for the boundary conditions illustrated by area II in Fig. 4. The parent circles were chosen as the circle inscribed in an equilateral triangle (R_2), the circle obtained by filling the interstice thus formed (R_3), and the side of the triangle which may be regarded as the third parent circle (R_1) having an infinite radius. Because the triangle is equilateral, $R_2/R_3 = 1/3$. The radii of the zeroth, first and any subsequent generation can be calculated from equation (2) by letting the parent radius equal infinity when the new circle touches the straight line. Figs. 2 and 3 show that the two distributions do not differ widely in spite of the difference in the boundary conditions.

The essential independence of the distributions of circle radii from the boundary conditions is seen more clearly when one examines the behaviour of the first and second movements of the distributions. We denote any radius of a circle of the Kth generation by $r(_K)$ and the first moment of the logarithms of all circle radii within the Kth generation by $\langle \ln r_{(K)} \rangle$. The first moments were calculated as a function of K for the six different boundary conditions listed in Table 1 together with the radii of the respective parent circles. The radius R_4 of the zeroth generation was taken to be unity in all cases.

Table 1. RADII OF PARENT CIRCLES FOR BOUNDARY CONDITIONS OF CIRCLE SIZE DISTRIBUTIONS

	Boundary condition	R_1	R_2	R_3	R_4
I	Ideal tricuspid interstice	24·14	8·35	2·89	1·0
II	Subcell of equilateral triangle	∞	7·47	2·49	1·0
III	Uniform tricuspid interstice	6·46	6·46	6·46	1·0
IV	Subcell of uniform tricuspid interstice	45·75	7·07	2·87	1·0
V	Irregular tricuspid interstice	12·58	6·15	4·31	1·0
VI	Equilateral triangle	∞	∞	∞	1·0

Boundary condition I, referred to as ideal tricuspid, and condition II, subcell of equilateral triangle, have already been discussed. They yield the distributions of fourth generation circle radii shown in Figs. 2 and 3 respectively. Condition III is given by the uniform tricuspid area formed from three circles of equal radii ($R_1 = R_2 = R_3$). Condition IV is the subcell of the uniform tricuspid formed from one circle each of the parent, zeroth and first order generation of the uniform tricuspid area III. Such a subcell of an irregular tricuspid is shown as area IV in Fig. 1. Condition IV is derived from a uniform tricuspid interstice. Condition V is the irregular tricuspid area formed by three parent circles with arbitrarily chosen radii. Finally, boundary condition VI is represented by an equilateral triangle the side of which may be regarded as circles of infinite radii. Here the zeroth generation circle with radius R_4 is the circle inscribed in the triangle, the sides of which must equal $2\sqrt{3} = 3{\cdot}46$ to give $R_4 = 1$.

In Fig. 5 $\langle \ln r(_K) \rangle$ is plotted against K for the six boundary conditions. For the circle radii distributions derived from the boundary conditions I–V the mean, $\langle \ln r(_K) \rangle$, is a linear function of K for $K > 0$ with slope

$$\frac{\mathrm{d}M_1(K)}{\mathrm{d}K} = \frac{\mathrm{d}\langle \ln r_{(K)} \rangle}{\mathrm{d}K} = -0{\cdot}91 \qquad (3)$$

The curve for the triangular area VI differs slightly but has the same slope for $K \geq 2$.

The constancy of the decrease of the average logarithmic circle radius with K is an interesting feature and warrants a little more scrutiny. The constancy is a consequence of the property of equation (2) to yield values of R_4/R_3 in a very narrow range for widely varying ratios of R_2/R_1 and R_3/R_2. If we let

$$\ln y(K) = \langle \ln r_{(K)} \rangle - \langle \ln r_{(K-1)} \rangle \qquad (4)$$

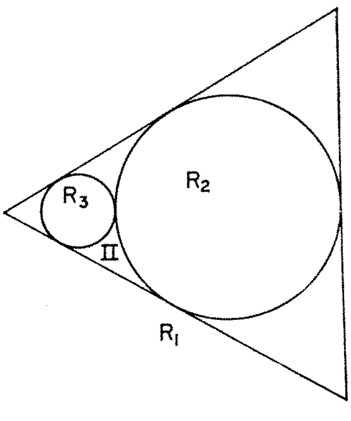


Fig. 4. Subcell of equilateral triangle.

then the ratio R_4/R_3 can be denoted by $y(0)$. In Fig. 6 the functions $y(0)$ and $y(1)$ are plotted against R_3/R_2 with R_2/R_1 as parameter. With R_3/R_2 and R_2/R_1 independently assuming values between 0 and 1, all possible shapes of tricuspid areas can be represented. It is seen that $y(0)$, that is, the ratio R_4/R_3, is confined to the narrow band of values shown by the shaded area in Fig. 6. The average ratio $y(1)$ of first order circle radii to the zeroth order circle radius (dotted line in Fig. 6) has a value of $y(1) \simeq 0{\cdot}402 \pm 0{\cdot}004$ and is therefore practically independent of R_2/R_1 and R_3/R_2. Further computations have shown that

$$\lim_{K \to \infty} y(K) = 0{\cdot}4028 \tag{5}$$

This explains the constant change of $\langle \ln r_{(K)} \rangle$ with K. Equations (3) and (5) are in agreement because $\ln 0{\cdot}4028 = -0{\cdot}90942$.

Fig. 6 also gives information about the second moment of the circle size distribution, $M_2(K)$, which is defined as

$$M_2(K) = \langle (\ln R_{(K)} - \overline{\ln R_{(K)}})^2 \rangle \tag{6}$$

The decrease in circle size per generation will vary within a narrow range of values only, so it is obvious that the increase in the second moment is also restricted. Calculations of the second moments of the circle size distributions for the areas I to VI showed that

$$\left(\frac{dM_2(K)}{dK}\right)_{K \geq 3} = 0{\cdot}052 \tag{7}$$

It should be noted that a generation of circle sizes is a complete distribution and not a random sample of a large distribution. Hence, we are interested in

$$s_0^2 = \frac{1}{N} \sum_1^N (x_i - \mu)^2 \tag{8}$$

and not in

$$s^2 = \frac{1}{N-1} \sum_1^N (x_i - M)^2 \tag{9}$$

where the x_i's form the distribution, μ is the true mean, s is the standard deviation and M is the experimental mean[10].

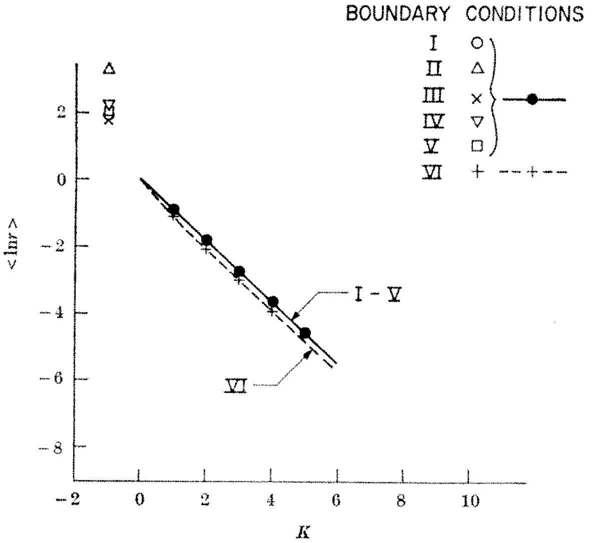


Fig. 5. Average logarithmic circle radius as a function of K for all six boundary conditions.

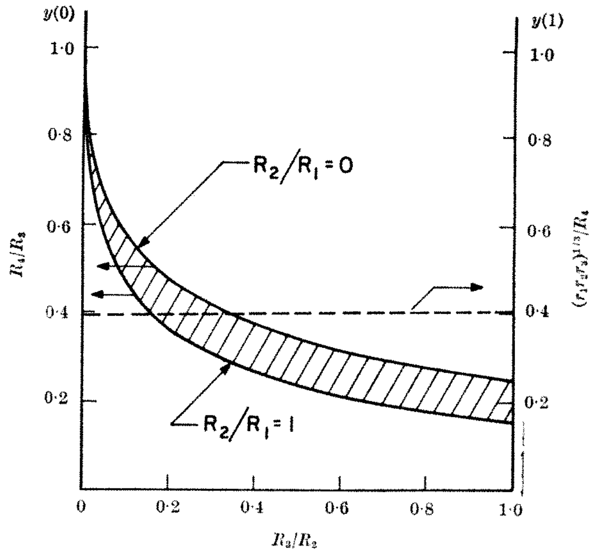


Fig. 6. Influence of R_2/R_1 and R_3/R_2 on the ratios $y(0)$ and $y(1)$ of zero- and first-order circle radii. Left hand ordinate and shaded area: ratio $R_4/R_3(y(0))$; right hand ordinate: geometric mean of first order circle radii ($y(1)$).

Analytic Expression

The circle size distributions in osculatory packing are weak functions of the boundary conditions and thus share important features. We can therefore construct an analytic expression that provides a good approximation to the osculatory distribution, regardless of the boundary condition. We use the fact, demonstrated above, that the decrease of the first moment of $\ln r_{(K)}$ with K approaches the constant value 0·90942, while the increase of the second moment with K likewise approaches the constant value 0·052. To derive the desired relation it is convenient to choose the boundary condition represented by the ideal tricuspid (condition I).

An approximation to $r_{(K)}$ with the aforementioned properties of the first and second moment can be derived from the identity

$$r_{(K)} = r_{ijk \ldots x} = R_4 \frac{r_i}{R_4} \frac{r_{ij}}{r_i} \frac{r_{ijk}}{r_{ij}} \cdots \frac{r_{ijk \ldots x}}{r_{ijk \ldots}} \tag{10}$$
$$(i, j, \ldots x = 1,2,3)$$

where the number of the indices $i, j, k \ldots x$ is equal to K. We stipulate that the ratios r_i/R_4, r_{ij}/r_i, and so on, can assume three distinct values c_1, c_2 and c_3 only. Equation (10) then becomes

$$r_{(K)} = r_{ijk \ldots x} = R_4 c_i c_j c_k \ldots c_x \; i, j, k \ldots = 1,2,3 \tag{11}$$

The assumption of distinct coefficients which repeat themselves within each generation is not unreasonable. There is indeed a solution of equation (2) with $R_2/R_1 = R_3/R_2 = R_4/R_3$, and the ratio was determined numerically to be 0·3460. The tricuspid with parent circles fulfilling these relations is precisely the ideal tricuspid, which was thus termed because the radii R_1 to R_4, and subsequently the smallest radii from each generation, $r_3, r_{33}, r_{333} \ldots$, form a geometric series with common factor 0·3460. Even if R_1 to R_4 do not fulfil the condition of constant ratios, the series of smallest circles from each generation will rapidly approach this factor. We will therefore arbitrarily choose one of the coefficients, c_3, to be equal to 0·3460. The coefficients c_1 and c_2 are determined so as to give the required behaviour of the first moment, $M_1(K)$, and the second moment, $M_2(K)$, of $\ln r_{(K)}$.

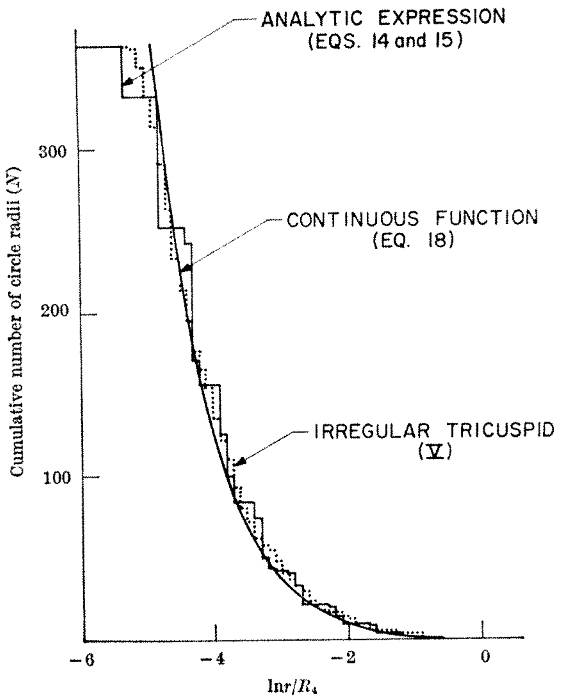


Fig. 7. Comparison of different cumulative distributions of circle radii.

We must have

$$\frac{\mathrm{d}M_1(K)}{\mathrm{d}K} = \frac{1}{3}\ln c_1c_2c_3 = -0{\cdot}90942 \qquad (12)$$

from the first condition, and

$$\frac{\mathrm{d}M_2(K)}{\mathrm{d}K} = \frac{1}{3}[(\ln c_1)^2+(\ln c_2)^2+(\ln c_3)^2]-\frac{1}{9}(\ln c_1c_2c_3)^2 = 0{\cdot}052 \qquad (13)$$

from the second. Using the value 0·3460 for c_3, equations (12) and (13) can be solved to yield $c_1=0{\cdot}555$ and $c_2=0{\cdot}340$. Equation (7) can now be replaced by

$$r_{ijk\ \ldots\ x}=R_4c_1{}^{\alpha}c_2{}^{\beta}c_3{}^{\gamma}=r(\alpha,\beta,\gamma) \qquad (14)$$

where α, β and γ take on any integral values subject to the restriction $\alpha+\beta+\gamma=K$. The multiplicity of any specific value $r(\alpha,\beta,\gamma)$ is given by the permutation

$$P(\alpha,\beta,\gamma) = \frac{K!}{\alpha!\,\beta!\,\gamma!} \qquad (15)$$

Equation (15) describes the probability distribution of the Kth order circle radii and together with equation (14) constitutes the desired analytic expression. The cumulative distribution of circle radii from all generations can be obtained by adding the contributions from each generation as calculated from equations (14) and (15). The result is shown in Fig. 7. The stepped solid line and the dotted line represent the cumulative distribution calculated from the analytic expression, and the cumulative distribution obtained from boundary condition V (the most commonly occurring irregular tricuspid), respectively, up to and including the radii of the circles of the fifth order.

The cumulative distribution function can also be derived directly from the knowledge that the number of radii within the jth generation is equal to 3^j and that

$$\langle \ln r_{(j)}\rangle = \ln R_4 - 0{\cdot}90942j \qquad (16)$$

We assume that within the jth generation half of the radii are smaller than $\langle \ln r_{(j)}\rangle$. Then for all points ln $r=\langle \ln r_{(j)}\rangle$, the total number of circles with radii between r and R_4 will be exactly

$$Z(j)=1+3+3^2\ \ldots\ +3^{j-1}+\frac{1}{2}3^j \qquad (17)$$

Using the summation formula of a geometric series, and expressing j by ln r as in equation (16), we obtain the number of radii per interstice with a radius of r or larger than r as

$$Z(\ln r)=3^{-(\ln r/R_4)/0{\cdot}90942}-0{\cdot}5=(r/R_4)^{-1{\cdot}2080}-0{\cdot}5 \qquad (18)$$

The function $Z(\ln r)$ is also shown in Fig. 7, where it is represented by the unbroken solid line. It provides a very satisfactory representation of the actual distribution for the irregular tricuspid area. (It should be possible to obtain the asymptotic distribution of ln r also from Hirst's[8] Lemma 7 together with the results of Furstenberg and Kesten[11].)

The differential distribution of circle radii is derived from equation (18) as

$$n(r)\mathrm{d}r = \frac{\mathrm{d}Z(\ln r)}{\mathrm{d}r} = -1{\cdot}2080\,(r/R_4)^{-2{\cdot}2080}\,\mathrm{d}(r/R_4) \qquad (19)$$

It is evident that the smaller circles will be more numerous, for their number is increasing with a constant power of 2·2080 and does not depend on the boundary conditions.

From equation (18) an empirical value can also be obtained for Melzak's[7] exponent of osculatory packing S, for which his theorem 3 supplies the bounds $1{\cdot}035<S<1{\cdot}999971$. Based on the calculations of Gilbert[6], Melzak has suggested a value in the neighbourhood of 1·3 for S. Because equation (18) is the more satisfactory the larger the number of circles, 1·208 seems to be a good empirical value for S.

Optimal Circle Size Distribution

The derivation of equations (14) and (15) assumed that the largest circle radii in each generation decrease from generation to generation by a factor of $c_1=0{\cdot}555$. If equation (19) is a good approximation to the circle size distribution in osculatory packing, we shall not expect the Kth generation to contain any circle with a radius larger than $R_4c_1{}^K$. The osculatory packing of K-1 generations of circles therefore yields the optimal cumulative distribution for $c_1{}^K<r/R_4<1$, because the cumulative distribution of circles between these limits will not be changed by packing the Kth and any further generations of circles. The optimal covering of a given area is therefore also a weak function of the boundary conditions and can be approximated closely by equation (19).

This research was supported by the Air Force Rocket Propulsion Laboratory, Research and Technology Division, Edwards, California, Air Force Systems Command, US Air Force.

Received July 14; revised November 11, 1969.

[1] Fejes Toth, L., *Acta Mathematica Acad. Sci., Hungaricae*, **4**, 103 (1953).
[2] Fejes Toth, L., *Lagerungen in der Ebene, auf der Kugel, und im Raum* (Springer Verlag, Berlin/Heidelberg, 1953).
[3] Florian, A., *Circolo Mathematico di Palermo, Rendiconti*, **9**, 300 (1960).
[4] Rogers, C. A., *Packing and Covering* (Cambridge University Press, 1964).
[5] Gardner, M., *Sci. Amer.*, **202**, 174 (1960), and **218**, 130 (1968).
[6] Gilbert, E. N., *Canad. J. Math.*, **16**, 286 (1964).
[7] Melzak, Z. A., *Canad. J. Math.*, **18**, 838 (1966).
[8] Hirst, K. E., *J. London Math. Soc.*, **42**, 281 (1967).
[9] Soddy, F., *Nature*, **137**, 1021 (1936).
[10] Ebert, H. (editor), *Physikalisches Taschenbuch*, 114 (F. Vieweg und Sohn, Braunschweig, 1962).
[11] Furstenberg, H., and Kesten, H., *Ann. Math. Stat.*, **31**, 457 (1960).

LETTERS TO THE EDITOR

PHYSICAL SCIENCES

Short-term Periodic Variations following an Optical Flare of Sco X-1

We have recently carried out a detailed investigation of the "flickering" of the optical source associated with Sco X-1 using the technique of power spectrum analysis to search for periodicities in the photometric data of Sandage *et al.*[1]. In common with others[2,3], we have found no evidence for stable periodic structure when the time series resulting from a whole night's observations is analysed. But we have also studied shorter stretches of data (500 to 900 consecutive data points) chosen so that no large-scale flaring occurred during these periods. Many of these short runs seem to contain periodic structure at one or more frequencies, corresponding to components with amplitudes in the range 0·1 to 0·5 per cent of the mean intensity over the appropriate interval. The results of this work (unpublished) have encouraged us to look more closely at certain regions of the data.

It is natural to suppose that the larger-scale flaring of the source may influence the small-scale flickering, and in the data which we have available there is one instance of a particularly large and well defined flare which occurred just before data point 610 (in the notation of Sandage *et al.*) on the night of April 3, 1967. Our present investigation has therefore concentrated on the fairly smooth stretch of data immediately following this flare, in order to find any detectable influence of the flare on the flickering. We have already discussed the technique of power spectrum analysis in another context[4], and the application here differs only in detail from that already described. The data were analysed in stretches of 500 data points with 100 lags, so that the bandwidth, using a "hamming" window, is 0·0025 Hz. In each case the autocovariance function was "detrended" using a least-squares approximation in order to remove the effects of drift in the mean intensity level, and the raw time series themselves were prewhitened using a simple difference filter with constant 0·9. Each data point corresponds to an integration time of 2 s within a sampling interval of 5 s (ref. 1). Figs. 1–4 show the resulting power spectra for data points 610–1,109, 810–1,309, 1,010–1,509 and 1,210–1,709, respectively, from which it is clear that the flare immediately preceding has had a pronounced effect on the smaller and more rapid variation.

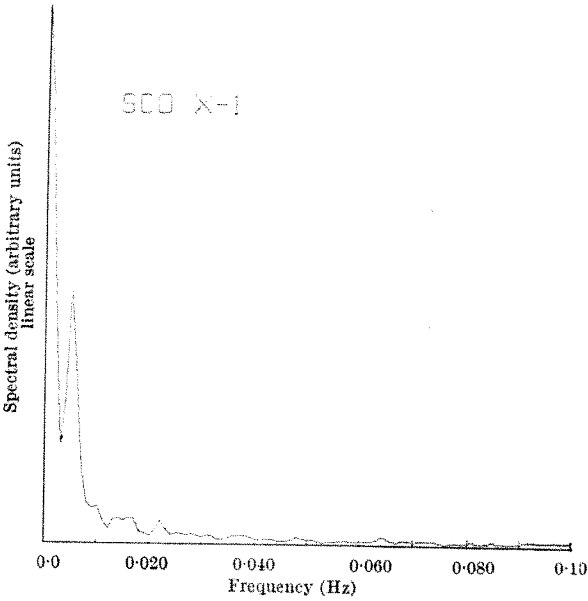


Fig. 2. Power spectrum obtained from data points 810–1,309 (post-flare).

Just after the flare (Fig. 1) we see that a fairly broad band of frequencies centred on 0·006 Hz (corresponding to a

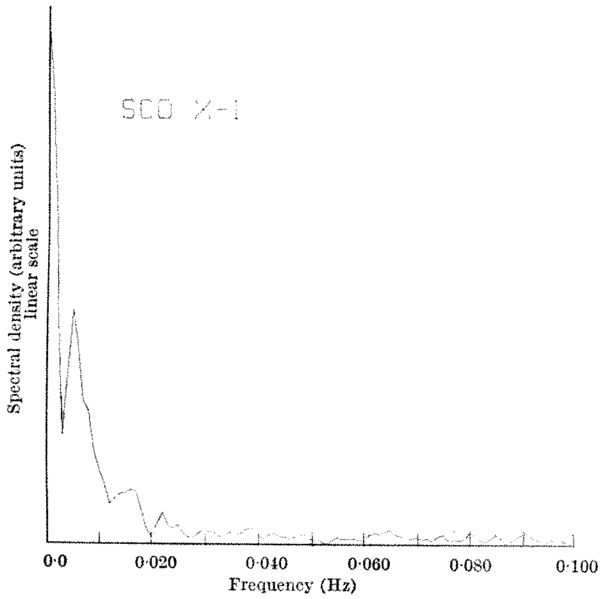


Fig. 1. Power spectrum obtained from data points 610–1,109 (post-flare).

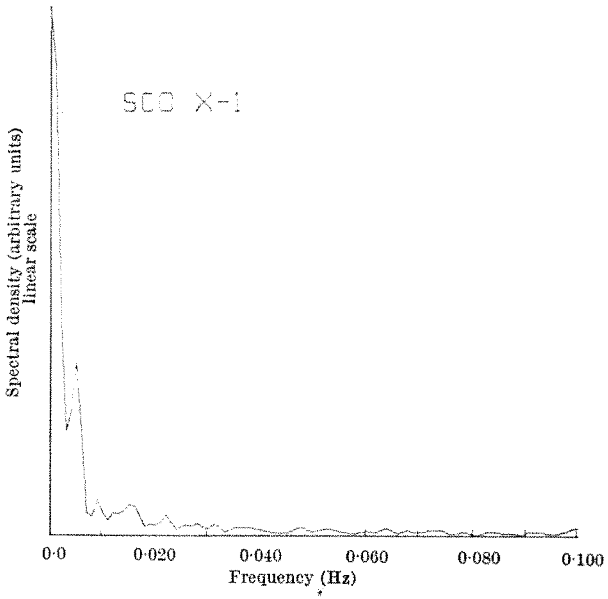


Fig. 3. Power spectrum obtained from data points 1,010–1,509 (post-flare).

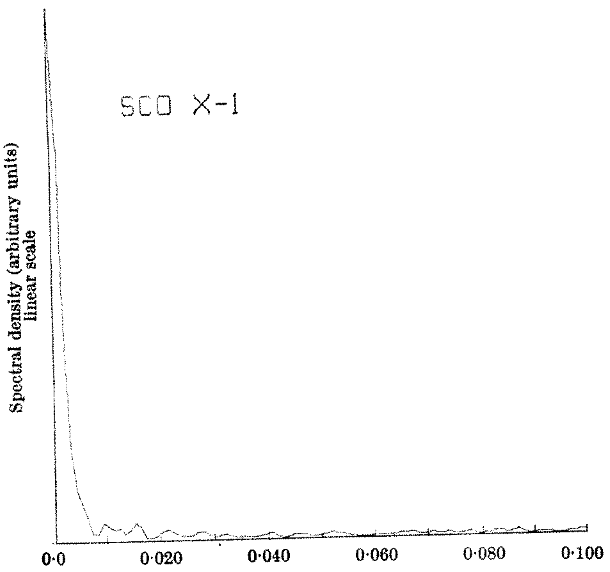


Fig. 4. Power spectrum obtained from data points 1,210–1,709 (post-flare).

period of ~170 s) has been excited. Shortly after this (Fig. 2) the height of the peak at 0·006 Hz is even greater, but it is rather more sharply defined, suggesting a relaxation of the periodic variation into a "fundamental" mode in the absence of any continuous excitation. Subsequently, the energy associated with this variation is rapidly dissipated (Fig. 3) until some 1,000 data points (~80 min) after the flare it is no longer detectable (Fig. 4), and the power spectrum has the same overall appearance as that for data points 1–500 immediately preceding the flare (Fig. 5). The largest peaks (comparing with Figs. 1 and 2) seem to represent an oscillation with amplitude ~1 per cent of the mean optical intensity of the source at this time, because a calibration sinusoidal variation of this magnitude superimposed on the original time series produces a comparably sized peak in the power spectrum.

It is not clear how energy is transferred from the flare to produce periodic flickering, so discussion of possible models to account for this behaviour must await completion of the more detailed study of the optical variation of Sco X-1 currently being prepared. Here we wish only to emphasize the reality of the large peak at 0·006 Hz, the height of which above the background noise may be estimated from comparison with Figs. 4 and 5, where this peak is totally absent.

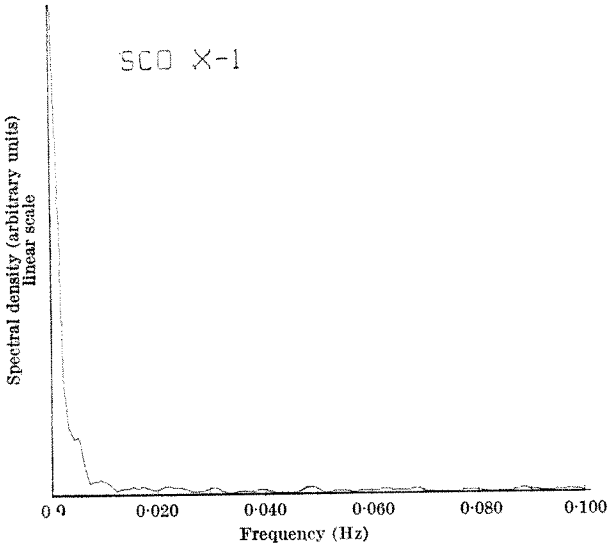


Fig. 5. Power spectrum obtained from data points 1–500 (pre-flare).

We thank Drs J. Kristian and A. Sandage for providing their photometric observations of Sco X-1 as a deck of punched cards, and Professor J. W. Tukey for discussions concerning details of the power spectrum analysis. J. R. G. holds an Isaac Newton studentship from the University of Cambridge and a minor studentship from University College, Cambridge.

J. R. Gribbin
P. A. Feldman
S. H. Plagemann

Institute of Theoretical Astronomy,
Madingley Road,
Cambridge.

Received March 2, 1970.

[1] Sandage, A., Westphal, J. A., and Kristian, J., *Astrophys. J.*, **156**, 927 (1969).
[2] Westphal, J. A., Sandage, A., and Kristian, J., *Astrophys. J.*, **154**, 139 (1968).
[3] Seeds, M. A., *Astrophys. J. Lett.*, **159**, L121 (1970).
[4] Plagemann, S. H., Feldman, P. A., and Gribbin, J. R., *Nature*, **224**, 875 (1969).

Improved Parameters for Seven Pulsars

Positions, pulse periods and mean pulse energies are important parameters of pulsars. For many pulsars, particularly those discovered at the Molonglo Radio Observatory, the right ascension measurement has small error limits while the declination is not well determined. Observations at 410 MHz using the 300 foot transit telescope of the National Radio Astronomy Observatory have therefore been undertaken to determine more accurate declinations and periods for several pulsars. Mean pulse energies at 410 MHz have also been measured.

The observations were made during the period November 17–23, 1969, using a dual channel receiver with two feeds spaced by one beamwidth (35′ arc) in declination. The feeds, which were linearly polarized with position angle 90°, could be displaced off axis in the east–west direction, allowing the source to be tracked for up to 40 min. The detected outputs from the two channels were continuously sampled at 5 ms intervals by a digital computer and stored on magnetic tape. The i.f. bandwidth was 5·0 MHz for low dispersion pulsars and 0·5 MHz for those pulsars where the dispersion broadening was greater than 5 ms at 5·0 MHz bandwidth.

In subsequent analysis the data were averaged in 2 min blocks and the pulse profile obtained for each 2 min average. The source declination was calculated from the ratio of pulse amplitude in the north and south beams. Pulse arrival times were determined by fitting a mean pulse profile to each 2 min average. These arrival times were reduced to the barycentre, using an ephemeris generated at Lincoln Laboratory, Massachusetts Institute of Technology, and kindly supplied by Dr I. I. Shapiro, and periods calculated by making a least squares fit of a straight line to the data. Mean pulse energies were determined by averaging all data obtained, usually recorded on four or more days with 20 to 40 min of data each day. The effective pulse width, defined as the pulse energy divided by peak flux density, was also calculated.

Results for pulsars 0031 – 07 (ref. 1), 1237 + 25 (ref. 2), 1706 – 16 (ref. 3), 1818 – 04 (ref. 4), 1911 – 04 (ref. 3), 2022 + 51 (ref. 5) and 2218 + 47 (ref. 6) are given in Table 1. Right ascensions are taken from the original references. Periods are given in units of atomic (A.1) seconds and errors quoted are the sum of the r.m.s. timing errors

Table 1. PARAMETERS OF SEVEN PULSARS

PSR	R.A. (1950·0)				Dec. (1950·0)		Period (A.1) s	Error (ns)	Mean residual (ms)	Mean energy (10^{-26} J m^{-2} Hz^{-1})	Effective pulse width (ms)
	h	m	s	s	°	′ ″					
0031 – 07	00	31	37	± 1	– 07	37·0 ± 3·0	0·942 950 780	11	2·90	0·045	60
1237 + 25	12	37	17	± 10	25	11·5 ± 2·0	1·382 448 573	58	0·85	0·07	44
1706 – 16	17	06	35	± 2	– 16	37·0 ± 3·0	0·653 050 423	12	1·24	0·02	14
1818 – 04	18	18	14	± 1	– 04	28·0 ± 3·0	0·598 072 601	12	0·60	0·095	19
1911 – 04	19	11	15	± 1	– 04	47·0 ± 3·0	0·825 933 659	15	0·76	0·16	14
2022 + 51	20	22	20	± 60	51	44·5 ± 2·0	0·529 195 240	67	1·33	0·09	26
2218 + 47	22	18	18	± 60	47	40·0 ± 2·5	0·538 467 383	37	0·59	0·055	12

and errors resulting from position uncertainties in right ascension and declination, these errors being considered independent. In all cases the quoted errors are dominated by the position uncertainty. The mean timing residual, that is, the mean of the unsigned deviations of observed arrival times from the fitted straight line, is also given in Table 1.

The National Radio Astronomy Observatory is operated by Associated Universities, Inc., under contract with the US National Science Foundation.

R. N. MANCHESTER

National Radio Astronomy Observatory,
Green Bank, West Virginia.

Received February 16, 1970.

[1] Large, M. I., Vaughan, A. E., and Wielebinski, R., *Astrophys. Lett.*, **3**, 123 (1969).

[2] Lang, K. R., and Bosque, B., *IAU Circ. No. 2137* (1969).

[3] Large, M. I., Vaughan, A. E., and Wielebinski, R., *Nature*, **223**, 1249 (1969).

[4] Vaughan, A. E., Large, M. I., and Wielebinski, R., *Nature*, **222**, 963 (1969).

[5] Davies, J. G., *Nature*, **224**, 214 (1969).

[6] Taylor, J. H., and Huguenin, C. R., *Nature*, **221**, 816 (1969).

X-ray Scattering by Grains in the Direction of the Crab Pulsar and Sco XR-1

MOST of the X-ray emission from the Crab Nebula is from a diffuse cloud approximately 100″ in size[1,2], and about 10 per cent of the total X-ray flux is in pulsed form[3,4], presumably from the optical pulsar. Slysh[5] suggests that all of the X-ray flux originates in pulsed form at the pulsar and that scattering by grains washes out the time coherence and produces the observed angular size. This article is a discussion of the hypothesis of Slysh from the observational point of view.

Three parameters are needed to specify the X-ray scattering from grains: the number of scattering grains, N; the average size of these grains, a_0; and the grain material. X-rays undergo Thompson scattering by electrons in the grains, and the grain material specifies the number density of electrons. This is roughly similar for most possible materials, and, so for a first order approximation, we need only specify N and a_0. The observational data give us the ratio of scattered to unscattered photons and the scattering angle of the radiation. In principle, then, both N and a_0 can be determined.

In order to scatter 90 per cent of the source flux as required by the pulsar source hypothesis, the X-ray scattering depth, τ_X, must be 2·4. Overbeck[6] shows that for a given wavelength, λ, essentially all the scattered X-rays fall within a cone with half angle $\theta \simeq \theta_c/2$, where $\theta_c = \lambda/a_0$. The data on the size of the X-ray source in the Crab[2] can be used to find the average grain size once the effective wavelength of the measurement is known. Using parameters appropriate to this measurement, we have determined this wavelength from the relation

$$\langle E \rangle = \frac{\int E[\varepsilon(E)S(E)E^{-2}]\mathrm{d}E}{\int[\varepsilon(E)S(E)E^{-2}]\mathrm{d}E} \qquad (1)$$

where $\varepsilon(E)$ is the detection efficiency of the counter, $S(E)$ is the source spectrum of the Crab Nebula, and the factor E^{-2} accounts for the energy dependence of the efficiency of grain scattering. We find that $\langle E \rangle \simeq 5$ Å, and hence that $a_0 = 1$ µm. This is almost seven times the average grain size required to produce the scattering law which is observed in the visible[7].

A second test of the Slysh hypothesis uses the source position obtained on the same flight. To first order, X-rays scattered by a cloud would appear to be distributed with circular symmetry about the central source. As Overbeck points out, however, the alignment of elongated grains might produce an effect similar to Fraunhofer diffraction which would destroy this symmetry. Further, nonuniformities in the distribution of grains (say, by the chance occurrence of a cloud edge coinciding with the line of sight of the source) would move the centroid of the X-ray distribution away from the line of sight of the source. We have not tried to evaluate the effect of elongated grains because of our poor knowledge of grain shapes. We have, however, evaluated the effect of an off-axis distribution of grains on the position of the X-ray centroid. Observationally, Oda *et al.*[2] concluded that the X-ray centroid of the Crab was displaced 22″ from the position of the optical pulsar. Because the one sigma observational errors were about of this order, this discussion is perhaps most useful as an indication of the magnitude of the expected effect.

Suppose that along the line of sight to the X-ray source there is a mean number of clouds per unit volume, N, each having a radius s. We are concerned with cloud edges which lie within some angle θ to the line of sight for which each cloud at a distance r contributes an annular cross-section of $4\pi r\theta s$. The expected number of edges intercepted is the integral of the probability cross-section along the line of sight

$$N = \int_0^R 4\pi r\theta s\, N\mathrm{d}r = 2\pi s\theta\, NR^2 \qquad (2)$$

Using $s = 7·5$ pc and $N = 3 \times 10^{-5}$ pc^{-3} (ref. 8), we find the most probable number of edge intercepts is 0·6 for $\theta = 22''$ and $R = 2$ kpc. Variations about the mean occur and are given by Poisson's probability law. For our case, the probabilities of zero, one and two edges are 0·55, 0·33 and 0·10. More than two edges have a probability of occurrence of only 0·02.

If the mean visual absorption per cloud is 0·25 magnitudes[8], then there are about eight clouds in the line of sight to the Crab. Hence, an average cloud will scatter at most 11 per cent of the source flux, while even fewer X-rays will be scattered if the cloud fails to fill the cone of sight. Consequently, one cloud edge cannot displace the observed brightness centroid by more than 11 per cent of the 22″ angle within which scattering is appreciable. Two edges could produce a 22 per cent displacement but only if they were aligned. The net result is a displacement of only two or three arc seconds, which cannot explain the off-axis displacement observed.

X-ray data on Sco XR-1 are sufficiently precise to be applied to a discussion of the Slysh hypothesis. Both τ_X and the visual absorption, A_V, are proportional to the number of intervening grains. Assuming that the grains are similar in the direction of the Crab and Sco XR-1, we can form the relation:

$$\tau_X(\text{Sco XR-1}) = \tau_X\,(\text{Crab})\,\frac{A_V\,(\text{Sco})}{A_V\,(\text{Crab})} \qquad (3)$$

We have already concluded that τ_X (Crab) is 2·4 if the pulsar source hypothesis is valid. For the Crab A_V falls in the range from 1·6 to 1·9 (ref. 9). Values obtained for the extinction in the direction of Sco XR-1 vary from a low of 0·39 based on Eggen's work[10] to an upper limit of 2 deduced by Johnson[11]. We are rather sceptical of the lower value. It has long been known that the Upper Scorpius region is heavily reddened. The extensive data of Gutierrez-Moreno and Moreno[12] indicate that at a distance modulus of 6·0 to 6·5 in the general direction of Sco XR-1, the colour excess will be larger than 0·28. We attach greatest weight to the work of Sandage *et al.*[13], who concluded from an analysis of the reddening of seven stars within 9′ of Sco XR-1 that the excess of colour is approximately 0·23 over the distance interval between 100 and 400 pc. We conclude that τ_X (Sco XR-1) as determined from equation (3) will fall between 0·9 and 1·05.

To compare this result with data on the size of Sco XR-1 (ref. 2), we generate a synthetic X-ray distribution with 55 per cent of the flux scattered and the remainder in the line of sight corresponding to $\tau_X = 0{\cdot}9$. The angular distribution of the scattered radiation can be obtained from Overbeck's curves once the characteristic scattering angle is determined. Evaluating relation (1) using the spectrum of Sco XR-1 and with the appropriate instrumental parameters, and choosing $a_0 = 0{\cdot}2$ μm for compatibility with optical reddening data, we find $\theta_c \simeq 8{\cdot}5'$. We then sum the direct and scattered components of the flux and compare the resultant distribution with the experimental results. As opposed to the instrumental width of about 78″ which was observed, this distribution would have been detected as an extended source with a diameter of more than 160″. Repeating this procedure for a number of different X-ray scattering depths, we find that with τ_X (Sco) as low as 0·6, the effect of scattering would still have been detected. We therefore conclude that the X-ray scattering hypothesis for the Crab is at variance with the X-ray data obtained on Sco XR-1.

The final point considered in this analysis was the amount of optical reddening that would be produced in the direction of the Crab Nebula by those grains needed to produce the required X-ray scattering. We first picked a grain material and characteristic size distribution. Using Overbeck's work, we then derived the number of these grains necessary to produce the required X-ray scattering. Using these parameters with the work of Van de Hulst[14], we then computed the amount of optical extinction. For ease of computation, a size distribution of the form $\mathrm{d}n/\mathrm{d}a \sim 1/a_0 \exp\,(-a^2/2a_0^2)$ was used which is similar but not identical to typical distributions discussed by Greenberg[7]. This procedure was repeated for a range of average grain sizes for each of a number of different grain materials. In Fig. 1 we have plotted A_V for the required number of grains against the average grain size for several different materials. We have used one composite grain: a graphite core 0·05 μm in radius surrounded by an ice mantle of sufficient thickness to produce the grain size indicated. We also computed the reddening for single-sized grains of SiO_2 to determine the effect of a grain distribution on the optical reddening. We have drawn a line on this plot at $A_V = 1{\cdot}9$ which corresponds to the largest reddening in the range cited earlier for the Crab Nebula. The reddening predicted for all grain materials except iron is in clear violation of the observations for all reasonable grain sizes. Iron grains with an average size of 0·3 μm will fit the observations, but few would consider this a viable grain model. We conclude that the optical effects of any grains sufficient to produce the observed X-ray scattering are incompatible with the reddening data on the nebula. This is unquestionably the strongest argument presented in this discussion.

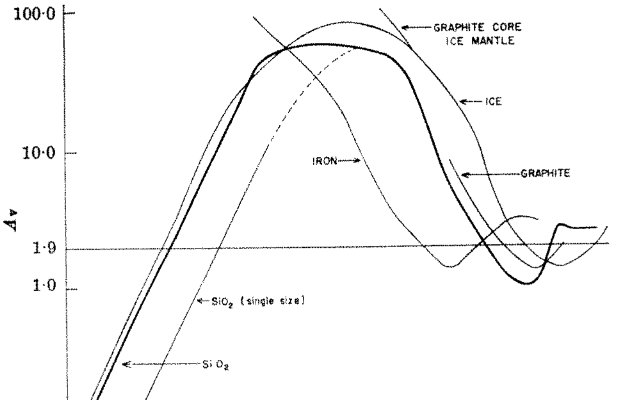


Fig. 1. Optical extinction in the direction of the Crab Nebula plotted against average grain size for those grains required to produce the observed X-ray effects from the pulsar. An upper limit to the extinction which is observed is indicated by a line at $A_V = 1{\cdot}9$.

Intuitively one might hope that X-ray scattering would be dependent on smaller grains than optical scattering and hence could provide data on the distribution of sizes of grains which is now unattainable. X-ray scattering efficiencies do indeed become larger than optical scattering efficiencies for grain sizes smaller than about 0·005 μm. The X-ray scattering cross-section per gram of grain material, however, is proportional to the grain radius for small grain sizes. Hence with 0·005 μm grains, fifty times more material would be required to produce scattering equivalent to that produced by 0·25 μm grains.

We have examined the relatively crude data which are now available on Sco XR-1 to see what information they can give about the larger grains. To do this, we first use the optical data available on Sco XR-1 with the relationship $A_V = 1{\cdot}086\ \tau_V = 1{\cdot}086\ NQ_V\pi a_0^2$ to obtain a value of N in terms of a_0. Here we use a value of 3 for Q_V, the optical scattering efficiency, in anticipation of our final result. We then use this N with the upper limits on τ_X obtained previously to obtain an upper limit on the value of σ_X. Van de Hulst[14] has shown that for X-rays the scattering efficiency is given by

$$Q_X = \frac{\sigma_X}{\pi a_0^2} = \frac{\rho^2}{2} = \frac{(2a_0\rho_e r_e\lambda)^2}{2}$$

where ρ is the phase shift across the grain, ρ_e is the number density of electrons in the grain and r_e is the classical electron radius. Our derived upper limit to σ_X can then be used in this formalism to obtain an upper limit on a_0 once we specify the grain material. Using Q_X (effective) $= 2Q_X(a_0)$ which is appropriate for X-rays, we find $a_0 \leq 1{\cdot}6$ μm for ice particles and $a_0 \leq 0{\cdot}13$ μm for iron grains. Hence although the existing X-ray data are clearly not sufficient to affect our present ideas concerning grains, we are not far from the point where this work will directly impact this topic.

Clearly much information about interstellar grains will be obtained from observations of those X-ray objects with optical counterparts in which both reddening and X-ray scattering can be observed. In principle there will be more observational data (τ_X, θ_c, A_V, and reddening as a function of wavelength) than grain parameters to be specified (N, a_0 and composition). Unfortunately, for most grain materials the optical scattering efficiency is between five and ten times larger than the X-ray scattering efficiency for grain sizes from 0·1 to 1 μm. Hence most objects in which X-ray scattering is pronounced are likely to be totally obscured in the visible. Multisized grain distributions have, however, been proposed with some components centred around sizes larger than 1 μm. For grains of this size the optical and X-ray scattering efficiencies of most materials are roughly similar and hence this may provide a means of evaluating the multicomponent grain size models proposed.

This work was supported by the National Aeronautics and Space Administration.

C. S. Bowyer

Department of Astronomy and
Space Science Laboratory,

J. Mack
M. Lampton

Space Science Laboratory,
University of California,
Berkeley, California.

Received January 27; revised February 23, 1970.

[1] Bowyer, C. S., Byram, E. T., Chubb, T. A., and Friedman, H., *Science*, **146**, 912 (1964).

[2] Oda, M., *et al.*, *Astrophys. J.*, **148**, L5 (1967).

[3] Fritz, G., Henry, R. C., Meekins, J. F., Chubb, T. A., and Friedman, H., *Science*, **164**, 709 (1969).

[4] Bradt, H., *et al.*, *Nature*, **224**, 728 (1969).

[5] Slysh, V. I., *Nature*, **224**, 159 (1969).

[6] Overbeck, J. W., *Astrophys. J.*, **141**, 864 (1965).

[7] Greenberg, J. M., *Stars and Stellar Systems*, **7** (edit. by Kuiper, G. P., and Middlehurst, B. M.) (Univ. of Chicago Press, 1968).

[8] Allen, C. W., *Astrophysical Quantities* (Athlone Press, London, 1963).

[9] Minkowski, R., *Stars and Stellar Systems*, **7** (edit. by Kuiper, G. P., and Middlehurst, B. M.) (Univ. of Chicago Press, 1968).

[10] Eggen, O. J., *Astrophys. J.*, **158**, L31 (1969).

[11] Johnson, H. M., *Astrophys. J.*, **144**, 635 (1966).

[12] Gutierrez-Moreno, A., and Moreno, H., *Astrophys. J. Suppl.*, **15**, 459 (1968).

[13] Sandage, A. R., *et al.*, *Astrophys. J.*, **146**, 316 (1966).

[14] Van de Hulst, H. C., *Light Scattering by Small Particles* (John Wiley, New York, 1957).

Magnets in Electromagnetic Theory

Two recent communications on electromagnetism[1,2] exemplify the two basic approaches to the problem. It is possible to regard E, D, H and B as labels identifying different properties which it is convenient to distinguish, and in the main this is the approach taken by Rosser[1]. On the other hand, it is possible to look for physical realities corresponding to each of these terms and to examine various relationships to see whether these correspond with "reality". This is basically the approach of Stopes-Roe[2].

The first approach is quite unexceptional but is rarely carried out consistently. It is, for example, usually assumed as *a priori* "fact" that charge is a fixed entity and that the movement of a real charge from one medium to another does not in any way affect the magnitude of the charge. I know of no experiment which proves this convenient assumption. There is no doubt that electrostatic calculations would be very much more difficult in a system in which the magnitude of a charge was a function of the medium (for example, $q=q_0\,\varepsilon^{-1/2}$, ε being the dielectric constant of the medium). This is a sufficiently good reason for defining a charge as being an entity which is independent of the medium.

The corresponding case of the magnetic dipole is rarely, if ever, considered in terms of convenience, but reference is made to hypothetic or real experiments. The results of these experiments are always open to different interpretations and no conclusive proof of one interpretation or another is ever produced. For example, experiments are made with long thin "hard" magnets which it is assumed may be taken to represent accurately the properties of elementary magnetic dipoles. This, however, is open to the objection that this form of magnet is that most influenced by demagnetizing effects. The classical ring magnet with a small gap is least affected by demagnetizing effects.

Experiments made with ring magnets would tend to show that "real" magnets produce a constant flux (constant B), but thin magnets in the same type of experiment will appear to produce a constant H.

It is further assumed that because a current carrying solenoid produces some magnetic effects like those of a permanent magnet then the latter "is" a system of atomic currents.

A solenoid is usually connected to a source to supply the current in it, however. In this case when the external medium is changed (even if the solenoid core is an evacuated enclosure in which the medium does not penetrate) energy is taken from or given to the source.

A permanent magnet, however, has no such external source from which it can draw energy and in this it differs from the hypothetical solenoid.

This objection can be overcome by postulating a superconducting solenoid carrying a current as being the equivalent of a permanent magnet. This concept avoids the problem of interchange of energy with a source but introduces another basic difficulty. A superconducting ring has an infinite current induced in it if the magnetic flux linking with it changes. If, therefore, the medium around such a solenoid is changed the superconductor prevents any change of magnetic flux through it. Compensating changes occur in the current circulating in the solenoid.

This type of solenoid therefore has the property that the magnetic flux linking it is independent of the medium. It is therefore the equivalent of a magnet of constant B (unlike the ordinary constant current solenoid which is the equivalent of a magnet of constant H).

The superconducting solenoid cannot be described by writing its magnetic moment as $m=IA$ because the current I involved is not a constant but is a function of the medium, falling if the permeability of the surroundings increases, and vice versa.

A magnet made up of superconducting elements would probably act more like real permanent magnets than any of the other models proposed. It would be a constant flux magnet (and therefore would be a direct analogue of the constant electrostatic charge which is generally favoured) and its properties would be very similar to those of single-crystal magnetic materials.

If, therefore, it is considered necessary to invent atomic concepts to account for macroscopic behaviour—instead of accepting E, D, H and B as convenient definitions—it seems more logical to take an elementary permanent magnet as a constant flux device and not as a constant H device.

W. F. Lovering

Department of Electrical and Control Engineering,
University of Surrey.

Received November 26, 1969.

[1] Rosser, W. G. V., *Nature*, **224**, 577 (1969).

[2] Stopes-Roe, H. V., *Nature*, **224**, 579 (1969).

Derivation of Rate Equations used in Thermogravimetry

There have been many attempts to extract kinetic parameters, such as energy of activation or order of reaction, from dynamic thermogravimetric data[1-3]. The main reasons for using thermogravimetry (TG) instead of the more traditional isothermal methods are that it is unnecessary to know the time and temperature of the onset of decomposition, and with TG it is possible to use only one set of experimental data to derive the desired parameters. In other methods, several isothermal experiments must be performed at different temperatures to yield a value for the energy of activation. It should be noted, however, that some of the best TG methods (Friedman[4] and Osawa[5]) entail several experiments at different heating rates.

For homogeneous decomposition reactions which evolve volatile products and leave a residue, for example, the thermal decomposition of polymers, the thermobalance results are analysed in the following way

$$\frac{dC}{dt} = k.\ f(C) \tag{1}$$

where (dC/dt) is the rate of fractional conversion, k is defined as the rate constant and $f(C)$ is some function of the fractional conversion, frequently written as $(1 - C)^n$ where n is the apparent reaction order.

k is then related to the temperature (T) and the activation energy (E) by the Arrhenius equation

$$k = A \exp(-E/RT) \tag{2}$$

A is the pre-exponential factor and R the gas constant.

Finally equation (2) is substituted in equation (1) to give

$$\frac{dC}{dt} = A.\ \exp(-E/RT)\ .\ f(C) \tag{3}$$

We believe this step is incorrect. The substitution for k, the "rate constant", can only be made for an equation involving an isothermal rate, that is

$$\left(\frac{dC}{dt}\right)_T = A.\ \exp(-E/RT)\ .\ f(C) \tag{4}$$

It can also be shown that equation (3) is not, as it is generally assumed to be, equivalent to equation (4), except under a special condition. If C is considered to be a function of time and temperature only, we have

$$C = f(t,T) \tag{5}$$

On differentiating,

$$dC = \left(\frac{\partial C}{\partial t}\right)_T dt + \left(\frac{\partial C}{\partial T}\right)_t dT \tag{6}$$

and

$$\frac{dC}{dt} = \left(\frac{\partial C}{\partial t}\right)_T + \left(\frac{\partial C}{\partial T}\right)_t .\beta \tag{7}$$

β is the heating rate (dT/dt). For purposes of comparison with (4), equation (7) may be rewritten as

$$\frac{dC}{dt} = \left(\frac{dC}{dt}\right)_T + \left(\frac{dC}{dT}\right)_t .\beta \tag{8}$$

From equation (8) it can be seen that the thermogravimetric rate (dC/dt) is not equal to the isothermal rate $(dC/dt)_T$ except, of course, when the heating rate (β) is zero (isothermal conditions). Use of equation (3) is therefore an incorrect simplification of the true state of affairs. The correct equation should be

$$\frac{dC}{dt} = A \exp(-E/RT)\ .f(C) + \beta\left(\frac{dC}{dT}\right)_t \tag{9}$$

When $\beta = 0$ in equation (9), (dC/dt) becomes $(dC/dt)_T$ the isothermal rate and the use of equation (3) is permissible.

We have applied several of the more commonly used methods of analysis of programmed thermogravimetry[6] to the thermal degradation of a single polymer in standard conditions. We found very poor agreement for the numerical values of the kinetic parameters evaluated, which suggests that the basic equations may be inaccurate. We therefore consider that only isothermal methods can be used to evaluate the order of the reaction, the pre-exponential factor and the activation energy. From equation (4), it seems that the following method fulfils the requirement of not defining the starting time of the reaction.

Taking logarithms of equation (4) gives

$$\log_e\left(\frac{dC}{dt}\right)_T = \log_e A + \log_e f(C) - \frac{E}{RT} \tag{10}$$

To obtain E, the logarithm of the isothermal reaction rate should be plotted against $1/T$, the reciprocal temperature (K^{-1}), at constant conversion. The measurement of A and n would require an assumption of the value of $f(C)$. The method may be repeated for different conversions, building up a picture of the change of the kinetic parameters with the extent of reaction. This technique needs data from several experiments carried out at different isothermal temperatures.

In examples for which programmed thermogravimetry and isothermal analysis give similar values for the activation energy, it must be concluded that the second term, $\beta\ (dC/dT)_t$, is very small compared with the first and hence negligible error is introduced by neglecting it.

J. R. MacCallum
J. Tanner*

Department of Chemistry,
The Purdie Building,
The University,
St Andrews,
Scotland.

Received December 3, 1969.

---

* Present address: Simon Fraser University, Burnaby 2, BC, Canada.

[1] Flynn, J. H., and Wall, L. A., *J. Res. Nat. Bur. Stand.*, **70A**, 487 (1966).
[2] Doyle, C. D., *Techniques and Methods of Polymer Evaluation*, chap. 4 (Edward Arnold, London, 1966).
[3] Reich, L., and Levi, D. W., *Macromol. Revs.*, **1**, 173 (1967).
[4] Friedman, H. L., *J. Polymer Sci.* (C), **6**, 183 (1964).
[5] Osawa, T., *Bull. Chem. Soc. Japan*, **38**, 1881 (1965).
[6] MacCallum, J. R., and Tanner, J., *Europ. Polymer J.* (in the press).

Liquid Phase Inversion

The phase behaviour of the nitrogen–methane–ethane system in the vicinity of −255° F has been reported in an earlier communication[1]. The system has subsequently been studied at lower temperatures by lowering the freezing point of the isopentane bath liquid with propane gas. It was found that a liquid phase inversion took place between the temperatures −255° F and −263·3° F, the bottom liquid layer being the nitrogen-rich phase at −263·3° F and the ethane-rich phase at −255° F. Binodal curves for the saturated liquids at these two temperatures are shown in Fig. 1, in which BL denotes the bottom layer. The phase inversion temperatures together with the equilibrium liquid–liquid compositions obtained in this investigation are listed in Table 1. In all cases investigated, the nitrogen composition in the equilibrium vapour phase was greater than 97 per cent.

The liquid phase inversion temperature for the binary system nitrogen–ethane was found to be −263·3° F. A similar phase inversion was reported by Schindler, Swift and Kurata[2] for nitrogen–propane at approximately

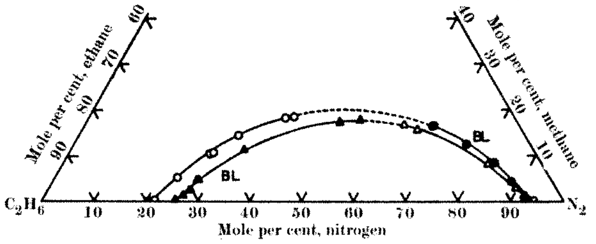


Fig. 1. Binodal curves for the nitrogen–methane–ethane system at two temperatures. ○ ●, −263·3° F; △ ▲, −255° F.

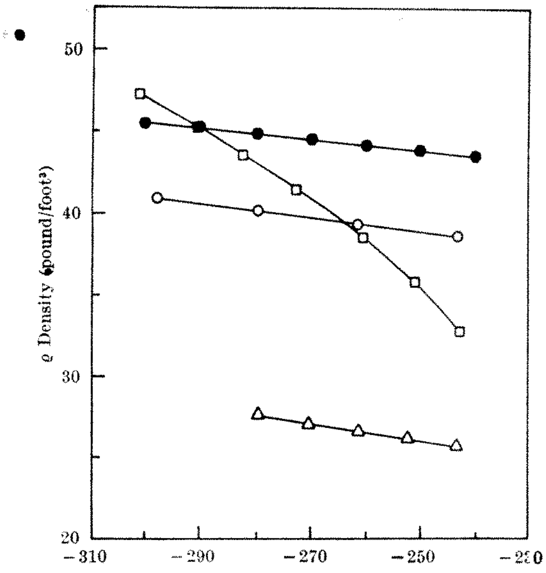


Fig. 2. Liquid densities of pure components. □, Nitrogen; △, methane; ○, ethane; ●, propane.

−292° F. These phase inversions are probably brought about by the decrease of liquid nitrogen density[3] with the increase of temperature taking place faster than for the paraffins (ref. 3 and unpublished results of J. Klosek and C. McKinley, presented at the International Conference on LNG, Chicago, April 1968) as shown in Fig. 2. This figure shows that the density curve for nitrogen crosses that for ethane at about −263° F, while it crosses that for propane at about −292° F.

Table 1. PHASE INVERSION TEMPERATURES AND LIQUID-LIQUID EQUILIBRIUM COMPOSITIONS

Temp. °F	Pressure psia	Liquid phase I (BL) N_2	C_1	C_2	Liquid phase II N_2	C_1	C_2
−263·6	190·0	0·2014		0·7986	0·9453		0·0547
	189·8	0·9238		0·0762	0·2228		0·7772
−258·9	200·8	0·2824	0·0960	0·6216	0·8396	0·0737	0·0867
	200·6	0·8276	0·0747	0·0977	0·2759	0·0938	0·6303
−255·9	208·5	0·3511	0·1487	0·5002	0·7443	0·1258	0·1299
	208·3	0·7392	0·1271	0·1337	0·3401	0·1463	0·5126
−255·4	213·0	0·4829	0·1850	0·3318	0·6324	0·1651	0·2025
	212·3	0·6266	0·1662	0·2072	0·4829	0·1848	0·3223

We reported the formation of a third liquid layer for the system nitrogen–methane–ethane in our previous communication[1]. The results of this investigation indicate that the composition of the top (third) layer is virtually identical with that of the bottom layer, and that there are only two liquid phases present because the relative amount of the top and the bottom layers can be manipulated by a sudden change of the vapour recirculation rate. The formation of the third liquid layer seems to be connected with the liquid phase inversion effect, and could be brought about by slight temperature non-homogeneities in the apparatus in the vicinity of a phase inversion point.

We thank Dr M. B. King of the University of Birmingham for his valuable comments on the manuscript.

BENJAMIN C.-Y. LU
PAUL YU
DAVID P. L. POON

Department of Chemical Engineering,
University of Ottawa,
Ottawa, Ontario, Canada.

Received July 22, 1969; revised January 12, 1970.

[1] Lu, B. C.-Y., Yu, P., and Poon, D. P. L., *Nature*, **222**, 768 (1969).
[2] Schindler, D. L., Swift, G. W., and Kurata, F., *Hydrocarbon Proc. Pet. Ref.* **45**, 205 (1966).
[3] Din, F., *Thermodynamic Functions of Gases*, **3**, 139 and 172 (Butterworths, London, 1961).

Pyrolysis of Early Pre-Cambrian Onverwacht Organic Matter ($>3\times10^9$ yr old)

CARBONACEOUS matter and microstructures of unknown origin in the oldest known sedimentary rocks (part of the Onverwacht Series in South Africa; $>3\cdot0\times10^9$ yr old) have created considerable interest (unpublished work of B. N., L. A. Nagy, C. G. Engel, A. E. J. Engel, G. O. W. Kremp and C. M. Drew (1967), and refs. 1–5). Because these rocks may be as old as $\sim3\cdot5\times10^9$ yr, while the generally accepted age of the Earth is $4\cdot5\times10^9$ yr, carbon compounds in the Onverwacht Sediments may be derived from some of the first living matter on Earth or perhaps from prebiological substances. Most sedimentary rocks are porous, and even the Onverwacht carbonaceous and argillaceous chert has been found to exhibit a small degree of liquid permeability[6]. Part of the soluble organic compounds could therefore have been brought in and deposited in the Onverwacht by percolating solutions at a later geological age. But, on the other hand, the insoluble organic polymeric substance, or kerogen, seems to be basically indigenous and most of it, if not all, was deposited with the rocks in Onverwacht times. We have therefore analysed the Onverwacht kerogen, hoping that this might elucidate the nature of the carbon compounds that were present on the Earth $\sim3\cdot5\times10^9$ yr ago.

The Onverwacht Series of rocks are part of the Swaziland System of the Barberton Mountain Land in South Africa. It consists basically of mafic, ultramafic and felsic lavas and pyroclasts with subordinate amounts of various sediments, such as cherts, shales and carbonate rocks. The overlying (younger) Fig Tree and Moodies series are composed mostly of sedimentary rocks. The Onverwacht has suffered considerable metamorphism in places, while at other locations the metamorphic overprint appears to be less severe. Recent work by Viljoen and Viljoen[7] led to the subdivision of the Onverwacht into six formations: The Sandspruit (oldest), Theespruit, Komati, Hoogenoeg, Kromberg and Swartkoppie (youngest), and they found that the Onverwacht is 50,000 feet thick stratigraphically. One of the sedimentary rock samples analysed in our study came from the upper part of the 6,200 foot thick Theespruit formation which overlies the 7,000 foot thick basal Sandspruit. This is a sample of the oldest known sediment on Earth. Other sediments analysed came from the upper Onverwacht, (the upper and middle portions of the 6,300 foot thick Kromberg formation), and one sample came from the overlying Fig Tree Series.

Finely ground samples, 100–200 mg, were pyrolysed in a modified Hamilton pyrolysis unit for 5 min at 500° C under a stream of helium (~5 ml./min). The pyrolysis unit consisted of a quartz tube, externally heated to 500° C, followed by an oven and heated transfer line maintained at 200° C. The heated transfer line was directly connected to a Perkin-Elmer model 226 gas chromatographic unit which in turn was connected through a Watson-Biemann molecular separator to a Hitachi RMU-6E single-focusing mass spectrometer. The heated transfer line from the pyrolysis unit to the gas chromatograph contained a liquid nitrogen trap in which the products accumulated during pyrolysis but which did not interfere with the helium flow. After pyrolysis, the trap was rapidly heated to 250°–300° C in an adjustable oven which resulted in the instantaneous introduction of the products into the gas chromatograph. Reproducibility of the runs was good. Most runs were made on a polyphenyl ether-coated capillary column (50 feet × 0·02 inch internal diameter), others were also run on Apiezon L (100 feet × 0·02 inch internal diameter) and Carbowax K-20*M* (50 feet × 0·02 inch internal diameter) capillary columns.

Simmonds *et al.*[8] have shown by a rather different technique that pyrolysis can be used to study early

Pre-Cambrian sedimentary organic matter; they used a Fig Tree rock sample. Fig. 1 shows gas chromatographic traces of the pyrolysis products of the Onverwacht and Fig Tree sediments from our study. These traces represent the total pyrolysable organic contents of the rocks, including all soluble organic matter; the latter, however, seems to be present in trace quantities only. Elementary analyses showed that these Onverwacht samples contained about 1 per cent total carbon, but available data suggest that the soluble organic substances in these rocks were in the <1 p.p.m. range. Table 1 shows the compounds that have been identified by mass spectrometry in the four samples shown in Fig. 1.

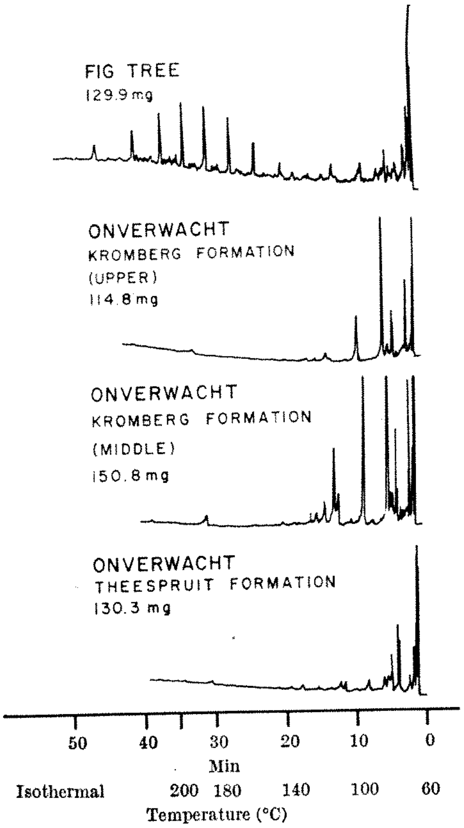


Fig. 1

Fig. 1 and Table 1 indicate that the Onverwacht samples contain mainly aromatic degradation products of the kerogen, while the Fig Tree sample shows an abundance of *n*-alkanes, which are usually thought to be of biological origin. It has been shown, for example, by Levy and Paul[9], that *n*-alkanes are not substantially degraded by the experimental conditions used in this pyrolysis study. It is difficult to account for an aromatic-type kerogen from biological sources in early Pre-Cambrian times, long before the appearance of the abundant aromatic biochemical, lignin. However tempting it may be to suggest that life first arose in Fig Tree times, it is not possible to draw this conclusion until samples from the entire Fig Tree and Onverwacht have been studied and compared, and, most important, until the relative metamorphic effects (which may lead to aromatization) on the Fig Tree and Onverwacht strata are compared and evaluated. In this context the microstructures found in the Onverwacht are morphologically better developed than those in the Fig Tree Series studied so far; the latter are thought by some investigators to be fossil algae. In conclusion, the origin of the aromatic kerogen is still unknown, although it has been suggested[10] that such a composition might be derived from living systems.

Table 1

Saturated hydrocarbons	Mono-unsaturated hydrocarbons	Poly-unsaturated hydrocarbons	Aromatic hydrocarbons	Hetero-atomic compounds
		Fig Tree		
Methane	Ethylene	C_5 Diene	Benzene	CO_2
Ethane	Propylene	C_6 Diene	Toluene	COS
Propane	2 Butenes	C_7 Diene	Ethyl benzene	SO_2
Butane	4 Pentenes		*o*, *m* and *p*-Xylene	CS_2
Pentane	2 Hexenes		Styrene	Water
Hexane	2 C_7 Alkenes		3 C_3 Alkyl benzenes	Methanol
C_7 branched	6 C_8 Alkenes		Methylstyrene	Thiophene
Heptane	5 C_9 Alkenes		3 C_4 Alkyl benzenes	
C_8 branched	2 C_{10} Alkenes		3 C_5 Alkyl benzenes	
Octane	C_{11} Alkene		3 C_6 Alkyl benzenes	
C_9 branched			Naphthalene	
Nonane			2 C_7 Alkyl benzenes	
Decane			2-Methyl naphthalene	
Undecane			1-Methyl naphthalene	
Dodecane			Diphenyl	
C_{13} branched				
Tridecane				
2 C_{14} branched				
Tetradecane				
3 C_{15} branched				
Pentadecane				
3 C_{16} branched				
Hexadecane				
2 C_{17} branched				
Heptadecane				
3 C_{18} branched				
Octadecane				
Pristane				
Phytane				
2 C_{19} branched				
Nonadecane				
		Onverwacht		
Methane	Ethylene	C_5 Diene	Benzene	CO_2
Ethane	Propylene	C_6 Diene	Toluene	SO_2
Propane	2 Butenes		Ethylbenzene	Water
Butane	2 Pentenes		*o*, *m* and *p*-Xylene	Methanol
Nonane	1 Hexene		3 C_3 Alkyl benzenes	Acetaldehyde
Decane	1 Heptene		1 C_4 Alkyl benzene	Acetone
Undecane	4 Octenes		Naphthalene	Thiophene
			1- and 2-Methyl naphthalene	1- and 2-Methyl thiophene
			Diphenyl	Ethanenitrile

We thank Mrs Lois A. Nagy and Mrs Alice Lumpkins for their assistance in the experiments, Dr Harold C. Urey of the University of California for his interest and advice, and Dr D. A. Pretorius of the University of the Witwatersrand for discussions of the Onverwacht geological problems. This investigation was sponsored by NASA.

WARD M. SCOTT
VINCENT E. MODZELESKI
BARTHOLOMEW NAGY

Department of Geochronology,
University of Arizona,
Tucson, Arizona 85721.

Received December 22, 1969.

[1] Nagy, B., and Urey, H. C., *Eighth COSPAR Meeting, Committee on Space Research Established by the International Council of Scientific Unions, Reprint No. L.2.6 Tokyo, Japan* (May 9–21, 1968).

[2] Nagy, B., Nagy, L. A., Bitz, M. C., Engel, C. G., and Engel, A. E. J., *Abstracts, Fourth Internat. Meeting Organic Geochem.*, 23 (Amsterdam, 1968).

[3] Engel, A. E. J., Nagy, B., Nagy, L. A., Engel, C. G., Kremp, G. O. W., and Drew, C. M., *Science*, **161**, 1005 (1968).

[4] Nagy, B., and Nagy, L. A., *Nature*, **223**, 1226 (1969).

[5] Schopf, J. W., *Biol. Rev.* (in the press).

[6] Nagy, B., *Geochim. Cosmochim. Acta* (in the press).

[7] Viljoen, M. J., and Viljoen, R. P., in *African Magmatism and Tectonics* (edit. by Clifford, T. N., and Glass, I.) (Oliver and Boyd, Edinburgh, 1969).

[8] Simmonds, P. G., Shulman, G. P., and Stembridge, C. H., *J. Chrom. Sci.*, **7**, 36 (1969).

[9] Levy, E. J., and Paul, D. G., *J. Gas Chromatog.*, **5**, 136 (1967).

[10] Brooks, J., and Shaw, G., *Nature*, **223**, 756 (1969).

BIOLOGICAL SCIENCES

Observations on the Primary Structure of the 23S Ribosomal RNA from *E. coli*

An understanding of the primary structures of the large ribosomal RNAs should lead to greater insight into the structure of ribosomes and the way in which they work. Nucleotide sequences within the 16S RNA of *Escherichia coli* have recently been studied[1], but little is known about the primary structure of the 23S RNA (refs. 2–5). Two sorts of observations have been reported, however, which might be of general significance in considering the structure and evolution of this molecule: (*a*) The 23S and 16S ribosomal RNAs of *E. coli* have been found to compete with each other in hybridizing with *E. coli* DNA (refs. 6–9). This would indicate that they share some nucleotide sequences in common. (*b*) It has been suggested that the nucleotide sequences in the neighbourhood of the methylated nucleotides each occur twice within the 23S RNA (refs. 2, 3), implying that sequence repetition could be widespread.

One of us[3] has already pointed out that fingerprints of the T_1 ribonuclease digestion products of 16S and 23S RNAs are very different, thus indicating dissimilar primary structures. The sequences of the three largest methylated oligonucleotides[2,3] and of the 3′-terminal decanucleotide[4] of the 23S RNA display no striking homologies with any of the products of digestion with T_1 ribonuclease from the 16S RNA (ref. 1). Inspection of the fingerprints of the 16S and 23S RNAs reveals, moreover, that a greater variety of large oligonucleotides are released from the 23S RNA than from the 16S RNA, by the action of T_1 ribonuclease. The 23S RNA is twice as large as the 16S RNA (ref. 10), and has a very similar composition[11], so it is unlikely to consist of two similar or identical halves[2,9].

We have attempted to resolve these questions by studying the nucleotide sequences of a further twenty-four products arising from digestion of the molecule with T_1 ribonuclease. We have determined the complete nucleotide sequences of sixteen of these products. The sequences of the remainder were sufficiently defined to be useful for purposes of comparison with 16S RNA sequences. We have also determined the relative yield of these oligonucleotides after digestion of the 23S RNA.

We have used the methods for fractionating radioactive oligonucleotides introduced by Sanger *et al.*[12] (Fig. 1). A fingerprint of the digestion products is shown in Fig. 1, and nucleotide sequences are listed in Table 1, together with suggested frequencies of occurrence within the molecule.

On the basis of their nucleotide sequences and frequencies (see below), these products contain more than 300 nucleotides, corresponding to about 10 per cent of the molecule. Presumably these products arise from a series of points widely spread through the polynucleotide chain. If any parts of the 23S RNA sequence resemble sections of the 16*S* RNA, at least a few of these products would be expected to display significant nucleotide sequence homologies with the T_1 ribonuclease products arising from the 16S RNA (ref. 1). In fact, there seem to be no significant homologies with any of the 16S RNA sequences. We believe therefore that the inferences drawn from the hybridization studies are incorrect, and that the 23S and 16S ribosomal RNAs are genetically unrelated.

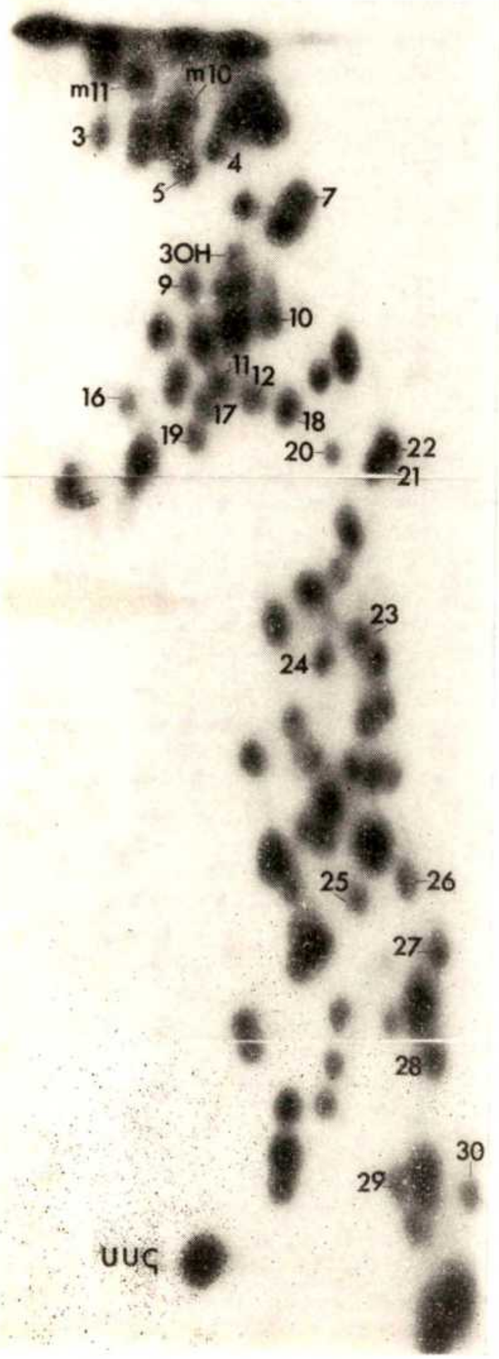


Fig. 1

Fig. 1. Fractionation of a T_1 ribonuclease digest of the 23S RNA. ^{32}P-labelled 23S RNA was prepared from *E. coli* MRE 600 in the way previously described[3]. It was digested with T_1 ribonuclease and bacterial alkaline phosphatase[3,14]. About 0·05 mg of the digest (specific radioactivity, 6 mCi/mg) was fractionated by high voltage electrophoresis on cellulose acetate (5 per cent acetic acid—7 M urea, direction right to left) and then on DEAE-paper (7 per cent formic acid, top to bottom), in the way described by Sanger *et al.*[12]. To study some of the larger oligonucleotides it was necessary to carry out electrophoresis for a long time in the second dimension (with the oligonucleotide UUG migrating about 75 cm from the origin) to obtain satisfactory separations of the large numbers of products. From their appearances on the fingerprints, we thought a considerable number of the spots were likely to be pure. From these we selected a group of spots at random, to examine further. Nucleotide sequence analysis of these products was carried out using the techniques described by Sanger and his colleagues[12,14]. The products resulting from treatment of the spots with pancreatic ribonuclease and venom phosphodiesterase were determined. In most instances it was necessary also to carry out partial digestion with venom phosphodiesterase to determine the complete nucleotide sequence. We used the following conditions: 0·01 ml. of a venom phosphodiesterase solution (0·02 mg/ml. enzyme; 0·02 M Tris, *p*H 8·5; 0·01 M Mg^{2+}); digestion for 10 min at room temperature. The products were fractionated on DEAE-paper at *p*H 1·9. These products were characterized by hydrolysis with pancreatic ribonuclease, venom phosphodiesterase or alkali. Those oligonucleotides which were incompletely analysed generally did not digest well in these conditions. The molar ratios of the oligonucleotides were determined by cutting out the spots and determining their radioactivities by liquid scintillation counting. Two well resolved fingerprints derived from different preparations of the 23S RNA were used for this. The mean values are reported in Table 1. The differences between the two determinations were generally less than 10 per cent.

Our quantitative studies show that in T_1 ribonuclease digests the methylated products m10 and m11 arise in twice the amounts of many of the other oligonucleotides, including the presumed 3′-terminal fragment (3OH). Two other oligonucleotides (3 and 24) are present in similar quantities to the methylated products, and spot 15 is probably also released in equimolar amounts with these products. Spots 5 and 18 display a sequence homology of ten nucleotides, interrupted by a single pyrimidine change. The chance of encountering this in such a study is somewhat less than 1 : 1,000 (see footnote to Table 1). Spots 20, 27 and 28 occur in small amounts relative to the other products, perhaps because of point ambiguities within the molecule at these positions.

Table 1. NUCLEOTIDE SEQUENCES AND SUGGESTED MOLAR FREQUENCIES OF SOME T_1 RIBONUCLEASE PRODUCTS FROM THE 23S RIBOSOMAL RNA

Spot	Sequence	Observed molar ratio	Suggested molar frequency
	Complete sequences		
m10*	GACAUAUm^{1}GΨTG	2·00	2
m11*	GAAATUCCUUG	1·8	2
3OH*	GCUUAACCUU	0·8	1
3	GUUAAUUG	1·8	2
5	GUUCUCCCUG	1·2	1
9	GUAAUAUG	1·3	1
10	GCUAAUCCUCG	1·2	1
11	GUAUCCUCG	1·1	1
12	GUCCCUUG	1·3	1
15	GAAAUAAUG	1·7	2 ?
16	GUUUAG	1·3	1
18	GUUCUCCCCG	0·9	1
19	GUUCCUG	1·3	1
21	GACUUACCAACCG	0·9	1
22	GACCCAUCCUCCAAG	0·9	1
24	GCUAAAUG	1·8	2
26	GAAAAUCAAG	0·8	1
29	GAUAAAG	1·1	1
30	GACCCCCUAAG	0·8	1
	Partial sequences		
4	G(AU,C_3,U)UUG	0·8	1
7	GAU(AAAU,AAC,C_2,U)G	0·8	1
17	G(U_2,C)CCUG	1·1	1
20	GC(AAU,AAU,C)G	0·5	?
23	GC(C,AU)CUAAG	1·1	1
25	GU(AC,U)CCG	0·8	1
27	G(AAAC,AAU,C)CCCG	0·5	?
28	GU(AC,C_2)CCAAACCCG	0·5	?

* The sequences of products m10, m11 and 3OH have been previously reported[2,4].

The observed molar ratios were expressed relative to spot m10 being present twice within the 23S RNA (see text). An octanucleotide homology exists between spots 5 and 18. The octanucleotide sequences of 3 and 24 are probably present twice. The probability of encountering an octanucleotide repetition in a study of this size may be roughly calculated in the following way: the completely determined sequences (Nos. 3–30) encompass fifty octanucleotide runs, that is about 1/1,250 of the total number of possible octanucleotides, and therefore the chance of obtaining a repetition of an octanucleotide sequence will be less than 1/1,000.

The simplest explanation of these findings would be that most of the sequences examined here occur once within the 23S RNA, but the methylated sequences and some others are repeated. This explanation is compatible with earlier findings that the methylated sequences are duplicated in the molecule[2,3] and also with more recent measurements of the amount of methylated nucleotides released by alkaline hydrolysis of ^{32}P-labelled 23S RNA (ref. 13). If this model is correct, then the 23S RNA has some properties in common with the 16S RNA. The latter is also predominantly homogeneous, and contains a greater amount of sequence repetition than would be expected on a random basis.

Alternative explanations of these results would invoke extensive heterogeneity of the 23S RNA. For example, the methylated sequences and certain others would be postulated to be present once each in all 23S RNA molecules, and arise in molar amounts in enzymatic digests, but other sequences would differ between the hypothetical sub-species of the 23S RNA, and therefore be encountered in sub-molar amounts. This explanation is not compatible with quantitative findings on the level of methylation of the 23S RNA, but nevertheless it cannot be entirely ruled out in the absence of more extensive sequence analysis of this molecule.

This work was supported financially by the Centre National de la Recherche Scientifique, and by the Délégation à la Recherche Scientifique et Technique (Biologie Moléculaire). We acknowledge the technical collaboration of Mlle O. Blasi.

P. FELLNER
J. P. EBEL

Laboratoire de Chimie Biologique,
Faculté des Sciences,
Université de Strasbourg,
Rue René Descartes,
67-Strasbourg.

Received December 15, 1969.

[1] Fellner, P., Ehresmann, C., and Ebel, J. P., *Nature*, **225**, 26 (1970).

[2] Fellner, P., and Sanger, F., *Nature*, **219**, 236 (1968).

[3] Fellner, P., *Europ. J. Biochem.*, **11**, 12 (1969).

[4] Fellner, P., and Ebel, J. P., *FEBS Lett.*, **6**, 102 (1970).

[5] Takanami, M., *J. Mol. Biol.*, **29**, 323 (1967).

[6] Attardi, G., Huang, P. C., and Kabat, S., *Proc. US Nat. Acad. Sci.*, **53** 1490 (1965).

[7] Avery, R. J., and Midgley, J. E. M., *Biochem. J.*, **108**, 33P (1968).

[8] Mangiarotti, G., Apirion, D., Schlessinger, D., and Silengo, L., *Biochemistry*, **7**, 456 (1968).

[9] Avery, R. J., Midgley, J. E. M., and Pigott, G. H., *Biochem. J.*, **115**, 395 (1969).

[10] Stanley, W. M., and Bock, R. M., *Biochemistry*, **4**, 1302 (1965).

[11] Spahr, P. F., and Tissières, A., *J. Mol. Biol.*, **1**, 237 (1959).

[12] Sanger, F., Brownlee, G. G., and Barrell, B. G., *J. Mol. Biol.*, **13**, 373 (1965).

[13] Varicchio, F., and Forget, B., *J. Mol. Biol.* (in the press).

[14] Brownlee, G. G., and Sanger, F., *J. Mol. Biol.* **23**, 337 (1967).

Temperature Sensitive Alteration of 30S Subunits demonstrated by *in vitro* Reassociation of Functional Ribosomes

RECONSTITUTED ribosomes which lack some ribosomal proteins were found to be inactive in catalysing poly-U-dependent phenylalanine incorporation[1]. A similar result was previously reported resulting from an alteration of the 50S ribosomal subunit by a temperature sensitive mutation[2]. Temperature sensitive mutants of *Escherichia coli* K12 defective in protein synthesis have been isolated in this laboratory, and I report here, for the first time, that mutant strain C1716 was much less active in poly-U-dependent polyphenylalanine synthesis due to an alteration of the 30S ribosomal subunit.

Mutant C1716 was isolated from the cells surviving treatment with 100 µg/ml. streptomycin for 1 h at 42° C. One might expect to find streptomycin resistant mutants of the kind that have already been extensively studied. One also expects to isolate bacteria that grow at low temperature but are sensitive to streptomycin, that are not killed by streptomycin at elevated temperature, and that do not grow at high temperature with or without streptomycin. On these assumptions, *E. coli*, K12 strain HfrH 3000 thy$^-$ met$^-$ growing exponentially at 35° C in L-broth[3] was exposed to N-methyl-N′-nitro-N-nitrosoguanidine, 100 µg/ml. for 40 min at 35° C. Cells were washed twice and grown for three generations at 25° C. The culture was then diluted, and the cells were grown at 43° C for 1 h in the presence of streptomycin sulphate, 100 µg/ml. Using the replica plating technique, 270 temperature sensitive (*ts*) mutants were isolated from a total of 5,000 clones examined. More *ts* mutants were isolated than I could investigate. Four out of thirty-six *ts* strains tested for poly-U-directed polyphenylalanine synthesis were defective at 42° C. One *ts* strain showed a mutation at the streptomycin locus[4].

Strain C1716 grew at 30° C, but growth was immediately arrested when the temperature was raised to 42° C. A temperature shift to 42° C caused protein and RNA synthesis in the intact cells to stop. *In vitro* incorporation of phenylalanine directed by poly-U was also inactive at 42° C with the extracts obtained from the mutant. Kinetics of phenylalanine incorporation showed that the inhibition of peptide synthesis started within 5 min of incubation at 42° C, whereas the incorporation at 30° C continued for 30 min (Fig. 1).

Table 1. POLY-U-DEPENDENT PHENYLALANINE INCORPORATION BY THE WILD TYPE STRAIN AND C1716

Ribosomes	Concentration of Mg^{2+} (mM)	30° C (c.p.m.)	42° C (c.p.m.)	Ratio of 42° C/30° C
HfrH 3000	10	2,310	1,050	0·32
C1716		2,010	192	0·095
HfrH 3000	15	5,140	5,760	1·1
C1716		3,960	704	0·18

The ribosomes and the supernatant fractions were isolated by the method of Nirenberg and Matthaei[5]. The assay system contained 0·1 mole Tris HCl, *p*H 7·8, 20 mmoles NH_4Cl, 10 or 15 mmoles magnesium acetate, 1 mmole ATP, 0·6 mmoles GTP, 1·8 mmoles mercaptoethanol, 0·2 mmole unlabelled amino-acids minus phenylalanine, 4 mmoles phosphoenol pyruvate, 20 µg/ml. pyruvate kinase, 0·5 µCi/ml. of ^{14}C-phenylalanine (100 mCi/mmole, New England Nuclear Co.), 100 µg/ml. *E. coli* K12 *t*RNA (Calbiochem), 100 µg/ml. poly-U (Sigma Co.), 2 $A_{260\ nm}$ units of ribosomes and 120 µg of the supernatant fraction protein of the wild type cells in a total volume of 0·2 ml. Reaction mixtures were incubated at 30° C and 42° C for 10 min. The reaction was stopped by addition of 3 ml. of 5 per cent trichloroacetic acid. The precipitate was heated in a 90° C water bath for 15 min, brought to room temperature and filtered on a glass filter GF/C (Whatman). The filter was dried, and the radioactivity was measured in a Packard liquid scintillation counter in the toluene-omnifluor (New England Nuclear Co.) system.

Experiments, comparing the ribosomes isolated from the wild type strain and the mutant, were performed using the soluble enzyme fraction obtained from the wild type strain. Ribosomes from strain C1716 were much less active in catalysing poly-U-dependent polyphenylalanine synthesis than ribosomes from the wild type at 42° C (Table 1). The difference between both strains was more drastic at 15 mM magnesium concentration. Even at 30° C, activity of the ribosomes was different when equal amounts of wild type and mutant ribosomes (on an $A_{260\ nm}$ basis) were assayed (Table 1). Other experiments showed that the soluble enzyme fraction from strain C1716 was equivalent to that of the wild type. The data indicate that strain C1716 has a *ts* mutation in the ribosomes. The ribosomes from the mutant were incubated at 55° C for 20 min in buffer to determine whether or not they irreversibly lose the capacity to function in subsequent peptide synthesis. After the heat treatment there was no significant reduction in ribosomal function when tested at 30° C.

Table 2. POLY-U-DEPENDENT-PHENYLALANINE INCORPORATION OF RECONSTITUTED RIBOSOMES

Ribosomes		Radioactivity (c.p.m.) at	
50S	30S	30° C	41° C
W*	—	184	42
M*	—	206	50
—	W	157	60
—	M	67	62
W	W	3,290	1,770
W	M	2,600	296
M	W	3,140	1,980
M	M	2,300	304

The 50S and 30S subunits of ribosomes were prepared by the method of Traub *et al.*[6]. Reaction mixture contained 0·05 moles Tris HCl, *p*H 7·8, 20 mmoles NH_4Cl, 10 mmoles Mg acetate, 1 mmole ATP, 0·6 mmoles GTP, 1·8 mmoles mercaptoethanol, 0·2 mmoles unlabelled amino-acids minus phenylalanine, 4 mmoles phosphoenol pyruvate, 20 µg/ml. pyruvate kinase, 0·75 µCi/ml. of ^{14}C-phenylalanine (100 mCi/mmole), 200 µg/ml. tRNA, 100 µg/ml. of poly-U, 2$A_{260\ nm}$ units of 50S, 1 $A_{260\ nm}$ units of 30S and 70 µg of supernatant fraction protein of wild type cells in a total volume of 0·2 ml. Reaction mixtures were incubated for 20 min at 30° C or 41° C. Detailed procedures are described in Table 1. Data are the average of two separate experiments.

* W indicates subunit obtained from the wild type cells, and M indicates subunit from the mutant cells.

To determine the site of inactivation within the ribosomes the 50S and 30S ribosomal subunits were prepared from C1716 and the wild type strain, and then hybrid 70S ribosomes were reassociated from these 50S and 30S subunits in all combinations (Table 2). Results using these hybrid ribosomes indicated that the source of the 50S ribosomal subunits did not have any influence on polyphenylalanine synthesis. In contrast, ribosomes reassociated from mutant 30S subunits with either wild type or mutant 50S subunits were much less active in poly-U-dependent polyphenylalanine synthesis when compared with the activities of reassociated ribosomes that contained wild type 30S subunits at a restricted temperature.

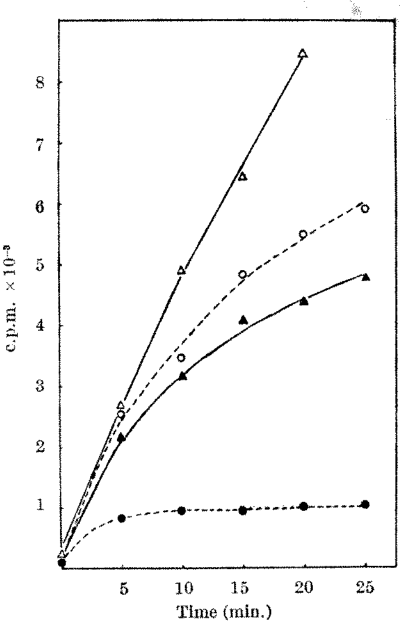


Fig. 1. Kinetics of poly-U-directed phenylalanine incorporation by the wild type and mutant cells extracts. 30S fractions were obtained by the method of Nirenberg and Matthaei[5]. The assay of incorporation was performed as described in Table 1, except that reaction mixtures contained 1 $A_{260\ nm}$ unit of 30S fractions per 0·2 ml. of the reaction mixture instead of ribosomes and soluble enzyme fraction. Open circles indicate incorporation by the mutant extracts at 30° C; closed circles by the mutant at 41° C; open triangles by the wild type at 30° C; closed triangles by the wild type at 41° C.

Thus the mutation in strain C1716 occurred in a cistron that was responsible for the synthesis of one of the 30S ribosomal components and alteration of this cistron caused an inhibition of protein synthesis at high temperature.

SOO-SANG KANG

Department of Microbiology,
University of Chicago,
Chicago, Illinois 60637.

Received October 20; revised December 16, 1969.

[1] Traub, P., and Nomura, M., *J. Mol. Biol.*, **34**, 575 (1968); Traub, P., and Nomura, M., *ibid.*, **34**, 595 (1968).
[2] Apirion, D., *J. Mol. Biol.*, **16**, 285 (1966).
[3] Lennox, E. S., *Virology*, **1**, 190 (1955).
[4] Kang, S., *Proc. US Nat. Acad. Sci.* (in the press).
[5] Nirenberg, M. W., and Matthaei, J. H., *Proc. US Nat. Acad. Sci.*, **48**, 684 (1961).
[6] Traub, P., Nomura, M., and Tu, L., *J. Mol. Biol.*, **19**, 215 (1966).

Differences in E and S Chains from Isoenzymes of Horse Liver Alcohol Dehydrogenase

THREE isoenzymes of horse liver alcohol dehydrogenase, $LADH_{EE}$, $LADH_{ES}$ and $LADH_{SS}$, are formed by the possible dimeric combinations of two different protein chains, the E-chain and the S-chain[1].

The structures of the two types of chains have been compared[2] and were shown to be similar, suggesting a common genetic origin. Three amino-acid substitutions were then found, all compatible with one-base mutations. This was performed by comparisons chiefly between the EE and ES isoenzymes, for only small amounts of the pure SS form were available. The latter has since been crystallized (Å. Åkeson and G. Lundquist, to be published) and purified in greater amounts. Tryptic digests of ^{14}C-carboxymethylated EE and SS isoenzymes have now been extensively compared by multidimensional "fingerprinting" techniques similar to those used with the ES isoenzyme[2]. The peptide differences can be seen more distinctly with the two homogeneous isoenzymes than with the EE and hybrid ES forms previously compared.

More differences between the two types of chains have been found so that six mutations are now known (Table 1). The first three mutations have been detected previously[2]. Their numerical positions and those of the fourth and fifth mutations are now exactly known from the amino-acid sequence of the E-chain (H. J., submitted for publication). The position of the sixth mutation is still somewhat uncertain, for the structure of this part of the protein chain is not definitely established. The peptide from the S-chain containing the fifth mutation (a basic heptapeptide) is difficult to purify; only a small yield was available. Although sufficient material for complete sequence analysis was not obtained, it was possible to determine the total composition. From this and the electrophoretic charge of the peptide, the mutation in Table 1 is deduced on the assumption that the peptide contains only one substitution. All other mutations have been proved by analyses of both compositions and complete amino-acid sequences of differing peptides. Although five of the mutations are compatible with one-base mutations, the fifth requires a change of two bases.

Table 1. MUTATION SITES

Mutation number	Position along the protein chain	Residue in E-chain	Residue in S-chain
1	17	Glu	Gln
2	94	Thr	Ile
3	101	Arg	Ser
4	110	Phe	Leu
5*	115	Asp	Ser*
6	366†	Glu	Lys

* Based on total composition only (see text).
† Preliminary number.

The six mutations now known render the S-chain more positive than the E-chain by three charged units around neutral *p*H. This explains the electrophoretic properties of the isoenzymes[1] and is in keeping with the differences in *pI*[2] between them. Before, when only the three first mutations were known[2], these facts could not be accounted for.

Of the six mutations, four are closely situated at the region around position number 100 of the chains. In addition, these four substitutions are for both smaller and more hydrophobic residues in the S-chain when compared with the similar portions of the E-chain. This fact and the known difference in substrate specificity between the chains (the S-chain is active towards steroid substrates but the E-chain is not[1]) might suggest that the mutation-rich region around position 100 is close to the substrate binding site. The need for more space and hydrophobicity at this site of the S-chain, as compared with that of the E-chain, might then possibly be explained by these mutations, although changes in the three-dimensional folding of the chains are, of course, alternative explanations.

I thank Professor H. Theorell and Dr Å. Åkeson for help and interest and for supplying the pure isoenzymes. This work was supported by a grant from the Swedish Medical Research Council to Professor Theorell.

HANS JÖRNVALL

Medicinska Nobelinstitutet,
Biokemiska Avdelningen,
Karolinska Institutet,
S-104 01 Stockholm 60.

Received December 19, 1969.

[1] Theorell, H., in *Alfred Benzon Symp. 1968* (Munksgaard, Copenhagen, in the press).
[2] Jörnvall, H., *Biochem. Biophys. Res. Commun.*, **35**, 542 (1969).

Size-specific Metal Complexing Sites in Native Collagen

THERE is evidence that metal ions are involved, directly and indirectly, in the structure and function of the collagens[1–11]. The direct interactions between collagen proteins and metal ions must be elucidated in order to understand the roles of these ions in connective tissues. One approach is to study the metal binding properties of relatively intact collagen matrices in model systems consisting of water solutions of several ionic salts. Previous experiments have involved the binding of a few ions to purified reconstituted calf-skin collagen[12], egg shell matrix[13], gelatine[14] and bivalent amino-acids[15].

We report here the results of experiments in which whole sections of decalcified bone matrix and tendon were exposed to twenty-four metallic ions.

Table 1. EXPERIMENTAL CONDITIONS DURING EXPOSURE TO IONIC SOLUTIONS

	Experiment 1	Experiment 2
*p*H	1+6·3 2+6·4 3+3·5 Adjusted NH_4OH	5·4; Adjusted NaOH
Ionic strength	0·011–0·016 M	0·16 M
Indiv. ion conc.	0·3–3·0 mM	4·0 mM
Sample/solution (w/v)	0·05 g/100 ml.	0·2 g/100 ml.
Exposure	3 days	2 days
Ions 1^+	Li, Na, Ag, K, Rb, Cs	Li, Na, Ag, K, Rb, Cs
2^+	Be, Mg, Sr, Ba, Mn, Zn, Cu, Cd, Hg, Pb	Be, Mg, Ca, Sr, Ba, Zn, Cu, Cd, Co, Ni, Pb
3^+	Al, Ga, Cr, Fe, Bi	

Small sections of normal human tibial cortex and human Achilles tendon were demineralized for 5 days (5 per cent formic acid, 25° C), washed and stored under distilled deionized water until use. Other tendon sections were only washed. The samples were immersed in compound equimolar nitrate salt solutions at room temperature (Table 1), and washed in three changes of water for 1 h. In the first experiment, demineralized bone and washed tendon (in the same vessel) were exposed to compound solutions of either 1^+, 2^+ or 3^+ ions. Demineralized tendon was included in the divalent ion solution. In experiment 2, demineralized bone and demineralized tendon (in separate vessels) were exposed to solutions containing many 1^+ and 2^+ ions mixed together. Relative uptakes were determined by measuring the resulting metal concentrations in the substrates, using d.c. arc emission spectroscopy[8,9]. Control concentrations in unexposed tissues were generally well below induced concentrations. Comparison of preliminary uptake with time showed that equilibrium was reached within 12 h of exposure.

Table 2. CONCENTRATIONS OF IONS BOUND TO COLLAGEN SAMPLES

		Experiment 1			Experiment 2	
		Demin. bone	Demin. tendon	Washed tendon	Demin. bone	Demin. tendon
1^+	Li	–		–	?	?
	Na	–		–	–	+
	Ag	+ + + +		+ + + +	+ + +	+ + + +
	K	–		–	–	+
	Rb	–		–	–	+
	Cs	–		–	–	+
2^+	Be	+	+	+	+ + +	+ +
	Mg	+	+	–	+	+
	Cu	+ +	+ +	+ +	+ + + +	+ + +
	Zn	+ +	+	+ +	+ + + +	+
	Mn	+	+	+		
	Cd	+	–	–	+	+
	Hg	–	–	–		
	Sr	–	–	–	+	+ +
	Pb	+ + + +	+	+	+ + + +	+ + + +
	Ba	–	–	–	+	+
	Ca				+	+
	Co				+	+
	Ni				+	+
3^+	Al	+		+ +		
	Ga	+		+ + +		
	Fe	+		+ + + +		
	Cr	+		+ + +		
	Bi	+		+ + + +		

– Indicates less than 0·1 μmoles/g of metal in dry tissue.
+ Indicates 0·1–10 μmoles/g of metal in dry tissue.
+ + Indicates 10–30 μmoles/g of metal in dry tissue.
+ + + Indicates 30–50 μmoles/g of metal in dry tissue.
+ + + + Indicates 50 or more μmoles/g of metal in dry tissue.

The concentrations of metal ions bound to the substrates are outlined in Table 2. Differences in Pb^{2+} and 3^+ ion uptakes in the first experiment may reflect structural or conformational differences between the bone and tendon collagen, as prepared here. When plotted as a function of the ionic radii of the metal ions[16], the preferentially bound ions fell into at least two size categories, 0·65 – 0·75 Å and 1·2 – 1·3 Å (Fig. 1). These categories were to a certain extent independent of the specific ions involved, the charges on the ions, and the experimental conditions used. It may also be significant that the "preferred sizes" differed approximately by a multiple of two. To our knowledge, these are the first such size-specific interactions observed for collagen matrices.

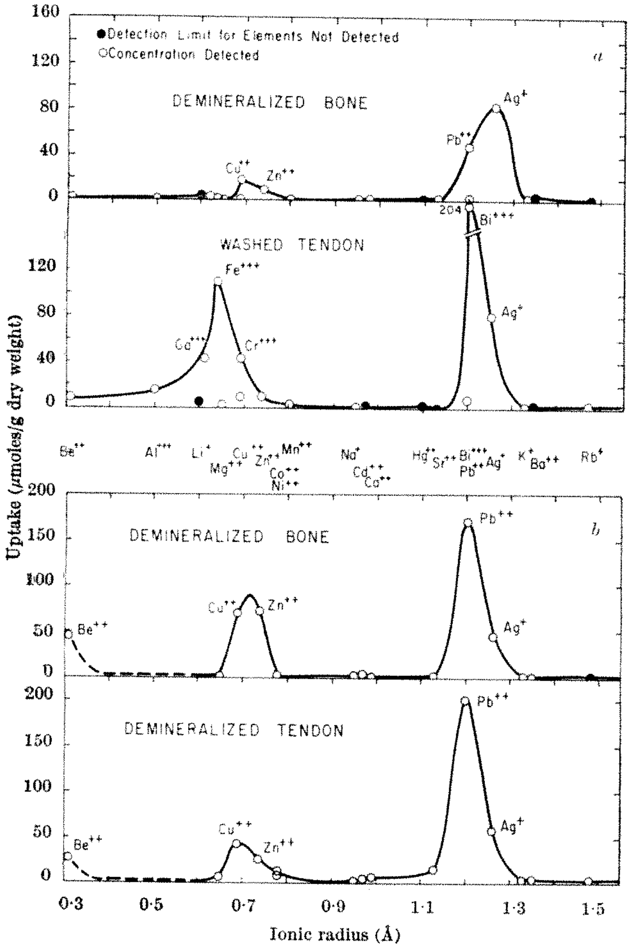


Fig. 1. The ion uptake (μmoles/g dry weight of tissue) as a function of ionic radius for (a) demineralized bone and washed tendon in experiment 1, and (b) demineralized bone and demineralized tendon in experiment 2. The uptake of divalent ions in demineralized tendon in experiment 1 (not shown) was identical to the results for washed tendon shown above. Trivalent ions were omitted in experiment 2 to maintain the pH of the hetero-ionic solutions above 5. The two preferred size regions are evident as are differences between the ion selectivities of bone and tendon matrices in experiment 1.

For chelate complexes involving a given donor and several different metal ions, it has been shown that the logs of the formation constants are a linear function of the total ionization potentials of the ions[17]. Furthermore, in the conditions of a large excess of several metal ions in equimolar concentrations, the log of the metal ion concentration measured in the substrate should also be proportional to the ionization potential[18] if a coordination complex is formed.

Thus the general linearity in Figs. 2 and 3 is strong evidence for coordination bonding (or chelation) in these collagen matrices, and that most of the ions were bound to a single type of donor group. Many

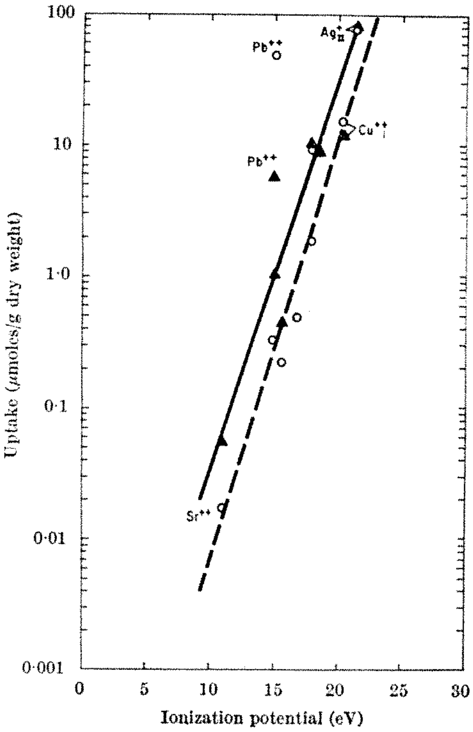


Fig. 2. The ion uptakes in experiment 1 (μmoles/g dry weight of tissue) plotted against the total ionization potentials of the ions on a semi-log scale. The trivalent data are omitted because of the extremely different experimental conditions obtaining in that part of the experiment. The divalent uptakes in decalcified tendon (not shown) were similar to those for washed tendon. The second ionization potential of Ag was used in this plot. The concentrations bound to the tendon matrix were higher than that for demineralized bone matrix, although the slopes were nearly identical. The Pb concentrations clearly stray from the rest. ○, Decalcified bones; ▲, washed tendon.

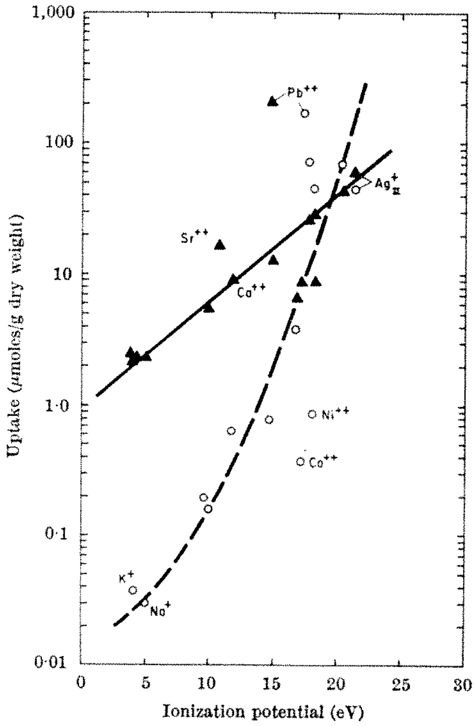


Fig. 3. The ion uptakes in experiment 2 (μmoles/g dry weight of tissue) plotted against the total ionization potentials on a semi-log scale. Ag is plotted using its second ionization potential. As in Fig. 2, the tendon matrix bound many ions more avidly than demineralized bone, but with a considerably smaller slope. The slope for decalcified bone is generally similar to that in the previous experiment; the considerable scatter and curvature in this experiment probably reflects the higher ionic strength and lower pH used. The Pb, Co and Ni uptakes stray from the bulk of the data. ○, Decalcified bone; ▲, decalcified tendon.

of the strongly bound ions (1+ and 2+) readily coordinate to donor nitrogens[19,20], so the bonding may be primarily to uncharged amino, imidazole or guanidino groups in the substrates, and may be largely ion-polar or covalent in character[20]. Although titratable, the charged carboxyls are only partially available for binding in native collagen due to internal charge compensation at medium *p*H[21]. The binding of trivalent ions in experiment 1 at low *p*H, however, may have been to the latter site[22].

Others have suggested that metallic coordination complex formation in cartilage[23], elastin[11,24] and egg shell matrix[13] is important in the primary calcification process. Similar complexes may be involved in the calcification (or lack of it) in bone and tendon matrices.

This work was supported in part by US Public Health Service grants and by the Veterans Administration Research Service.

JOSEPH A. SPADARO
ROBERT O. BECKER

Veterans Administration Hospital,
Syracuse, NY.

CHARLES H. BACHMAN

Department of Physics,
Syracuse University,
Syracuse, NY.

Received October 24; revised December 18, 1969.

[1] Chvapil, M., and Hurych, J., *International Review of Connective Tissue Research* (edit. by Hall, D. A.), **4** (Academic Press, New York and London, 1968).
[2] Bornstein, P., Kang, A. H., and Piez, K. A., *Proc. US Nat. Acad. Sci.*, **55**, 475 (1966).
[3] O'Dell, B. L., *et al.*, *Nature*, **209**, 401 (1966).
[4] Prockop, D. J., and Juva, K., *Proc. US Nat. Acad. Sci.*, **53**, 661 (1965).
[5] Urist, M. R., and Abernethy, J. L., *Clin. Ortho. Rel. Res.*, **51**, 255 (1967).
[6] Steven, F. S., *Biochim. Biophys. Acta*, **140**, 552 (1967).
[7] Sobel, A. E., and Hanok, A., *J. Biol. Chem.*, **197**, 669 (1952).
[8] Becker, R. O., Spadaro, J. A., and Berg, E. W., *J. Bone Joint Surg.*, **50-A**, 326 (1968).
[9] Ellis, E. H., Spadaro, J. A., and Becker, R. O., *Clin. Ortho. Rel. Res.*, **65**, 195 (1969).
[10] Wadkins, C. L., *Calc. Tiss. Res.*, **2**, 214 (1968).
[11] Schiffman, E., Corcoran, B. A., and Martin, G. R., *Arch. Biochem. Biophys.*, **115**, 87 (1966).
[12] Weinstock, A., King, P. C., and Wuthier, R. E., *Biochem. J.*, **102**, 983 (1967).
[13] Simkiss, K., and Tyler, C., *Quart. J. Microsc. Sci.*, **99**, 5 (1958).
[14] Bello, J., *Biochim. Biophys. Acta*, **109**, 250 (1965).
[15] Albert, A., *Biochem. J.*, **47**, 531 (1950).
[16] Pauling, L., *The Nature of the Chemical Bond* (Cornell University Press, Ithaca, 1960).
[17] Chaberek, S., and Martell, A. E., *Organic Sequestering Agents.* 69, 112 (John Wiley and Sons, Inc., New York, 1959).
[18] Ionization data from: *Handbook of Chemistry and Physics* (edit. by West, R. C.) (Chemical Rubber Co., Cleveland, 1968).
[19] Keller, R. N., and Parry, R. W., *The Chemistry of Coordination Compounds* (edit. by Bailar, J. C.), chap. 4 (Reinhold Publishing Co., New York, 1956).
[20] Dwyer, F. P., and Mellor, D. P., *Chelating Agents and Metal Chelates*, chap. 2 (Academic Press, New York and London 1964).
[21] Veis, A., *Treatise on Collagen* (edit. by Ramachandran), **1** (Academic Press, London and New York, 1967).
[22] Rosmus, J., *et al.*, *Experientia*, **23**, 898 (1967).
[23] Dunstone, J. R., *Biochem. J.*, **72**, 465 (1959).
[24] Molinari-Tosatti, *et al.*, *Clac. Tiss. Res.*, **2** (suppl.), 88 (1968).

Genetic Control of Deficiencies in γG Subclasses observed among Families with Hypogammaglobulinaemia

SEVERAL research units have studied the genetic mechanisms controlling the synthesis of immunoglobulins and its alteration in hypogammaglobulinaemia. Fudenberg *et al.*[1] investigated Gm and Inv groups in families with agammaglobulinaemia, and suggested the involvement of two genes in the synthesis of an immunoglobulin: one controlling the synthesis of the heavy chains, the other that of the light chains. Burtin *et al.*[2] applied the same principle in their studies on "non sex-linked atypic hypogammaglobulinaemia", and postulated the existence of abnormal alleles leading to an impaired synthesis of the heavy chains of one or several immunoglobulins.

Since then, the heavy chains of γG have been subdivided into four subclasses: γG1, γG2, γG3 and γG4[3,4]. The Gm factors were located on these subclasses as follows: the γG1 carry Gm factors 1, 2, 4, 8, 9, 17, 18 and 22; the γG2 carry factors Gm 8 and 23. The Gm factors 5, 6, 10, 11, 14, 15, 16 and 21 are located on the heavy γG3 chains. So far the γG4 have not been assigned a genetic marker. Independent genetic control of the synthesis of each γG subclass could be determined by a study of these Gm groups in families with one or several cases of immunoglobulin deficiency. Previously[5] we have shown a selective lack of some subclasses of γG in apparently healthy members of a family with two cases of

Table 1. Gm PHENOTYPES, INHIBITION TITRES AND IMMUNOGLOBULIN LEVELS IN MEMBERS OF THE B 492 FAMILY

Family relationship	γG1				γG2 γG1	γG3		Immunoglobulin levels (mg/ml.)		
	Gm (1,	2,	17)	Gm (4)	Gm (8)	Gm (5)	Gm (21)	γG	γA	γM
Control	1/512	1/512	1/2,048	–	–	–	1/512			
Control	–	–	–	1/128	1/64	1/512	–			
II 3 Pat. aunt	+	+	+	+	1/4	1/2	1/8	1,030	251	35
II 4 Pat. aunt	+	–	+	+	1/4	1/32	1/16	823	123	109
II 5 Pat. aunt	+	–	+	+	1/8	1/8	1/4	1,030	303	159
II a Mother	–	–	–	1/4	1/8	1/64	–	167	23	31
II b Mat. uncle	–	–	–	+	1/8	1/2	–	978	209	86
II c Mat. uncle	–	–	–	+	1/2	1/2	–	1,103	251	95
II d Mat. uncle	–	–	–	+	1/4	1/32	–	843	211	99
II e Mat. aunt	–	–	–	+	+	+	–	930	204	113
III 1 Pat. cousin	–	–	–	+	+	+	–	797	207	54
III 2 Pat. cousin	+	+	+	+	+	+	+	1,200	266	77
III 5 Pat. cousin	+	+	+	–	–	–	+	1,210	229	83
III 6 Brother	–	–	–	1/16	+	1/8	–	1,066	210	92
III 8 Proband	1/16	–	1/64	1/8	–	1/8	1/4	29	0	0
III a Mat. cousin	–	–	–	+	1/4	1/4	–	533	40	35
III b Mat. cousin	–	–	–	+	+	+	–	1,007	90	85
III d Mat. cousin	–	–	–	+	1/4	1/32	–	741	142	86
IV 1 Pat. sec. cousin	+	–	+	+	+	+	+	1,323	106	15
IV 2 Pat. sec. cousin	–	–	–	+	+	+	–	1,040	134	21
IV 4 Pat. sec. cousin	–	–	–	+	–	+	–	853	149	41
IV 5 Pat. sec. cousin	+	+	+	–	–	–	+	1,250	181	88

–, No inhibition. +, The inhibiting power is similar to that of the control serum.

Table 2. Gm PHENOTYPES, PROBABLE Gm STRUCTURE GENES AND REGULATORY GENES IN FAMILY U 204

Family member	Gm phenotypes	Unusual structure; gene alternative	Regulatory gene alternative	
			Structural genes	Regulatory genes
Father 2,392	Gm (1, –2, 4, 5, 8, 17, 21)	$Gm^{1,17,21}/Gm^{4,5,8}$	$Gm^{1,17,21}/Gm^{4,5,8}$	*G1, G2, G3*
Mother 2,393	Gm (1, 2, –4, 5*, 8*, 17, –21)	$Gm^{1,2}/Gm^{5,8}$	$Gm^{1,2,17,21}/Gm^{4,5,8}$	*G1, g1, G2, g2, G3, g3*
Children 2,394 II–1	Gm (1*, 2*, 4*, 5*, 8*, 17*, –21)	$Gm^{1,2,17}/Gm^{4,5,8}$	$Gm^{1,2,17,21}/Gm^{4,5,8}$	*G1, g1, G2, g2, G3, g3*
2,395 II–2	Gm (1, 2, 4*, 5*, 8*, 17, –21)	$Gm^{1,2,17}/Gm^{4,5,8}$	$Gm^{1,2,17,21}/Gm^{4,5,8}$	*G1, g1, G2, g2, G3, g3*
2,396 II–3	Gm (1, –2, 4, 5, 8, 17, 21)	$Gm^{1,17,21}/Gm^{4,5,8}$	$Gm^{1,17,21}/Gm^{4,5,8}$	*G1, G2, G3*

* Weakened inhibiting power as compared with the control serum.

ataxia telangiectasia. We report here the determination of Gm phenotypes in five families, one or more members of each having a deficit in one or several immunoglobulins. We measured the Gm factors 1, 2, 4, 5, 8, 17 and 21 and Inv factors 1 and 2 by their inhibiting power, and the concentration of γG, γA and γM globulins by the Mancini method.

The first family (B 492) was that of a 39 year old woman who in 1967 developed diarrhoea, arthralgia, furunculosis and tuberculous lymphadenitis. Her serum and the sera of nineteen relatives were studied. Table 1 gives the Gm titres and the levels of immunoglobulins in these sera. It shows that the proband is agammaglobulinaemic, and that her mother has unusually small amounts of the three immunoglobulins. Both have a weak expression of all Gm groups. In other members of the family, the inhibiting power of some Gm factors is clearly abnormally low. The results of the study on Gm factors indicate that the γG2 and γG3 are the ones most often decreased. This deficiency is genetically transmitted, as is shown in Table 1. It exists in the men II*b* and II*c* and their respective sons III*a* and III*d*. It contrasts with the value of total γG which is normal or only slightly lowered in these sera. This could be explained by the relatively small amounts of γG2 and γG3, which represent only 15 per cent and 10 per cent respectively of the total γG.

In each of the four other families we investigated, the Gm allotypes indicated a deficit in one or more γG subclasses in the proband's serum and in some clinically normal relatives. They have immunoglobulins which are quantitatively normal, or slightly deficient in one or more classes. These observations favour strongly the hypothesis of a genetic mechanism of "non sex-linked atypic hypogammaglobulinaemia".

Does this genetic mechanism involve structural or regulatory genes ? The observation of unusual phenotypes in some members of these families could favour the first alternative, as suggested by Yount *et al.*[6], who made the same statement. These phenotypes are very rare in Caucasians, for example, Gm (1, 2, −4, 5, −8, 17, 21) or Gm (1, 2, −4, 5, −8, 17, −21). Moreover, they could lead to an exclusion of paternity or maternity. For example, in family U 204 (Table 2), child II–3 has the Gm (1, 17, 21) group inherited from the father, and the Gm (4, 5, 8) group which should have come from the maternal chromosome. But the mother has only the phenotype Gm (5, 8) and lacks Gm (4). Does that mean exclusion of maternity ?

The other alternative is that these abnormal phenotypes correspond to an abnormally weak expression of normal structure genes, arising from a lesion of regulatory genes. Let us suppose the existence of regulatory genes $G1$, $G2$, $G3$, controlling the synthesis of the $\gamma 1$, $\gamma 2$, $\gamma 3$ chains. The presence of $g1$, $g2$, $g3$ alleles in simple or double doses would result in partial or total inhibition of the expression of structural genes. In family U 204, the mother having a deficit in Gm (5) and (8), and absence of Gm (4) and (21), would carry abnormal $g1$, $g2$ and $g3$ alleles. She is probably heterozygous, because her child II–3 has a normal phenotype. The decrease of Gm (4, 5, 8) in children II–1 and II–2, contrasting with a normal level of these factors in their father, could be explained by the heterozygous state $G1g1$, $G2g2$, $G3g3$ in the children. Accordingly, the father would have normal regulatory genes. Thus the transmission of regulatory genes would be independent from that of structural genes. It has not previously been possible to establish a selective deficit of γG4 because of the lack of a genetic marker for this subclass.

In conclusion, we have demonstrated the existence of independent genetic mechanisms controlling the synthesis of the heavy chains of each γG subclass. These mechanisms are altered in patients with non sex-linked hypogammaglobulinaemia, and in some of their relatives. The deficit may or may not be revealed by a decrease in total γG. A lesion of the regulatory rather than the structural genes seems to be responsible for this phenomenon.

LILIANE RIVAT

Research Group U78 of the INSERM,
76F Bois Guillaume.

C. ROPARTZ

Centre Départemental de Transfusion
Sanguine et de Génétique Humaine,
76F Bois Guillaume.

P. BURTIN

Institut de Recherches Scientifiques sur le Cancer,
Centre National de la Recherche Scientifique,
94F Villejuif.

A. CRUCHAUD

Department of Medicine,
University Medical School,
Hopital Cantonal,
Geneva.

Received April 15; revised November 20, 1969.

[1] Fudenberg, H. H., Heremans, J. F., and Franklin, E. C., *Ann. Inst. Pasteur*, **104**, 155 (1963).

[2] Burtin, P., Buffe, D., and Grabar, P., *Ann. Inst. Pasteur*, **106**, 519 (1964).

[3] Grey, H. M., and Kunkel, H. G., *J. Exp. Med.*, **120**, 253 (1964).

[4] Terry, W. D., and Fahey, J. L., *Science*, **146**, 400 (1964).

[5] Rivat, L., Ropartz, C., Burtin, P., and Karitzky, D., *Vox Sang.*, **17**, 5 (1969).

[6] Yount, W. J., Hong, R., Seligmann, M., and Kunkel, H. G., *J. Clin. Invest.*, **48**, 92*a* (1969).

Demonstration in Sarcoma Patients of Anti-tumour Antibodies which Fix Only Human Complement

SPECIFIC anti-tumour antibodies have now been demonstrated in human sera using several different serological techniques[1-5]. Except in the case of Burkitt's lymphoma[6,7], however, specific anti-tumour antibodies have not been consistently detectable with the complement fixation test. Our recent immunofluorescence studies have demonstrated specific antisarcoma antibody in the serum of patients with this disease[8]. In attempting to detect these antisarcoma antibodies by complement fixation, it was noted that they could be demonstrated only if cells derived from cultured human sarcoma cells were used as antigen and fresh human serum was used as a source of complement. This report describes the conditions necessary for the demonstration of these anti-tumour antibodies in human sera.

The complement fixation test was a modification of the microtitre technique described by Sever[9]. Isotonic vernal buffered saline (pH 7·4) was used as diluent throughout. The haemolytic system was a 0·5 per cent suspension of washed sheep red blood cells (SRBC) sensitized with an equal volume of rabbit anti-SRBC haemolysin, 1 : 2,000, at 37° C for 20 min. To ensure that all sera tested as sources of complement contained equivalent amounts of complement, dilutions of each were made so that two 100 per cent haemolytic units were contained in each 0·025 ml. of serum.

A human liposarcoma (SA-1) cell line was used as antigen[10,11]. Cells were thrice frozen at −190° C and thawed at 37° C. The suspension was then sonicated with a probe sonicator for 20 s/ml. of material and stored at −190° C. Four units of antigen were used in each test as determined by a box titration against a high titre serum from a patient with osteosarcoma which had previously been demonstrated to contain antibody by immunofluorescence.

Antigen prepared from tissue culture cells, antibody (sarcoma patient's heat inactivated serum) and two units of complement were added to the microplates, sealed and incubated at 37° C for 1 h. The haemolytic system was then added and incubated for an additional hour at 37° C. The plates were centrifuged and the test scored on a scale from 0 to 4, the scores corresponding to the size of the red-cell button and the amount of complement fixed. Each test included a complement titration, a complement titration in the presence of antigen, and a control for anti-complementary activity of the serum and antigen.

Table 1. THE ABILITY OF HUMAN ANTISARCOMA ANTIBODIES TO FIX VARIOUS TYPES OF COMPLEMENT

Source of complement	Serum dilution containing two units of complement/0·025 ml.	Antisarcoma titre* (1)	(2)	(3)
Rabbit A	1/8	0	0	0
Rabbit B	1/9	0	0	0
Rabbit C	1/9·5	0	0	0
Rabbit pooled (6)	1/8	0	0	0
Guinea-pig pooled (6)	1/80	0	0	0
Gelada baboon pooled (3)	1/11	0	0	0
Baboon (Papio) pooled (3)	1/8	—	—	—
Chimpanzee pooled (3)	1/5	—	—	—
Human sera AB^1 +	1/8	0	0	0
Human sera AB^2 +	1/10	0	0	0
Human sera A +	1/9	1/4	1/16	1/8
Human sera B +	1/8·5	1/4	1/8	1/4
Human sera C −	1/8	1/8	1/16	1/16
Pooled human sera (8)	1/8·5	1/4	1/8	1/8
Human cord sera pooled (10)	1/8	1/32	1/128	1/64

* Antigen was a 1 : 8 dilution of a 20 per cent suspension of SA-1 (liposarcoma) cells. The sera were from patients with (1) chondrosarcoma; (2) fibrosarcoma and (3) osteosarcoma. Sera marked "—" were unsuitable as a source of complement because they contained natural anti-human antibody.

Various types of fresh animal and human sera were examined as a potential source of complement. An aliquot of each serum was first heat-inactivated and tested for antibody activity against the SA-1 liposarcoma antigen using human cord serum as a complement source. If no antibody was detected, dilutions of each serum were made and complement was titrated at each dilution. The anti-sarcoma antibody titres of patients with skeletal and soft tissue sarcomas are listed in Table 1 along with the source of complement used. Pooled samples of Papio (baboon) and chimpanzee serum were found to react with HeLa and SA-1 antigen complement to a titre of 1/8 and therefore were not useful as a source of complement. No antisarcoma antibody was detectable in sera from rabbit, guinea-pig or gelada baboon (*Theropithecus gelada*), but each serum was found to be ineffective as a source of complement for the human sarcoma antigen–antibody system. When individual human sera with no detectable anti-sarcoma activity were tested, a considerable variation in their ability to act as sources of complement was noted. In most cases some complement fixation was evident. Pooled human umbilical cord serum was uniformly the best source of complement for the detection of these antisarcoma antibodies, for it never contained natural antibody to the antigen and always gave four to eight-fold higher titres than other types of human complement.

Preliminary studies also demonstrated the presence of antibodies in the sera of melanoma patients which react with a tissue culture cell line derived from a human malignant melanoma. These seem to be similar to the antisarcoma antibodies in that they, too, only fix human complement. Thus the complement fixation test using tissue culture cells from malignant tumours as antigen and human serum as a complement source may be valuable for the demonstration of anti-tumour antibodies in human sera.

F. R. EILBER
D. L. MORTON

Tumour Immunology Section,
Surgery Branch,
National Institutes of Health,
Bethesda, Maryland 20014.

Received September 12; revised December 22, 1969.

[1] Old, L. J., Boyse, E. A., Oettgen, H. F., deHarben, E., Geering, G., Williamson, B., and Clifford, P., *Proc. US Nat. Acad. Sci.*, **56**, 1699 (1966).
[2] Hellström, I. E., Hellström, K. E., Pierce, G. E., and Bill, A. H., *Proc. US Nat. Acad. Sci.*, **60**, 1231 (1968).
[3] Hellström, I. E., Hellström, K. E., Evans, C. A., Heppner, G. H., Pierce, G. E., and Yang, J. P. S., *Proc. US Nat. Acad. Sci.*, **62**, 362 (1969).
[4] Morton, D. L., Malgren, R. A., Holmes, E. C., and Ketcham, A. S., *Surgery*, **64**, 223 (1968).
[5] Klein, G., Clifford, P., Klein, E., Smith, R. J., Minowada, J., Kourisky, F. M., and Burchenal, J. H., *J. Nat. Cancer Inst.*, **39**, 1027 (1967).
[6] Armstrong, D., Henle, G., and Henle, W., *J. Bact.*, **91**, 1257 (1966).
[7] Gerber, P., and Birch, S., *Proc. US Nat. Acad. Sci.*, **58**, 478 (1967).
[8] Morton, D. L., and Malmgren, R. A., *Science*, **162**, 1279 (1968).
[9] Sever, J. L., *J. Immunol.*, **88**, 320 (1962).
[10] Morton, D. L., Malmgren, R. A., Hall, W. T., and Schidlovsky, G., *Surgery*, **66**, 152 (1969).
[11] Morton, D. L., Hall, W. T., and Malmgren, R. A., *Science*, **165**, 813 (1969).

Immunoglobulin G as Possible Precursor of Chemotactic Factor

FROM the site of an Arthus reaction or burn lesion, a chemical factor has been isolated ("leucoegressin") that causes the accumulation of polymorphonuclear leucocytes[1,2]. A possible precursor has, moreover, been located in the γ_2-globulin fraction of normal rabbit serum[3], because when six protein fractions such as albumin and α_1, α_2, β_1, β_2 (or γ_1) and γ_2-globulin fractions—obtained from normal rabbit serum by block electrophoresis on a 'Pevikon C-870' column—were each incubated with a purified SH-dependent protease[4,5] from inflammatory tissue, the γ_2-globulin fraction became strongly chemotactic for polymorphonuclear leucocytes. We now are able to report further information on its precursor. We have evidence that immunoglobulin G (IgG) gives rise to a chemotactic factor during these inflammatory processes, and it seems that the chemotactic factor has antigenic sites in common with IgG and can in fact be generated by the enzymatic treatment of fractionated IgG *in vitro*.

Table 1. EFFECT OF ANTI-IgG-ANTIBODY ON CHEMOTACTIC FACTOR

Samples (in lower compartment)	No. of PMNs migrated (per 5 fields)
Chemotactic factor + phosphate buffer	211
Anti-IgG-antibody + phosphate buffer	48
Chemotactic factor + anti-IgG-antibody (equal volumes)	4
Phosphate buffer	26

The materials used were chemotactic factor (100 μg in phosphate buffer, 0·067 M, *p*H 7·4) purified with CM-Sephadex rechromatography; and goat-IgG fraction (500 μg) precipitated at 33 per cent saturation with ammonium sulphate. All samples were filtered with 'Millipore' filter (pore size 0·3 μm) after the reaction.

Antisera against rabbit serum and rabbit IgG were prepared in goats with complete Freund's adjuvant. The sera were collected after several immunizations. Rabbit chemotactic factor was purified as already reported[1,2]. Agar immunoelectrophoresis (*p*H 8·2, *I* 0·05) gave a single precipitin line with goat anti-rabbit serum (Fig. 1). By using goat antiserum against rabbit IgG, partial identity of the chemotactic factor and IgG was revealed. When goat antibody fraction against rabbit IgG was reacted with chemotactic factor at 37° C for 30 min and the absorption product filtered on a 'Millipore' filter (0·3 μm) the chemotactic activity disappeared from the fluid phase (Table 1).

IgG was separated in the cold from normal rabbit serum according to the method of Kapusta and Halberstam[6], using sodium sulphate precipitation and DEAE-cellulose column chromatography. The isolated fraction was homogeneous as judged by immunoelectrophoresis against goat anti-rabbit serum. This IgG had no chemotactic activity, but after treatment with the purified inflammatory SH-dependent protease[4,5] exhibited chemotactic activity on polymorphonuclear leucocytes, as demonstrated by *in vitro* assay according to Boyden's

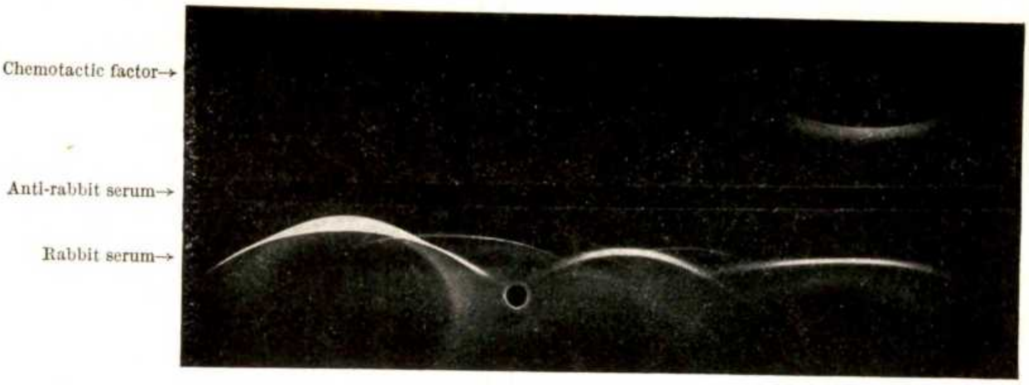


Fig. 1. Agar immunoelectrophoresis of chemotactic factor (pH 8·2, I 0·05, veronal-HCl buffer). The anode is to the right.

method[7] using 'Millipore' filters (DAWP013; pore size 0·65 μm) and a modified chamber containing two 1 ml. compartments. The test samples, in 0·067 M phosphate buffer (pH 7·4), were placed in the lower compartment; the 'Millipore' filter was placed on it, and rabbit polymorphonuclear leucocytes—collected from the peritoneal cavity by Hirsch's method[8] and suspended in Gey's buffered solution containing 2 per cent human serum albumin (pH 7·4)—were poured into the upper compartment. The cells that had passed through the filter were stained with haematoxylin and counted over five microscopic fields (10 × 40); the total counts were recorded (Table 2).

Table 2. ACTIVATION OF IgG WITH THE INFLAMMATORY SH-DEPENDENT PROTEASE (INFLAMMATORY PROTEASE)

Samples (in lower compartment)	No. of PMNs migrated (per 5 fields)
Rabbit IgG (100 μg) + cysteine (10^{-3} M) + inflammatory protease*	449
Rabbit IgG + cysteine + phosphate buffer	24
Cysteine + inflammatory protease	25
Phosphate buffer	18

* 0·02 absorbance units at 276 nm, estimated by the casein digestion method of Kunitz[5,6].

On the basis of these results, we suggest that the precursor of an inflammatory SH-dependent protease is activated in the process of inflammation which follows an antigen–antibody reaction or thermal trauma[1,9-12]. After the increase in vascular permeability has occurred[13-17], this SH-dependent protease converts the exuded IgG molecule to the chemotactic factor which causes the migration of polymorphonuclear leucocytes to the inflammatory lesion. Thus besides the well known specific part as antibody, the IgG molecule may play an immunologically non-specific part as the precursor of a chemotactic factor in inflammation.

Other species of immunoglobulin in the normal rabbit serum were present in amounts too small for the present assay, but we are performing the same type of experiment using IgG and other immunoglobulins of human serum.

We thank Dr M. Koono for a supply of purified inflammatory SH-dependent protease. This work was in part supported by grants from the Education Ministry of Japan.

M. YOSHINAGA
M. MAYUMI
S. YAMAMOTO
H. HAYASHI

Department of Pathology,
Kumamoto University Medical School,
Kumamoto, Japan.

Received September 30, 1969; revised January 21, 1970.

[1] Hayashi, H., *Trans. Soc. Pathol. Jap.*, **56**, 37 (1967) (in Japanese).
[2] Yoshida, K., Yoshinaga, M., and Hayashi, H., *Nature*, **218**, 977 (1968).
[3] Yoshinaga, M., Tashiro, A., Maeda, S., and Hayashi, H., *J. Jap. Biochem. Soc.*, **41**, 435 (1969) (in Japanese).
[4] Koono, M., Muto, M., and Hayashi, H., *Tohoku J. Exp. Med.*, **94**, 231 (1968).
[5] Hayashi, H., Koono, M., Yoshinaga, M., and Muto, M., in *Inflammation Biochemistry and Drug Interaction* (edit. by Bertelli, A.), 32 (Excerpta Medica Foundation, Amsterdam, 1969).
[6] Kapusta, M. A., and Halberstam, D., *Immunochemistry*, **5**, 293 (1964).
[7] Boyden, S., *J. Exp. Med.*, **115**, 453 (1962).
[8] Hirsch, J. G., *J. Exp. Med.*, **103**, 589 (1956).
[9] Hayashi, H., Tokuda, A., and Udaka, K., *J. Exp. Med.*, **112**, 237 (1960).
[10] Hayashi, H., in *Biochemistry of the Acute Allergic Reactions* (edit. by Austen, K. F., and Becker, E. L.), 141 (Blackwell, Oxford, 1968).
[11] Hayashi, H., Udaka, K., Miyoshi, M., and Kudo, S., *Lab. Invest.*, **14**, 665 (1965).
[12] Sakabe, S., *Mie-Igaku*, **6**, 138 (1962) (in Japanese).
[13] Hayashi, H., Yoshinaga, M., Koono, M., Miyoshi, M., and Matsumura, M., *Brit. J. Exp. Path.*, **45**, 419 (1964).
[14] Yoshinaga, M., Tasaki, I., and Hayashi, H., *Biochim. Biophys. Acta*, **127**, 172 (1966).
[15] Tasaki, I., Yoshinaga, M., and Hayashi, H., *Biochim. Biophys. Acta*, **130**, 260 (1966).
[16] Hayashi, H., Tasaki, I., and Yoshinaga, M., *Nature*, **215**, 759 (1967).
[17] Tasaki, I., Yoshinaga, M., and Hayashi, H., *Protein, Nucleic Acid, Enzyme (Tokyo)*, **14**, 1336 (1969) (in Japanese).

Effect of Antilymphocytic Serum on the Appearance of Reticular Neoplasms in SJL/J Mice

IT has been shown that BALB/c mice infected with Moloney leukaemogenic virus develop a higher incidence of leukaemias and lymphomas, after a shorter latent period, when they are treated with antilymphocytic serum (ALS) than in the absence of ALS[1,2]. We therefore considered it of interest to examine the effects of ALS on a strain of mice in which lymphomas occur naturally without infection by any exogenous virus. Mice of the strain SJL/J spontaneously develop a high incidence of reticular neoplasms[3-5], which are of the pleiomorphic type *B* described by Dunn[6]. In untreated mice, neoplasms begin to appear when they are about 1 year old and the cumulative incidence before death is over 70 per cent.

ALS was raised by two intravenous injections of 10^9 SJL/J or CBA thymus cells into New Zealand rabbits[7]. In some experiments the active immunoglobulin fraction (ALG) was prepared[8]. Sera were de-complemented at 56° C for 30 min and absorbed with SJL/J erythrocytes before use. Controls received normal rabbit serum (NRS) or the IgG fraction prepared from it (NRG). In one experiment ALS or NRS was administered to SJL/J mice aged 4–6 weeks. At least three subcutaneous injections were given, and animals were killed when aged 27–30 weeks for autopsy. Of eleven survivors receiving ALS, eight showed histological evidence of reticular neoplasia, with distortion of the normal structure of lymph nodes and spleen and proliferation of plasma cells, pleiomorphic lymphoid cells and reticulum cells. None of six controls that had received NRS examined histologically showed signs of neoplasia. This difference is significant ($P < 0·01$).

In a second experiment, newborn SJL/J mice were made tolerant to normal rabbit IgG[1] before receiving eight injections at twice-weekly intervals of 1 mg ALG or NRG. Two mice receiving ALG died with gross neoplasms before the thirtieth week, at which time all survivors were killed for autopsy. Of nine mice receiving ALG, seven had histological evidence of reticular neoplasia, as previously described, whereas none of seven survivors of the group receiving NRG showed signs of neoplasia. This difference is again statistically significant ($P < 0\cdot01$). Two of three attempts to transplant the neoplasms in syngeneic recipients were successful.

These results show that treatment with ALS or ALG shortens the latent period for appearance of reticular neoplasms in SJL/J mice. The most likely explanation seems to be suppression by ALS of an immunological surveillance mechanism which normally retards the appearance of malignancy in this strain of mice. It is not yet clear whether an oncogenic virus plays a major part in this system, but SJL/J tumours show individual antigenicity when transplanted into syngeneic recipients[9], and they may well provoke an immune response in the autochthonous host. Possibly the proliferative stimulus of ALS to primitive lymphoid cells, or other factors, is also relevant. The potentiation by ALS of a naturally occurring malignancy in mice may perhaps serve as a rather extreme model for the appearance of reticulum-cell sarcomas in some human patients receiving chronic immunosuppressive therapy[10-12].

N. A. BURSTEIN*

Laboratory of Carcinogenesis,
National Cancer Institute,
Bethesda, Maryland.

A. C. ALLISON

Clinical Research Centre Laboratories,
National Institute for Medical Research,
London NW7.

Received January 5, 1970.

---

* Present address: Department of Pathology, Massachusetts General Hospital, Boston.

[1] Allison, A. C., and Law, L. W., *Proc. Soc. Exp. Biol. and Med.*, **127**, 207 (1968).

[2] Law, L. W., Ting, R. C., and Allison, A. C., *Nature*, **220**, 611 (1968).

[3] Murphy, E. D., *Proc. Amer. Assoc. Cancer Res.*, **4**, 46 (1963).

[4] McIntyre, K. R., and Law, L. W., *J. Nat. Cancer Inst.*, **39**, 1197 (1967).

[5] Haran-Ghera, N., Kotler, M., and Meshorer, A., *J. Nat. Cancer Inst.*, **39**, 653 (1967).

[6] Dunn, T. B., *J. Nat. Cancer Inst.*, **14**, 1281 (1954).

[7] Levey, R. H., and Medawar, P. B., *Proc. US Nat. Acad. Sci.*, **56**, 1130 (1966).

[8] James, K., and Anderson, N. F., *Nature*, **213**, 1195 (1967).

[9] Wanebo, H., Carswell, E., Old, C. J., and Boyse, E. A., *Proc. Amer. Assoc. Cancer Res.*, **10**, 97 (1969).

[10] Deodhar, S. D., Kuklinca, A. G., Vidt, D. G., Robertson, A. L., and Hazard, J. B., *New Engl. J. Med.*, **280**, 1104 (1969).

[11] Penn, I., Brettschneider, L., and Starzl, T. E., *Transplant. Proc.*, **1**, 106 (1969).

[12] McKhann, C. F., *Transplantation*, **8**, 209 (1969).

Effect of Route of Administration of Antilymphocytic Globulin on Skin Allograft Survival in Rats

ADMINISTRATION of antilymphocytic serum (ALS) or globulin (ALG) by subcutaneous[1], intramuscular[2], intraperitoneal[3] or intravenous[4] injection has been shown to prolong survival of allografts in various experimental systems. No data seem to be available, however, concerning the relative effect of administering the same dose of ALG by different routes on a particular graft in one species. We have compared the effect of daily intramuscular, intraperitoneal and intravenous injection, and continuous intravenous infusion, of ALG on skin allograft survival in rats.

Table 1. IMMUNIZATION SCHEDULE OF HORSE TO PRODUCE ALG

Antigen : rat thoracic duct lymphocytes

Day	Cell No. $\times 10^9$	Cell viability (per cent)
0	1·02	93
8	1·50	90
14	1·5	87
22	1·02	89
28	1·16	90
35	1·44	95
50	1·70	98
133	3·26	96
174	1·12	81
335	1·00	85
468	1·36	93

ALS was prepared by immunizing a horse with rat thoracic duct lymphocytes as shown in Table 1. The cells were injected intravenously except for the second dose which was given subcutaneously. Adjuvants were not used. Anti-rat ALG was prepared from serum as previously described[5] and absorbed with rat erythrocytes until the haemagglutinin titre of a 1 g/100 ml. solution was not greater than four. The batch used in the present experiments was from blood collected 3 weeks after the last injection of antigen. The cytotoxic titre^{-1} (50 per cent end point) by the trypan blue method[6], using rat thoracic duct lymphocytes and guinea-pig complement, was 128.

Inbred male hooded rats, approximately six months old, were sub-divided into four groups. All received 1·5 ml. of a 1 g/100 ml. solution of ALG daily for 10 days, but the method of administration to the various groups differed as follows: I, intramuscular injection; II, intraperitoneal injection; III, intravenous injection; IV, continuous intravenous infusion.

Intravenous ALG administration was effected with a variable rate infusion pump (B. Braun, Melsungen, Germany) by the technique of Dalton, Touraine and Wilson[7]. In the case of the "intravenous infusion", ALG was given continuously for 10 days. The pump had a delivery rate of 2·4 ml./24 h, so the daily dose of 1·5 ml. ALG solution was made up to 2·4 ml. with isotonic saline solution. Because ALG deteriorates if left at room temperature, syringes were filled once or twice daily. In the case of the daily "intravenous injection", the pump was regulated to deliver the 1·5 ml. dose of ALG solution in 4 min. Isotonic saline solution alone was then infused continuously (2·4 ml./24 h) to maintain the patency of the intravascular catheters. A fifth group of similar rats which were given no ALG (the controls) were infused intravenously with 2·4 ml./24 h of isotonic saline.

The rats were grafted on the third day of ALG administration with split skin from adult male rats of an inbred Wistar strain[8]. The protective dressing over the graft was removed after 7 days. The two rat strains have been maintained in this department for many years by brother–sister mating, and the survival time of Wistar skin allografts to untreated hooded rats not subjected to the technique of continuous intravenous infusion is 8 days[3]. Rats were killed when their skin grafts were completely rejected; and their kidneys were prepared for histological examination.

Table 2. SURVIVAL TIME OF SKIN GRAFTS IN RATS GIVEN THE SAME DOSE OF ALG BY FOUR DIFFERENT METHODS

Group	Treatment	Skin graft survival individual rats (days)	Mean (days)
I	Intramuscular injection	10, 10, 11, 11, 11, 11, 11,	11
II	Intraperitoneal injection	10, 10, 11, 11, 11, 11, 22	12
III	Intravenous injection	17, 18, 23, 23, 31, 32, 77	32 (24)*
IV	Intravenous infusion	17, 21†, 22, 26, 28, 31, 33	25
V	Controls	8, 8, 8, 9, 9, 11, 11	9

* Mean excluding rat with atypical graft survival time of 77 days.

† Rat died although graft was not rejected.

By Student's *t* test, the statistical significances of the differences between the group means were as follows: I and II, not significant; I and II combined : III*, $P < 0\cdot001$; I and II combined : IV, $P < 0\cdot001$; III* : IV, not significant.

The results are shown in Table 2. It can be seen that skin graft survival was significantly longer when ALG was administered intravenously than when it was injected intramuscularly or intraperitoneally. The skin graft survival of one rat in group III was exceptionally long.

If this apparently atypical result is excluded from the group mean, there is no significant difference between the mean graft survival time when ALG was given daily by intravenous injection (group III) or by continuous infusion (group IV). No abnormality was detected in sections of kidney examined by light microscopy.

The greater effectiveness of ALG given intravenously is consistent with the view[9] that its immunosuppressive effect is mainly to be attributed to its action on circulating small lymphocytes. Possibly, when given by other routes, some of the antibody is fixed to tissue at the site of injection and fails to reach the circulation. Further evidence on this point, and an explanation of the lack of difference between the results of a single intravenous injection of ALG and continuous intravenous infusion, might be obtained by studying the residual antilymphocytic activity of serum obtained from animals bled at intervals after injection of ALG. Experiments along these lines are in progress.

From the point of view of clinical application, these results are encouraging because increasing use is being made of intravenous injection of ALG into patients in order to avoid the painful local reactions which often occur with intramuscular or subcutaneous injection[10]. By taking appropriate precautions, including preliminary intradermal tests, slow injection and simultaneous administration of corticosteroids and antihistamines, the risk of acute anaphylaxis can be greatly reduced. Whether or not the risk of subsequent renal insufficiency as a result of damage to glomerular basement membrane or deposition of antigen–antibody complexes will prove to be serious with intravenous administration remains to be seen. Survival experiments and electron microscopic studies will, it is hoped, provide an answer to this question.

R. G. DALTON
J. L. TOURAINE
N. F. ANDERSON
M. F. A. WOODRUFF

Department of Surgical Science,
University of Edinburgh.

Received December 3, 1969.

[1] Levey, R. H., and Medawar, P. B., *Ann. NY Acad. Sci.*, **129**, 164 (1966).
[2] Starzl, T. E., Marchiero, T. L., Porter, K. A., Iwasaki, Y., and Cerilli, G. J., *Surg. Gynec. Obstet.*, **124**, 301 (1967).
[3] Woodruff, M. F. A., and Anderson, N. F., *Ann. NY Acad. Sci.*, **120**, 119 (1964).
[4] Abaza, H. M., Nolan, B., Watt, J. G., and Woodruff, M. F. A., *Transplantation*, **4**, 618 (1966).
[5] James, K., Pullar, D. M., and James, V. S., *Clin. Exp. Immunol.*, **3**, 963 (1968).
[6] Abaza, H. M., and Woodruff, M. F. A., *Revue Francaise d'Etudes Clinique et Biologiques*, **11**, 821 (1966).
[7] Dalton, R. G., Touraine, J. L., and Wilson, T. R., *J. Lab. Clin. Med.*, **74**, 813 (1969).
[8] Woodruff, M. F. A., and Simpson, L. O., *Plast. Reconstr. Surg.*, **15**, 451 (1955).
[9] Denman, A. M., and Frenkel, E. P., *Immunology*, **14**, 107 (1968).
[10] Woodruff, M. F. A., *Antibiotica et Chemotherapia*, **15**, 234 (1969).

Effect of Adjuvants on the Immunosuppressive Properties of Antilymphocytic Globulin

IN recent years we have been primarily concerned with investigating factors affecting the immunosuppressive properties of antilymphocytic antibody. Detailed studies on humoral systems in rats have indicated that effective immunosuppression is dependent on the intactness of the antilymphocytic IgG molecule, the time of its administration in relation to the antigen, the strain of rats used and the antigen under test[1]. Because adjuvants are generally known to influence markedly the ontogeny of the humoral immune response, it seemed desirable to ascertain whether the ability of antilymphocytic antibody to suppress the humoral response (in this case, to bovine serum albumin) was also dependent on the adjuvant in which it was administered. These preliminary studies demonstrate that such a dependence exists.

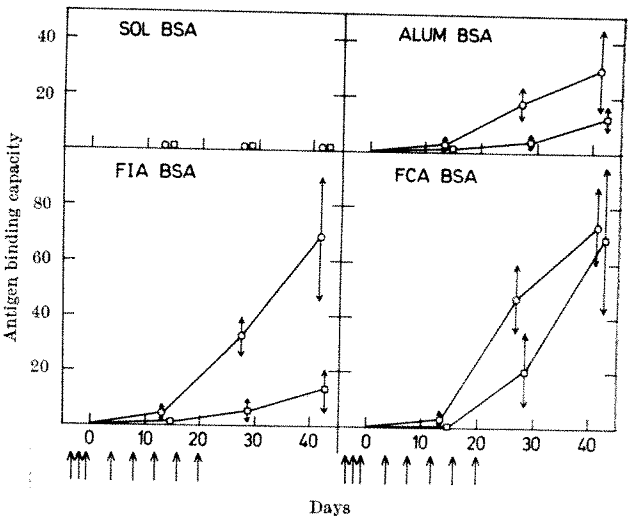


Fig. 1. The effect of antilymphocytic IgG on the response of hooded rats to bovine serum albumin. Note that in animals receiving the antigen in alum or Freund's incomplete adjuvant (FIA) there was a marked suppression of the humoral response throughout the experimental period. By contrast, animals receiving antigen in Freund's complete adjuvant (FCA) had fully recovered by day 42. ○, Normal IgG treatment; □, antilymphocytic IgG treatment.

The antilymphocytic serum was produced in horses using thoracic duct lymphocytes obtained from random bred Wistar rats. The immunization schedule and the preparation and properties of the normal horse and horse anti-rat lymphocyte IgG have been described in the preceding article[3]. The immunosuppressive studies were performed in groups (containing ten animals) of inbred hooded strain rats (sex female, age 5 months). The animals received intraperitoneal injections of 1 ml. of 2 per cent normal horse or antilymphocytic IgG on the days indicated in Fig. 1. On day 0 the rats were injected subcutaneously with 5 mg of bovine serum albumin (3X crystallized, Armour Pharmaceutical Co.). This was administered in soluble form (non-centrifuged in phosphate buffered saline) or as a suspension in 6·25 mg of alum ('Alhydrogel' supplied by the Supefos Export Co., Copenhagen) or in 1 ml. of Freund's complete or incomplete adjuvant (supplied by Difco, Detroit, Michigan).

The animals were bled by cardiac puncture on days 0, 14, 28 and 42. The antibody content of the sera obtained was assessed by the ammonium sulphate procedure of Farr[4], the results being expressed as antigen binding capacities (μg of BSA nitrogen bound by 1 ml. of rat serum). These values were estimated in a system containing 0·2 μg of bovine serum albumin nitrogen per test, the end point being the dilution of serum which bound 33 per cent of the test antigen. Standard antiserum and normal controls were used throughout these tests. Further details of the Farr and labelling procedures used are recorded elsewhere[2,4-6].

The mean antigen binding capacities observed are plotted together with their standard deviations (Fig. 1). A significant immune response was detected only in those animals receiving the test antigen incorporated in adjuvants. Furthermore, it will be noted that throughout the 6 weeks of the experiment the antilymphocytic IgG caused a significant suppression of the humoral response of animals injected with antigen incorporated in alum or Freund's incomplete adjuvant. By contrast, in the Freund's complete adjuvant group, the animals treated with antilymphocytic globulin quickly recovered their immunocompetence, the antigen binding capacities observed on day 42 being similar to those observed in animals treated with normal IgG. Thus the ability of antilymphocytic IgG to suppress the primary humoral response to bovine serum albumin is dependent on the adjuvant in which it is incorporated.

There are possible, though as yet untested, explanations of the above observations. Previous radioimmunoelectrophoretic studies on the immune response of rats to a variety of antigens, including bovine serum in Freund's complete adjuvant and bentonite, have clearly demonstrated the presence of five classes or sub-classes of antibody in this species, and shown that the relative distribution of antibody activity varies throughout the post-immunization period[7,8]. Furthermore, detailed investigations in mice have indicated that the quantity and quality (or class) of antibody evoked in response to antigens are dependent on the adjuvant in which it is incorporated[9,10]. On the basis of these observations it is claimed that adjuvants can markedly affect the expression of H chain genes[9], presumably by influencing the recruitment proliferation and/or maturation of specific antibody producing cells[10]. In the present studies, similar cytokinetic changes may have been occasioned by the mycobacterium component of the Freund's complete adjuvant. Thus an accelerated recovery of the antilymphocytic globulin treated cells or the selective proliferation of a population of antilymphocytic globulin refractive cells which produces antibody may have accompanied administration of this material. Such a population of cells might be responsible for the production of a select class or sub-class of antibody, and experiments are currently under way to test this hypothesis.

We thank Dr N. F. Anderson and Dr R. Dalton for producing the antilymphocytic serum, Mr C. Shepley for preparing the figure, and Professor M. F. A. Woodruff for his encouragement.

RANDALL A. ALLARDYCE
ROBERT D. HUNTER
KEITH JAMES

Department of Surgical Science,
University of Edinburgh Medical School,
Teviot Place,
Edinburgh.

Received December 5, 1969.

[1] James, K., *Fed. Proc.* (in the press).
[2] James, K., Pullar, D. M., and James, V. S., *Clin. Exp. Immunol.*, **3**, 963 (1968).
[3] Dalton, R. G., Touraine, J. L., Anderson, N. F., and Woodruff, M. F. A., *Nature*, **225**, 1140 (1970).
[4] Farr, R. S., *J. Infect. Dis.*, **103**, 239 (1958).
[5] Pinckard, R. N., Weir, D. M., and McBride, W. H., *Clin. Exp. Immunol.*, **2**, 331 (1967).
[6] Hunter, W. M., and Greenwood, F. C., *Nature*, **194**, 495 (1962).
[7] Banovitz, J., and Ishizaka, K., *Proc. Soc. Exp. Biol. and Med.*, **125**, 78 (1967).
[8] Bloch, K. J., Morse, H. C., and Austen, K. F., *J. Immunol.*, **101**, 650 (1968).
[9] Warner, N. L., Vaz, N. M., and Ovary, Z., *Immunology*, **14**, 725 (1968).
[10] Barth, W. F., McLaughlin, C. L., and Fahey, J. L., *J. Immunol.*, **95**, 781 (1965).

Adding a Ventricle instead of transplanting a Heart

IN heart transplant operations, as practised at present, an unpaired vital organ is excised and another of doubtful viability and doubtful compatibility is substituted. It is no surprise to find that these operations are looked on with some disfavour and have a high mortality rate and poor long term results.

Most patients considered for heart transplants suffer from coronary artery disease and their disability is the consequence of impairment of contraction of the left ventricle. An operation which mounted a donor left ventricle parallel to their own, sharing the work load between the two hearts, might be all they need.

I have shown the feasibility of such an operation experimentally. The donor heart is placed upside down next to the recipient heart with the two left atria adjacent. The atrial appendage and adjacent atrial walls are excised and the two atria joined together to form a common chamber. The donor aorta is anastomosed end-to-side to the descending thoracic aorta. Coronary sinus blood is returned to the circulation by making an atrial septal defect in the donor heart. The donor right ventricle is defunctioned by making the tricuspid valve incompetent. All vessels in the donor heart except the aorta are tied off.

I have implanted additional hearts in this way into seven dogs, and had six immediate survivors. Three died after 9, 9 and 6 days with rupture of the anastomoses from infection. In one the donor heart went into cardiac arrest even before being excised, and showed no signs of activity after the second day. At present one dog is alive after 9 days and another after 48 days. The latter is showing signs of rejection for the first time. Except in one case, the ventricles have been seen to eject blood against the blood pressure created by the recipient heart. The exception was in the donor heart which was in poor condition when implanted. On some occasions the blood pressure produced by the donor has been equal to that of the recipient heart. When the recipient heart is healthy, however, it is not possible to see the ejection from the donor heart on the blood pressure trace. The obvious next step is to impair the function of the recipient left ventricle and to see the extent to which the donor left ventricle is able to take over the load.

The arrangement has interesting experimental possibilities. Its great advantage is that rejection does not impair the blood supply to the transplanted heart; rejection can be studied without the accompanying cardiac failure and ischaemia which must occur if a single transplanted heart rejects. The reaction of a denervated heart to drugs can be compared with that of the normal heart.

It may be possible to consider this operation eventually for human transplants, for it has several theoretical advantages. First, the recipient's heart is not excised; second, the operation can be done without a heart–lung machine; third, rejection does not totally jeopardize the patient; and fourth, the donor heart, if rejected, could be excised and another substituted.

KENNETH BLOOR

Department of Surgery,
The Royal Infirmary,
Manchester M13 9WL.

Received January 26, 1970.

Some Properties of Angiotensin Converting Enzyme in the Lung *in vivo*

THE lung has many important metabolic functions[1,2] which include the selective activation or inactivation of endogenous peptides. Bradykinin is inactivated on transit through the pulmonary circulation[3,4], probably by at least two endopeptidases[5], but other peptides such as angiotensin II (refs. 6–10), polistes kinin[11] and eledoisin[3,4] pass through without loss.

The lungs also contain a highly active angiotensin converting enzyme[8,12–15]. This enzyme removes histidine and leucine from the C-terminal of angiotensin I, thereby generating angiotensin II, which is 10–50 times more potent physiologically. Converting enzyme was first isolated from plasma[16,17], but the activity of the plasma enzyme is too low to account for the rapid conversion of angiotensin I to II *in vivo*[12,13].

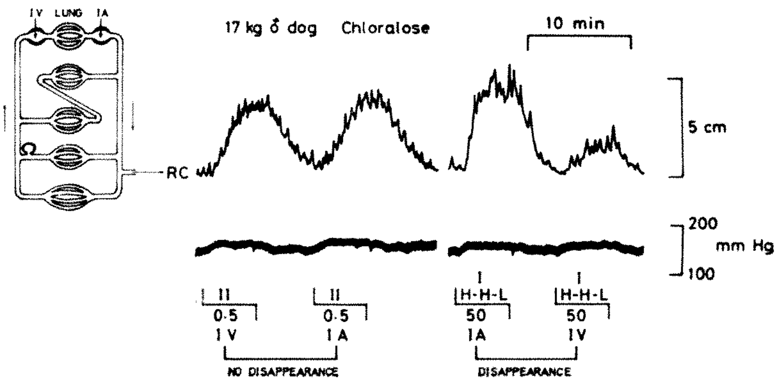


Fig. 1. Male dog (17 kg). The diagram illustrates the route of infusions: intravenous (IV) infusions were given into the femoral vein, intra-aortic (IA) infusions were given into the root of the aorta and blood at 10 ml./min was sampled continuously from the femoral artery for superfusion of the rat colon (RC). Intravenous and intra-arterial angiotensin II (0·5 μg/min) produced the same effects on the rat colon. HHL analogue (50 μg/min) intravenously gave a contraction very much smaller than that produced by 50 μg/min intra-arterially, showing inactivation in the pulmonary circulation. Time, 10 min; vertical scales, 5 cm and mm Hg.

The converting enzyme in lung may be an endopeptidase similar to that in plasma, or a carboxypeptidase which first cleaves the leucine and then the histidine from angiotensin I. The availability of an undecapeptide related to angiotensin but containing an extra histidine residue at position 10 ([Ile^5, His^9, His^{10}, Leu^{11}]-angiotensin II; HHL analogue) led us to test this substance for its ability to act at angiotensin receptors and also as a substrate for converting enzyme. The angiotensin-like activity of the HHL analogue was measured on rat colons[18], superfused[19] either with Krebs solution or with blood, using the blood-bathed organ technique[20], as previously described[12,13].

When superfused with Krebs solution, the rat colon preparations were contracted by infusions of angiotensin II (Hypertensin, Ciba) to give concentrations of 1–10 ng/ml. High concentrations (200–2,000 ng/ml.) of the HHL analogue were necessary to produce similar contractions (Table 1). Thus the HHL analogue had 0·5–0·6 per cent of the activity of angiotensin II in Krebs solution. In arterial blood of dog or cat (contact time 30 s), the relative activity was 1·0–1·2 per cent. After intravenous infusions, however, the relative activity on both blood pressure and the rat colon preparations was 0·2–0·3 per cent. Because the assay organs were bathed in carotid arterial blood, the reduction in the relative activity of the HHL analogue after intravenous infusion suggested a disappearance or inactivation of the peptide during passage through the lungs. In direct comparison, intravenous infusions of the HHL analogue (20–100 μg/min) induced a smaller contraction of the rat colon than that produced by the same rate of infusion into the root of the aorta. A comparison of the contractions showed that 40–50 per cent (five observations) of the HHL analogue disappeared in transit through the lungs of either dog or cat.

Table 1. RELATIVE ACTIVITY OF [ILE^5]-ANGIOTENSIN I AND THE HHL ANALOGUE ON THE RAT COLON IN KREBS SOLUTION, AFTER INCUBATION IN BLOOD FOR 30 S AND AFTER INTRAVENOUS INFUSIONS

	Angiotensin I (from ref. 13)	HHL analogue (per cent)
Krebs solution	2–10% (10)	0·5–0·6 (4)
Blood (30 s)	3–30% (11)	1·0–1·2 (8)
Intravenous	20–60% (10)	0·2–0·3 (6)

Activity is expressed as the range of percentage activity of angiotensin II amide.

The figures in parentheses refer to the number of observations made on the assay organs.

Fig. 1 illustrates this disappearance. Infusions of angiotensin II (0·5 μg/min) gave similar contractions of the blood-bathed rat colon when given either into the aorta or intravenously, showing that angiotensin II passed through the lungs without loss. An intravenous infusion of the HHL analogue (50 μg/min), however, was less potent than the intra-aortic infusion, showing that the HHL analogue was inactivated.

This inactivation of the HHL analogue is consistent with the converting enzyme of lung being an endopeptidase similar to that of plasma, for stepwise cleavage by a carboxypeptidase would have resulted in production of angiotensin II which is resistant to further breakdown by the lungs. This inactivation of the HHL analogue was unlikely to be caused by the action of an endopeptidase acting at any peptide links in the angiotensin II moiety of HHL because angiotensin II survives passage through the lungs. Possibly converting enzyme splits the His^9–His^{10} bond leaving [His^9]-angiotensin II, the activity of which could be very low.

Ferreira[21] has isolated from the venom of the South American snake *Bothrops jararaca* a mixture of peptides ("bradykinin potentiating factor", BPF) which inhibits kininases[3,22,23]. BPF also inhibits converting enzyme both in cell-free extracts of dog lung[24] and in isolated perfused lung[8]. We have now tested BPF as a converting enzyme inhibitor *in vivo*. Because of the scarcity of the extract this has been done in rats.

Six rats of either sex (250–300 g) were anaesthetized with 6 mg/100 g of pentobarbitone sodium intraperitoneally and given 4 mg/100 g of pentolinium tartrate subcutaneously. Cannulae were inserted into both external jugular veins and blood pressure was recorded from either the carotid or femoral artery. Intra-aortic injections were given through a fine polyethylene tubing inserted via the left carotid artery and advanced so that its tip lay above the aortic valves. Heparin (500 IU/100 g) was given intravenously and the mean blood pressure was recorded by a Statham *P23Db* transducer on a Beckman-Offner dynograph.

[Ile^5]-angiotensin I (10–100 ng) injected into the vein was twice as potent in raising the systemic blood pressure as when it was injected into the root of the aorta, confirming activation of intravenous angiotensin I in the lungs. Peripheral vascular beds inactivate about half of the angiotensin II reaching them[7–9], leaving the other half to recirculate. Thus even with 100 per cent conversion of angiotensin I to II in one transit through the lungs, the maximum possible difference between potencies of intravenous and intra-aortic injections would be two-fold.

The effect of BPF on the conversion of angiotensin I to II was examined in four other rats. An experiment is illustrated in Fig. 2. Panel *A* shows that 50 ng of angiotensin I produced a rise in blood pressure slightly greater than that produced by 20 ng of angiotensin II (a ratio of about 2 : 1). Panel *B* shows that during infusion of BPF (0·1 mg/min intravenously), although the pressor response to angiotensin II was unaffected, the response induced by angiotensin I was considerably reduced so that the ratio became about 15 : 1. Panel *C*, at a faster paper speed, also shows that the residual effect of angiotensin I in the presence of BPF is more prolonged than that of angiotensin II. Similar results were obtained in three more experiments. The inhibitory effect of BPF on the pressor activity of angiotensin I was rapidly reversible; within 5–10 min of stopping the infusion of BPF the pressor activity of angiotensin I had almost fully recovered.

These experiments show that BPF greatly reduces the hypertensive activity of angiotensin I *in vivo*. In view of the *in vitro* results with the same extract[8,24], it is reasonable to conclude that the reduction in activity is due to inhibition of lung converting enzyme. Experiments are in progress to determine whether the plasma converting enzyme is also inhibited by BPF.

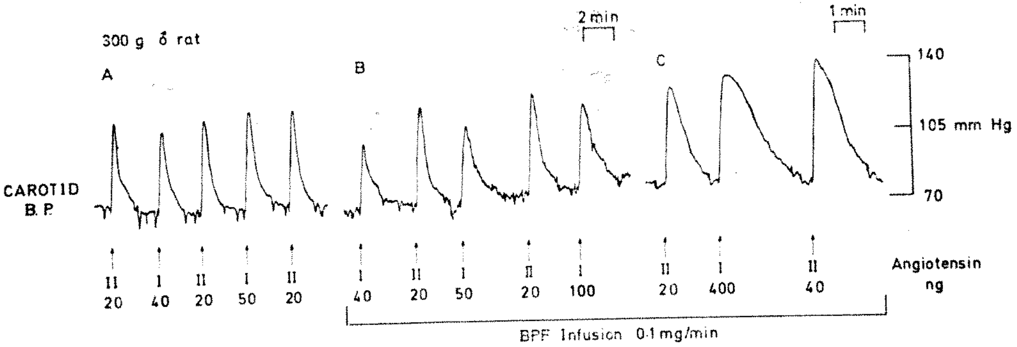


Fig. 2. Effects of BPF on the pressor responses to intravenous injections of angiotensin I and II in an anaesthetized rat (300 g). Panel *A* shows that angiotensin I was nearly half as potent as angiotensin II. The potency was reduced to about one-fifteenth of angiotensin II during BPF infusion (panels *B* and *C*). Time, 2 min in panel *A* and panel *B*; 1 min in panel *C*; vertical scales, mm Hg.

We thank Dr S. Wilkinson of the Wellcome Foundation for gifts of angiotensin I and the HHL analogue and Dr S. H. Ferreira for the gift of BPF. One of us (K. K. F. N.) was an overseas scholar of the University of Singapore. This work was supported by the MRC and the Wellcome Trust.

K. K. F. NG*
J. R. VANE

Department of Pharmacology,
Institute of Basic Medical Sciences,
Royal College of Surgeons of England,
Lincoln's Inn Fields,
London WC2.

Received January 9, 1970.

* Present address: Department of Pharmacology, University of Singapore, Singapore, 3.

[1] Vane, J. R., *Brit. J. Pharmacol.*, **35**, 209 (1969).
[2] Heinemann, H. O., and Fishman, A. P., *Physiol. Rev.*, **49**, 1 (1969).
[3] Ferreira, S. H., and Vane, J. R., *Brit. J. Pharmacol.*, **29**, 367 (1967).
[4] Biron, P., and Charbonneau, R., *Rev. Canad. Biol.*, **27**, 75 (1968).
[5] Ryan, J. W., Roblero, J., and Stewart, J. M., *Biochem. J.*, **110**, 795 (1968).
[6] Goffinet, J. A., and Mulrow, P. J., *Clin. Res.*, **11**, 408 (1965).
[7] Hodge, R. L., Ng, K. K. F., and Vane, J. R., *Nature*, **215**, 138 (1967).
[8] Bakhle, Y. S., Reynard, A. M., and Vane, J. R., *Nature*, **222**, 956 (1969).
[9] Biron, P., Meyer, P., and Pannisset, J. C., *Canad. J. Physiol. Pharmacol.*, **46**, 175 (1968).
[10] Leary, W. P., and Ledingham, J. G., *Nature*, **222**, 959 (1969).
[11] Stewart, J. M., *Fed. Proc.*, **27**, 63 (1968).
[12] Ng, K. K. F., and Vane, J. R., *Nature*, **216**, 762 (1967).
[13] Ng, K. K. F., and Vane, J. R., *Nature*, **218**, 144 (1968).
[14] Biron, P., and Huggins, C. G., *Life Sci.*, **7**, 965 (1968).
[15] Oparil, S., Sanders, C. A., and Haber, E., *Fed. Proc.*, **28**, 580 (1969).
[16] Skeggs, L. T., Marsh, W. H., Kahn, J. R., and Shumway, N. P., *J. Exp. Med.*, **99**, 275 (1954).
[17] Skeggs, L. T., Kahn, J. R., and Shumway, N. P., *J. Exp. Med.*, **103**, 295 (1956).
[18] Regoli, D., and Vane, J. R., *Brit. J. Pharmacol. Chemother.*, **23**, 351 (1964).
[19] Gaddum, J. H., *Brit. J. Pharmacol. Chemother.*, **8**, 321 (1953).
[20] Vane, J. R., *Brit. J. Pharmacol. Chemother.*, **23**, 360 (1964).
[21] Ferreira, S. H., *Brit. J. Pharmacol. Chemother.*, **24**, 163 (1965).
[22] Ferreira, S. H., and Roche e Silva, M., *Experientia*, **121**, 347 (1965).
[23] Ferreira, S. H., in *Hypotensive Peptides* (edit. by Erdös, G., Back, N., and Sicuteri, F.), 356 (Springer Verlag, New York, 1966).
[24] Bakhle, Y. S., *Nature*, **220**, 919 (1968).

Effects on Smooth Muscle Preparations of Unidentified Vasoactive Peptides from Intestine and Lung

EXTRACTS of lung[1,2] and of small intestine[3] from the pig contain previously unidentified vasoactive peptides which, when given intravenously, increase peripheral blood flow and cause a systemic hypotension, both effects being long lasting. Although the peptide fractions from the lung and small intestine are extractable by similar techniques, the vascular responses they elicit are somewhat different. One peptide from the intestine induced a quicker and more pronounced vasodilatation than either the second peptide from intestine or that from lung[3]. These substances have not yet been completely purified, and so their activity may be due to a single compound or to several different ones. Because of the short supply of extracted peptides, investigation of their biological activity has been limited largely to their peripheral vasodilator effect.

Superfusion[4] of several isolated organs in cascade[5,6] was used to clarify some of these points. We tested the activity of three peptide fractions, one from the lung and two from the gut, on a variety of isolated smooth muscle preparations to determine: (1) whether a particular tissue was suitable for detecting and assaying the peptides; (2) the activity of the peptides on different smooth muscle preparations; (3) the degree of similarity between the activity of the three peptide fractions; and (4) any resemblance between the actions of the peptides and those of known compounds. The activity of the following peptide preparations was investigated: (L) a desalted methanol extract from hog lung[1,2], (G 1) the peptide fraction of methanol extract of hog small intestine which, on chromatography on carboxymethyl cellulose (CMC),

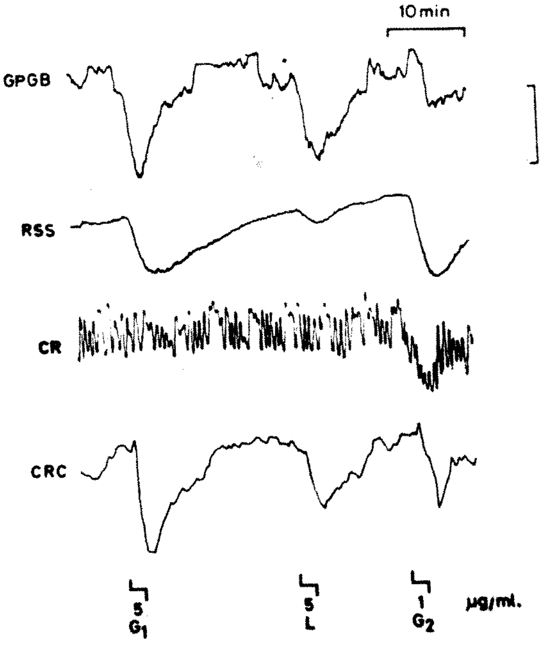


Fig. 1. A guinea-pig gall-bladder (GPGB), rat stomach strip (RSS), chick rectum (CR) and chick rectal caecum (CRC) were superfused with Krebs solution at 10 ml./min. Each of the three peptides G 1 (5 μg/ml.), L (5 μg/ml.) and G 2 (1 μg/ml.) relaxed these tissues, but the pattern of activity was different. Time 10 min, vertical scale 5 cm.

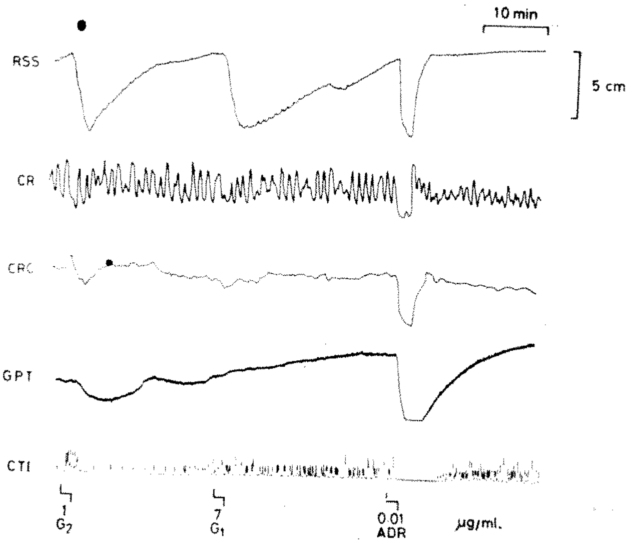


Fig. 2. A rat stomach strip (RSS), chick rectum (CR), chick rectal caecum (CRC), guinea-pig trachea (GPT) and cat terminal ileum (CTI) were superfused with Krebs solution at 10 ml./min. G 2 (1 µg/ml.) relaxed all tissues except the cat terminal ileum which contracted. G 1 (7 µg/ml.) was much less potent. The relative activities of G 1 and G 2 in the tissues were different from that of adrenaline (0·01 µg/ml.). Time 10 min, vertical scale 5 cm.

in phosphate buffer at pH 8, was eluted ahead of secretin[7] (pre-secretin gut fraction), and (G 2) the peptide fraction of the same extract which remained absorbed to the CMC column in the same conditions and was eluted with 0·2 M HCl (post-secretin gut fraction). Further purification of G 2 yielded another fraction labelled vaso-inhibitory peptide (VIP).

The lyophilized preparations to be tested were dissolved in physiological saline and, like the calibrating solutions, were added to the perfusing fluid by a constant flow infusion pump.

The isolated smooth muscle preparations were chosen from those in use in this laboratory. They were superfused at 10 ml./min with Krebs solution warmed to 37° C and bubbled with 5 per cent CO_2 in O_2. The tissues were arranged in two cascades, each containing two or three preparations[5,6]. The sensitivities of the preparations to histamine, adrenaline, and so on, were demonstrated and the three peptides were then tested. In the concentrations used (10^{-8}–2×10^{-6} g/ml.), the peptides had no action on nine of the fifteen preparations tested. These were the longitudinal muscles of the guinea-pig ileum, cat jejunum[8], rat colon[9], rat duodenum[10] and rat uterus; rat atria, spirally cut strips of rabbit aorta[11], longitudinal strips of rabbit portal vein[12] and duck pulmonary artery[13].

The peptides (10^{-7} g/ml.) were active on six tissues (Figs. 1 and 2), the rat stomach strip[14], chick rectum[15], chick rectal caecum[16], guinea-pig trachea (cut as a zig-zag) and longitudinal strips of guinea-pig gall-bladder and cat terminal ileum. The spectrum of activity distinguished them from the known substances tested, including bradykinin, angiotensins, secretin, prostaglandins, 5-hydroxytryptamine and the catecholamines. The most potent preparation was the post-secretin gut fraction (G 2) or VIP and the least potent was the pulmonary fraction (L). The impurity of the fractions may, however, have contributed to the variation in potency between them. All these preparations were relaxed by fraction G 2 except the cat terminal ileum which was contracted. This contraction was selectively abolished by 10^{-7} g/ml. of mepyramine, so it was probably due to slight contamination with histamine. This conclusion was further reinforced by use of the more purified fraction of G 2. VIP had about four times the relaxant potency of G 2 on the rat stomach strip and guinea-pig trachea, but none of the contractor activity on the cat terminal ileum (Fig. 3).

The other gut peptide (G 1) and the lung peptide (L) did not contract the cat terminal ileum, but they relaxed guinea-pig gall-bladder, rat stomach strip, chick rectum and rectal caecum. They had little or no action on the guinea-pig trachea. Thus even though the inhibitory reactions to the three peptide preparations were similar, they could be distinguished by their relative reactions on the different smooth muscle preparations. Doses of peptide fractions G 1 and G 2 (or VIP) inducing comparable relaxation of the rat stomach caused unequal relaxation of the chick rectum or of the chick rectal caecum (Figs. 1 and 2). Also, while giving an equivalent relaxation of the guinea-pig gall-bladder to that caused by G 1, a dose of L produced a much weaker relaxation of the rat stomach (Fig. 1). These differences suggest that the

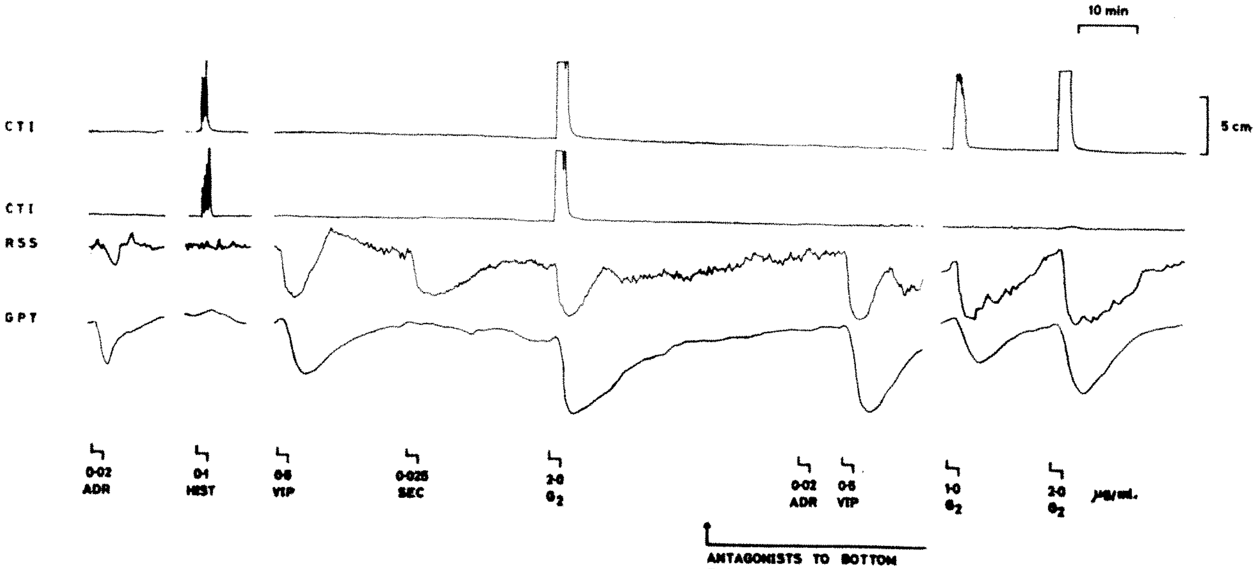


Fig. 3. Two cat terminal ileums (CTI), a rat stomach strip (RSS) and a guinea-pig trachea (GPT) were superfused with Krebs solution at 10 ml./min. The first two panels show that adrenaline (0·02 µg/ml.) relaxed the RSS and GPT whereas histamine (0·1 µg/ml.) contracted the CTI and GPT. VIP (0·5 µg/ml.) had about the same potency as G 2 (2 µg/ml.) on the RSS and GPT, but did not contract the CTI. Secretin (0·025 µg/ml.) relaxed only the RSS. The mixture of antagonists (for details, see text) was applied to the lower three tissues. It abolished the relaxant actions of adrenaline but did not affect the relaxant actions of VIP or G 2. The contraction of the CTI in response to G 2 was abolished. Time 10 min, vertical scale 5 cm.

active principles in the pulmonary and intestinal extracts are not identical.

The reactions of the isolated tissues distinguish the main activity of all of the peptide fractions tested from histamine (which contracts chick rectum, cat ileum and guinea-pig gall-bladder), bradykinin (which contracts rat uterus, rat stomach strip, cat jejunum and cat ileum), prostaglandins (which contract chick rectum, rat colon and rat stomach strip), and catecholamines (which stimulate atrial muscle, contract rabbit aorta, rabbit portal vein and duck pulmonary artery and relax rat colon, rat stomach and chick rectum). The effects of various antagonists further distinguished these peptides from known substances. In concentrations which blocked the effects of catecholamines, phenoxybenzamine (10^{-7} g/ml.) and propranolol (2×10^{-6} g/ml.) did not antagonize the effects of the peptides. When a combination of several antagonists was used (Fig. 3) the effects of G 2 and VIP were unaltered, except for the abolition of the response of the cat terminal ileum due to contamination of G 2 by histamine. The mixture of antagonists contained mepyramine maleate (10^{-7} g/ml.) to block the effects of histamine, methysergide bimaleate (2×10^{-7} g/ml.) to antagonize 5-hydroxytryptamine, hyoscine hydrobromide (10^{-7} g/ml.) to antagonize acetylcholine, phenoxybenzamine hydrochloride (10^{-7} g/ml.) and propranolol hydrochloride (2×10^{-6} g/ml.) to antagonize the α and β-effects of catecholamines.

Fig. 3 also shows that synthetic secretin differed from the G 2 and VIP extracts, for it relaxed only the rat stomach strip. The relaxation of the rat stomach strip by secretin was dose-dependent and occurred at low concentrations (10–100 ng/ml.). This unexpected effect may be useful as a bioassay for preparations of secretin. It also suggests that the inhibitory activity of secretin on intestinal muscle should be further investigated.

The demonstration that the three unidentified peptides have potent inhibitory effects on tracheal muscle and on several different intestinal smooth muscles, as well as vasodilator activity[1-3], makes it possible that the peptides have a physiological function. This might be either as a local hormone, affecting the smooth muscle of the organs containing them, or as a circulating hormone. Their inhibitory actions on vascular, respiratory and intestinal smooth muscle would certainly be prolonged if they were released into the blood stream.

One important feature of these results is their possible application for the assay of the active peptides during further purification. For instance, in the case of G 2 (or VIP), use of the rat stomach should provide an assay method which is many times more sensitive and also more specific than the method used so far, namely, intra-arterial injection in the dog hind limb. In further work, use of the blood-bathed organ technique[17] should afford opportunities for studying aspects of the metabolism and possible release of these newly identified agents *in vivo*.

We thank Dr M. Ondetti of the Squibb Institute for Medical Research for a sample of synthetic secretin and the Wellcome Trust, the Medical Research Council, the US Public Health Service, the American Heart and the National Tuberculosis and Respiratory Disease Associations for grants. S. I. S. is a recipient of a research career development award from NIH.

PRISCILLA J. PIPER
S. I. SAID*
J. R. VANE

Department of Pharmacology,
Institute of Basic Medical Sciences,
Royal College of Surgeons of England,
Lincoln's Inn Fields,
London WC2.

Received January 9, 1970.

* Present address: Department of Medicine, Medical College of Virginia, Richmond, Virginia.

[1] Said, S. I., and Mutt, V., *Scand. J. Clin. Lab. Invest.*, **24** (Suppl. 107), 51 (1969).
[2] Said, S. I., and Mutt, V., *Nature*, **224**, 699 (1969).
[3] Said, S. I., and Mutt, V., *Nature*, **225**, 863 (1970).
[4] Gaddum, J. H., *Brit. J. Pharmacol. Chemother.*, **8**, 321 (1953).
[5] Piper, Priscilla J., and Vane, J. R., *Nature*, **223**, 29 (1969).
[6] Gilmore, N., Vane, J. R., and Wyllie, J. H., *Nature*, **218**, 1135 (1968).
[7] Mutt, V., *Arkiv Kemi*, **15**, 69 (1959).
[8] Ferreira, S. H., and Vane, J. R., *Brit. J. Pharmacol. Chemother.*, **29**, 367 (1967).
[9] Regoli, D., and Vane, J. R., *Brit. J. Pharmacol. Chemother.*, **23**, 351 (1964).
[10] Horton, E. W., *Brit. J. Pharmacol. Chemother.*, **14**, 125 (1959).
[11] Furchgott, R. F., *Pharmacol. Rev.*, **7**, 183 (1955).
[12] Hughes, J., and Vane, J. R., *Brit. J. Pharmacol.*, **30**, 46 (1967).
[13] Vane, J. R., and Williams, I., *Brit. J. Pharmacol.*, **38**, 444*P* (1970).
[14] Vane, J. R., *Brit. J. Pharmacol. Chemother.*, **12**, 344 (1957).
[15] Mann, M., and West, G. B., *Brit. J. Pharmacol. Chemother.*, **5**, 173 (1950).
[16] Barsoum, G. S., and Gaddum, J. H., *J. Physiol.*, **85**, 1 (1935).
[17] Vane, J. R., *Brit. J. Pharmacol. Chemother.*, **23**, 360 (1964).

Magnetic Fields and the Number of Blood Platelets

INVESTIGATIONS of mice exposed to strong homogeneous magnetic fields have revealed that the number of megakaryocytes decreases in the bone marrow and increases in the spleen of the exposed animals[1]. It is currently assumed that platelets originate in the bone marrow when the cytoplasm of megakaryocytes becomes fragmented; the platelets then enter the blood stream. In mice the mean circulating life-span of platelets is approximately 4 days[2]. The purpose of the investigation we report here was to find out whether magnetic fields, of the same strength as those in which we have observed the change in the number of megakaryocytes in the bone marrow, would also alter the number of platelets in the circulating blood.

Female virgin DBA/J2 strain mice, 100–120 days old, were used in two experiments. Six mice in the first experiment, and five in the second, were placed for 20 days in a 9,000 oersted homogeneous, vertical magnetic field, while an equal number of mice were kept in identical "dummy magnets". Details of the magnet, the cage arrangement and so on can be found in ref. 1.

Blood was taken every 3–6 days (always at the same time of the day) from the tail vein of the mouse. From a sharp incision made with a razor blade, blood was removed with the help of a 13 μl. volume capillary syringe ('Unopette') and was immediately mixed with 1·3 cm^3 of 1 per cent ammonium oxalate, buffered to *p*H 6·5–6·8. After haemolysis of the red cells, the chamber of a haemocytometer was filled with the diluted blood sample and, when settling was completed, the number of blood platelets was counted with a phase contrast microscope (× 600, dark contrast objective). From each blood sample 200–400 platelets were counted. The standard error of the mean of a group of five or six mice was on average 4 per cent. Occasionally deviations from the average in a single animal amounted to 25 per cent.

Fig. 1 shows the relative change in the number of platelets: the average of the two magnet group values expressed as a percentage of the corresponding control group values. The mice kept in the magnetic field showed, in both experiments, as early as the third day of their exposure to the field, a 25 ± 3 per cent increase in the number of platelets over the value of their controls. From the tenth to the last day of their residence in the field, the difference between the magnet and control groups vanished. The observed variation in the number of platelets could be interpreted as a reaction of the organism to the stress of the magnetic field[3]. After removal of the mice from the magnetic field we see a repetition of the same pattern: an increase in platelet number. Further investigations will have to clarify whether the magnetic field merely changes the number of

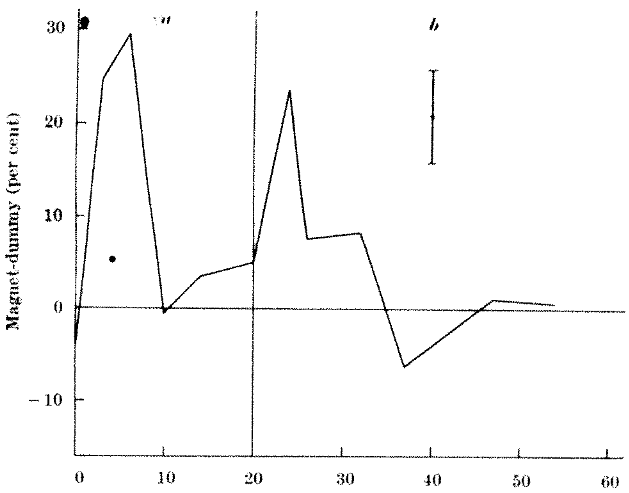


Fig. 1. Average platelet number of mice expressed as percentage of the control group values, during exposure to a magnetic field of 9,000 oersted (*a*) and after removal of the animals from the field (*b*). The bar shows the average standard error of one point.

blood platelets, or whether it also produces morphological alterations in them. Investigations in a magnetic field may contribute to the further elucidation of the clotting mechanism, and we may speculate that ultimately magnetic fields may be used to control some haemorrhagic disorders.

We cannot rule out the possibility that the beneficial effect we obtained by counteracting radiation syndromes[4], through pre-irradiation exposure of mice to magnetic fields, may have been caused partly by the increased number of platelets in the circulating blood after the magnetic exposure ended. The increased number of platelets may have reduced the death rate from intestinal haemorrhages.

This research was supported by a graduate research grant of the University of Illinois and by the Biomagnetic Research Foundation.

Madeleine F. Barnothy

College of Pharmacy,
University of Illinois,
Chicago, Illinois 60612.

Jeno M. Barnothy

Biomagnetic Research Foundation,
833 Lincoln Street,
Evanston, Illinois 60201.

Received October 20, 1969; revised January 5, 1970.

[1] Barnothy, M. F., and Sümegi, I., *Nature*, **221**, 270 (1969).

[2] Russel, E. S., and Bernstein, S. E., *Biology of the Laboratory Mouse*, 353 (McGraw-Hill, 1966).

[3] Barnothy, M. F., and Sümegi, I., in *Biological Effects of Magnetic Fields* (edit. by Barnothy, M. F.), **2**, 103 (Plenum Publ., 1969).

[4] Barnothy, M. F., and Barnothy, J. M., *Nature*, **181**, 1785 (1958).

Simultaneous Measurement of Cytochrome a_3 Reduction and Chemoreceptor Afferent Activity in the Carotid Body

The discharge frequency of carotid body chemoreceptors increases progressively as p_aO_2 is reduced below 250–150 mm Hg[1]. Chemical agents which either inhibit electron transport or uncouple oxidative phosphorylation in other tissues also increase chemoreceptor discharge[2,3]; however, the fall in pO_2 required to stimulate the receptors physiologically is much smaller than that required to influence mitochondrial respiration in other tissues[4,5]. It appears then that mitochondria of the carotid chemoreceptors must have unusual properties if they play a part in the oxygen related generation of chemoreceptor impulses. To evaluate the relationship between chemoreceptor discharge and respiratory chain activity we simultaneously measured the frequency of the potentials and the cytochrome a_3 reduction level in the carotid body. The organ was perfused with physiological saline *in vitro* because it must be both adequately oxygenated and free of haemoglobin to permit spectrophotometric measurement of cytochrome a_3 reduction.

Cats were anaesthetized with sodium pentobarbitone and the carotid bifurcation and sinus nerve exposed. The preparation was heparinized, and all arteries branching from the bifurcation were ligated. After stopping the heart with a large dose of anaesthetic, the carotid bifurcation was flushed free of blood with Locke's solution[6] injected into the common carotid artery. The bifurcation with the attached sinus nerve was removed and placed in oxygenated Locke's solution. All tissues surrounding the carotid body were removed to facilitate transmission of light and the adventitia stripped from the sinus wall to minimize baroreceptor afferent activity in the sinus nerve[7]. The preparation was transferred to a chamber—like that used to superfuse the carotid body[6,8]—which was provided with a second inlet tube with which the common carotid artery was cannulated so that the carotid body could be both perfused and superfused. Reservoirs containing Locke's solution plus dextran equilibrated with oxygen (100, 20, 12·8 and 5 per cent) and nitrogen (100 per cent) were placed above the chamber to provide a perfusion pressure of 130 mm Hg. Flow in the superfusion line was controlled with a clamp so that the level of solution in the chamber was just sufficient to cover the carotid body. The temperature of the bathing solution in the chamber was maintained at 36° C. Liquid paraffin was layered over the solution and the sinus nerve lifted into the paraffin and placed on a platinum electrode. Action potentials were amplified and displayed on an oscilloscope and also introduced into a pulse height selector. Pulses of constant amplitude and duration then triggered a rate meter, the output of which was recorded on a Grass polygraph.

The preparation was observed with a microscope equipped with a × 11 objective lens. A photomultiplier tube was introduced into the body tube of the microscope so that the end-window intercepted the light beam at a plane where a distinct image of the object was not formed. Transillumination was provided by rapidly alternating beams of light from two monochromators using the same incandescent lamp as a light source. The wavelength span of one beam (the measuring beam) was chosen to coincide with the absorption peak of a cytochrome or of haemoglobin; the other provided a reference signal. The latter was kept constant by feedback on the voltage to the dynode stages of the photomultiplier. Changes in light transmission were measured differentially between the two beams. This ensures elimination of artefactual amplification or attenuation of existing differences between the two light signals caused by large equal changes (for example, by light scattering) in the light beams during passage through the tissue. This technique is an adaptation and extension of dual-wavelength spectrophotometry introduced by Dr B. Chance.

Oxygen response curves were plotted from the ratemeter recordings of chemoreceptor discharge in four experiments. Discharge was maximum when the carotid body was perfused with solution equilibrated with N_2, and it was 11–20 per cent of maximum during perfusion with air equilibrated solution. The oxygen response curves of the carotid body perfused *in vitro* are similar to curves derived from comparable whole sinus nerve recordings in intact anaesthetized cats[9]. Slight differences in shape and position of the response curves may result from a lack of

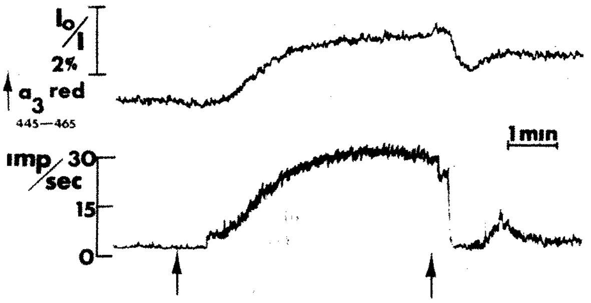


Fig. 1. Simultaneous measurement of cytochrome a_3 reduction and chemoreceptor activity in the carotid body. Upper: cytochrome a_3 reduction state measured at 445 nm–465 nm; calibration shows 2 per cent transmission. Lower: ratemeter recording of chemoreceptor afferent activity (impulses per s); time constant 2·0 s. At first arrow, perfusate equilibrated with 100 per cent O_2 replaced by perfusate equilibrated with 100 per cent N_2. At second arrow, N_2 perfusate replaced by solution equilibrated with 5 per cent O_2. Switching solutions produced an artefactual deflexion which precedes the onset of the maintained decline in both traces.

CO_2 in the perfusate and from the absence of a factor in blood other than haemoglobin which may influence receptor activity[3,10].

The reduction state of cytochrome a_3 was monitored on the basis of absorbance at 445 nm (measuring beam) minus that at 465 nm (reference beam). When perfusate equilibrated with 100 per cent O_2 was replaced by one equilibrated with 100 per cent N_2, there was an increase in $A_{445\ \mathrm{nm}}$ compared with $A_{465\ \mathrm{nm}}$ after a variable delay (Fig. 1, upper trace). We established that a compound with a maximum absorption at 445 nm is present in the carotid body by monitoring the optical signal at wavelengths between 410 nm and 465 nm while switching between 100 per cent O_2 and 100 per cent N_2 perfusates. The optical change does signal an increase in the level of reduction of cytochrome a_3. The records show typically an S-shaped curve for this reaction. When haemoglobin is present its deoxygenation can be measured in terms of $A_{437\ \mathrm{nm}}-A_{465\ \mathrm{nm}}$. On switching to solution equilibrated with N_2 the onset of the signal is much more sudden and its initial rate of rise is the maximal one. Thus the kinetics more closely resemble those of a first order reaction, and the half-maximum value is attained much earlier than with cytochrome a_3.

Surprisingly, there was a small (10–12 per cent) but reproducible increase in cytochrome a_3 reduction level when solution equilibrated with air was substituted for one equilibrated with 100 per cent O_2. The oxygen consumption of the saline perfused carotid body is about 1·5 ml./min/100 g (less than 20 per cent of that in the blood perfused organ[11]), and saline flow is often four to five times the blood flow of 40 µl./min[3]. For a carotid body weighing 2 mg the O_2 consumption is 3×10^{-5} ml./min. From the Fick relationship, an arteriovenous O_2 difference of 0·03 ml./100 ml. can be calculated at a saline flow of 100 µl./min. Thus the decrease in pO_2 along the carotid body capillaries is only 10 mm Hg. Because the pO_2 drop from capillaries to chemoreceptors is estimated to be approximately 10 mm Hg (ref. 5), the receptors must be exposed to a pO_2 only 20 mm Hg below the measured pO_2 of 142 mm Hg in the air equilibrated solution. The observation that the cytochrome a_3 reduction level is increased at approximately 120 mm Hg is not compatible with the current view that a high affinity of cytochrome a_3 for oxygen keeps a_3 in the oxidized state at pO_2 levels above about 5 mm Hg (see ref. 1).

The lower trace of Fig. 1 illustrates the change in chemoreceptor impulse frequency when a perfusate equilibrated with 100 per cent O_2 is replaced by one equilibrated with 100 per cent N_2. The simultaneous measurements of reduction level of cytochrome a_3 and chemoreceptor impulse frequency reveal that the onset of cytochrome a_3 reduction precedes slightly or is simultaneous with the rise in impulse frequency. Analysis of the continuous process demonstrated a close correlation between the time course of cytochrome a_3 reduction and increase in chemoreceptor impulse frequency (Fig. 2). In some experiments, however, impulse frequency increased more rapidly than cytochrome a_3 reduction level in the range between 12·8 and 5 per cent O_2.

Fig. 1 also shows the effect of changing from a nitrogen equilibrated solution to one equilibrated with 5 per cent O_2. At the first indication of increased cytochrome a_3 oxidation, there was a precipitous fall in impulse frequency followed by a rebound to an intermediate rate of discharge. We repeatedly observe this effect on switching from N_2 perfusate to solutions equilibrated at a higher pO_2. Fig. 2 (closed circles) correlates cytochrome a_3 reduction level and impulse frequency in the steady state during such stepwise increases in pO_2.

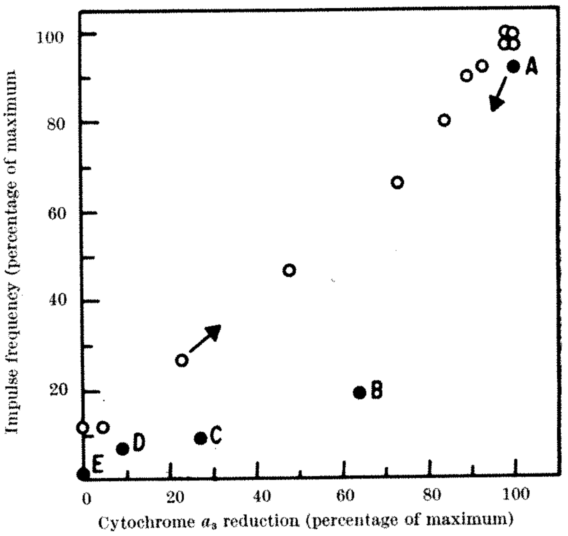


Fig. 2. Correlation between cytochrome a_3 reduction level and chemoreceptor activity during changes in pO_2 (graph derived from record shown in part in Fig. 1). ○, Continuous plot (20 s interval) of correlation on switching from 100 per cent O_2 to 100 per cent N_2. ●, Steady state plot during stepwise return to 100 per cent O_2. Point *A*, 100 per cent N_2; *B*, 5 per cent O_2; *C*, 12·8 per cent O_2; *D*, 20 per cent O_2; *E*, 100 per cent O_2. Maximum for cytochrome a_3 reduction and chemoreceptor impulse frequency is the value attained during perfusion with 100 per cent N_2 solution, and cytochrome a_3 is considered to be completely oxidized during perfusion with 100 per cent O_2 solution.

The short latency of the change in chemoreceptor firing rate with a change in cytochrome a_3 reduction level (especially towards increased oxidation) makes it extremely unlikely that impulse generation is mediated through the usual processes of intermediary metabolism, oxidative phosphorylation or ATP level as suggested by the results of experiments with metabolic inhibitors[2,3]. A more direct link with the reduction state of the members of the respiratory chain is indicated. In this regard the results are of some considerable interest because they also imply that the affinity of the cytochrome a_3 of the carotid body preparation is much lower than that revealed by studies on isolated, mammalian mitochondria.

This research was supported by grants from the US National Institute for Neurological Diseases and Stroke and the United Medical Research Foundation.

E. MILLS
F. F. JÖBSIS

Department of Physiology and Pharmacology,
Duke University Medical Center,
Durham, North Carolina 27706.

Received August 14; revised December 1, 1969.

[1] Biscoe, T. J., Sampson, S. R., and Purves, M. J., *Nature*, **215**, 654 (1967).
[2] Anichkiov, S. V., and Belenkii, M. L., *Pharmacology of the Carotid Body Chemoreceptors* (Pergamon, Oxford, 1968).
[3] Joels, N., and Neil, E., in *Proc. Wales Foundation Symp. on Arterial Chemoreceptors*, 153 (Blackwell, Oxford, 1968).

[4] Jöbsis, F. F., in *Handbook of Physiology, Section 3, Respiration*, **1**, 109 (Amer. Physiol. Soc., Washington, 1968).

[5] Forster, R. E., in *Proc. Wates Foundation Symp. on Arterial Chemoreceptors*, 115 (Blackwell, Oxford, 1968).

[6] Eyzaguirre, C., and Koyano, H., *J. Physiol.*, **178**, 385 (1965).

[7] Alvarez-Buylla, R., *Arch. Inst. Cardiol. Mexico*, **24**, 26 (1954).

[8] Eyzaguirre, C., and Lewin, J., *J. Physiol.*, **159**, 238 (1961).

[9] Hornbein, T. F., in *Proc. Wates Foundation Symp. on Arterial Chemoreceptors*, 153 (Blackwell, Oxford, 1968).

[10] Biscoe, T. J., and Taylor, A., *J. Physiol.*, **168**, 332 (1963).

[11] Fay, F. S., *Fed. Proc.*, **28**, 338 (1969).

Localization of Acetylcholinesterase by Immunofluorescence in Eel Electroplax

ACETYLCHOLINESTERASE (AChE), an important component of a variety of excitable membranes, seems to be associated with excitability[1]. Considering the possible role of AChE in the electrogenic action of cholinergic agonists, we prepared antibodies against AChE purified from the electric organ of *Electrophorus electricus*. This paper describes the cytological distribution of material reacting with a rabbit anti-AChE serum which, after absorption with eel serum, appears to be immunochemically homogeneous, in contrast to antisera recently obtained by others[2].

Purified AChE, a gift of W. Leuzinger and D. Nachmansohn (Columbia University), had been prepared following the method of Leuzinger and Baker[3]. The enzyme had a specific activity greater than 300 mmoles of acetylthiocholine/mg protein/h. A 1 mg solution, emulsified in Freund's complete adjuvant, was injected in a rabbit intramuscularly and intradermally; the injections were repeated a month later. Just before boosting, antibodies were already detectable. The antiserum was assayed with purified AChE (0·5 mg/ml. solution in 0·05 M phosphate buffer, *p*H 7·0) and with a commercially available AChE preparation (Sigma) 1·5 mg/ml. solution. (Specific activity: 80 mmoles of acetylthiocholine/mg protein/h.) Detection of antibodies directed toward AChE was made by the double diffusion technique of Ouchterlony. Only one precipitation band was found with purified AChE and it contained all the AChE activity detected with substrates for AChE, such as indoxylacetate or tetrazolium salts.

When commercial AChE was used, two bands of precipitation were seen, but only one of them showed an esterase activity. The enzymatically inactive band, which stained with amido black, seemed antigenically identical to a protein found in eel serum (Fig. 1). After absorption of anti-AChE rabbit serum with eel serum, this second band disappeared, leaving only the band bearing esterasic activity. This result was confirmed by immunoelectrophoresis[4]. With commercial AChE two bands were detected but only the more anionic band showed enzymatic activity.

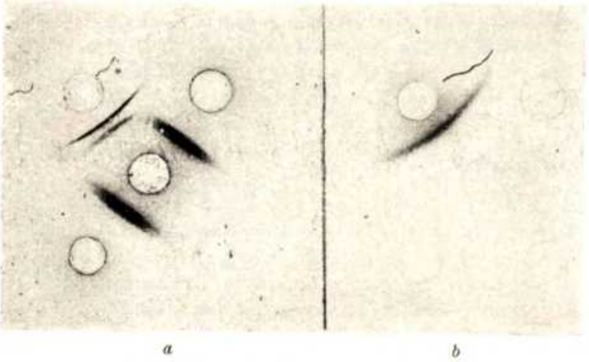


Fig. 1. Immunodiffusion (Ouchterlony) in 1·2 per cent ionagar gel and 0·025 M veronal buffer, *p*H 8·2. *a*, Protein stain (amido black); *b*, AChE stain (indoxylacetate). Centre well: antiserum to AChE. Upper left well: AChE sigma (1·5 mg/ml.). Upper right and bottom left well: eel serum.

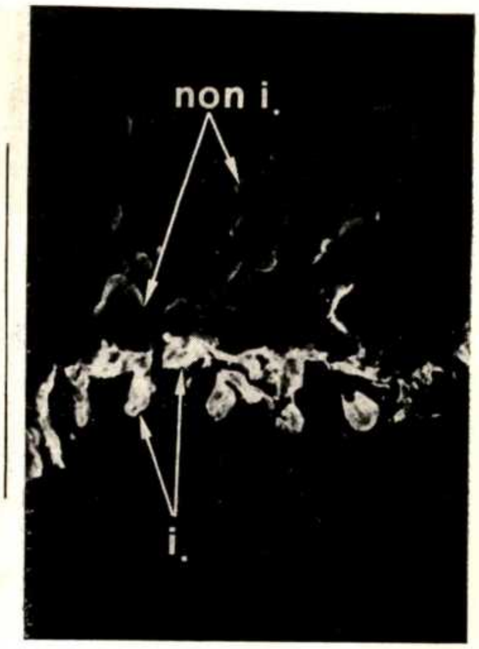


Fig. 2. Electroplax from the organ of Sachs, × 456. Frozen section, 16 μm in thickness. Indirect immunofluorescent method. Dilution of antiserum: 1/10. Leitz microscope with high pressure mercury lamp (exciter filter: BG 12, suppression filter: K 530). Bright field condenser. High speed Ektachrome film. Arrows *i* indicate the innervated side, arrows *non i* the non-innervated side. The vertical bar represents 100 μm.

In precipitation tests, after adding increasing amounts of immune serum to a constant dilution of AChE[5,6], residual enzymatic activity was measured following centrifugation[7]. It revealed that precipitation of AChE by the immune serum removed more than 85 per cent of the enzymatic activity of the AChE solution. In immunofluorescent studies, we used snap-frozen tissue because we observed thermal denaturation at 47° C, as demonstrated by a lateral shift in complement fixation curves, which indicates a conformational change[8]. Electroplaxes from Sachs's organ were cut along a plane parallel to the surface of the organ, in a cryostat at −20° C. Sections were exposed to a 1/10 dilution of immune serum (absorbed with eel serum) for 45 min and, after washing, to 1/10 or 1/20 dilution of fluorescein-conjugated anti-rabbit globulins, produced in goat (Hyland Laboratories). Alternate sections were stained by a modified thiocholine method for histochemical localization of AChE[9].

The innervated side appeared brightly fluorescent (Fig. 2), along the external infoldings of the membrane. This fluorescence contrasted with the appearance of the non-innervated side, although, on closer examination, parts of it exhibited a faint fluorescence. In comparison, the thiocholine method gave a distinct reaction, after a 2 min incubation, visible along the innervated side. After a 10 min incubation, a discrete reaction appeared on the non-innervated membrane, in agreement with the fluorescent data.

When these immunofluorescent results are compared with other histochemical studies made at the optical[10] or electron microscopical[11,12] level, it is clear that diffusion artefacts are less likely to take place with our use of immunochemical methods. Such a specific material demonstrated the presence of AChE not only in the innervated membrane, but also, at a much smaller degree, in the non-innervated side. Moreover, we recently observed fluorescing neural cells located in brain and cord of *Electrophorus*, after exposure to immune serum.

We thank Professor A. Lwoff and Dr A. Eyquem for support and Mlle Monique Huchet for technical assistance.

PHILIPPE BENDA
SHIGERU TSUJI*
JEAN DAUSSANT
JEAN-PIERRE CHANGEUX

Department of Cellular Biochemistry,
Institut Pasteur;
Department of Cytology*,
Faculté des Sciences, Paris.

Received September 25; revised December 4, 1969.

[1] Nachmanson, D., *Chemical and Molecular Basis of Nerve Activity* (Academic Press, New York, 1959).
[2] Williams, R. M., *Proc. US Nat. Acad. Sci.*, **62**, 1175 (1969).
[3] Leuzinger, W., and Baker, A. L., *Proc. US Nat. Acad. Sci.*, **57**, 446 (1967).
[4] Grabar, P., and Burtin, P., *Immunoelectrophoretic Analysis* (Elsevier, Amsterdam, 1964).
[5] Perrin, D., *Ann. NY Acad. Sci.*, **103**, 1058 (1963).
[6] Yanofsky, C., *Ann. NY Acad. Sci.*, **103**, 1067 (1963).
[7] Ellman, G. L., Courtney, K. D., Andres, jun., V., and Featherstone, R. M., *Biochem. Pharmacol.*, **7**, 88 (1961).
[8] Levine, L., *Handbook of Experimental Immunology* (edit. by Weir, D.), 707 (Blackwell, Oxford, 1967).
[9] Tsuji, S., *CR Acad. Sci.*, **267**, 801 (1968).
[10] Couceiro, A., and Freire, J., *Ann. Acad. Bras. Sci.*, **25**, 205 (1953).
[11] Bloom, F. E., and Barnett, R. J., *J. Cell Biol.*, **29**, 475 (1966).
[12] Changeux, J.-P., Gautron, J., Israel, M., and Podleski, T., *CR Acad. Sci.*, **269**, 1788 (1969).

Effect of Drugs on Red Cell Fragility

MANY drugs which affect the nervous system seem to be capable of altering the physical properties of cell membranes[1]. The concept of membrane stabilizing drugs has evolved since Spirtes and Guth[2] observed an inhibition of mitochondrial swelling in the presence of chlorpromazine. Recently, a wide range of local anaesthetics and central nervous system depressants have been shown to stabilize the red cell membrane[3]. If concurrent membrane changes in nerve cells occurred and produced a decreased ionic permeability of axons or an inhibition of transmitter release, this would result in a depression of neuronal activity. It is unlikely, however, that drugs which act on membranes have a similar action on all cells, and in some cases specific organelles may be selectively involved. For example, Tanaka and Tizuka[4] showed that some anti-inflammatory drugs decrease the permeability of the lysosomal membrane to acid hydrolases. Also, even though barbiturates decrease the excitability of pre and postsynaptic neuronal membrane, with low concentrations of the drug, the predominant effect appears to be an inhibition of release of neurotransmitter[5].

We compare here the red cell membrane stabilization properties of several types of drugs, including those known to stimulate brain activity as well as some cerebral depressants. The barbiturates were used in this experiment because they show a wide range of effect: barbituric and thiobarbituric acids are pharmacologically inactive, but C5 alkyl substituted barbiturates produce sedation if the alkyl side chain contains less than 6–7 carbon atoms. Barbiturates with larger side chains may induce epileptic fits.

The effect of drugs on red cell fragility was measured by the method of Seeman and Weinstein[3], except that the buffered hypotonic saline solution was adjusted to *p*H 6·5. This was found to give a more sensitive indication of membrane stabilization than by using the recommended *p*H of 7·0. Each drug was examined in ten-fold dilutions between 10^{-3} and 10^{-8} M inclusive. In the presence and absence of drug the degree of haemolysis was measured by estimating the absorbance of the supernatant at 570 nm 15 min after suspending the washed red cell in 66·5 mM NaCl in 10 mM phosphate buffer at 21° C. The results were expressed as ratios of control/test absorbance values. When drugs were found to protect against osmotic haemolysis this ratio was less than 1. Table 1 lists optimum concentrations of the drugs which produce protection. As previously demonstrated by Seeman and Weinstein[3], chlorpromazine showed considerable stabilizing effects. Prenylamine also showed a comparable degree of red cell stabilization. This drug exhibits reserpine-like effects in its ability to deplete amine stores in neurones[6], and its ability to stabilize the erythrocyte membrane is also similar to reserpine[3]. None of the other drugs tested produced similar degrees of membrane stabilization. Further, there was very little correlation between pharmacological activity and the effect on red cell haemolysis. This was particularly evident with barbituric acid and thiobarbituric acid, which are pharmacologically inactive. The stabilization effect which these barbiturates produced was small and similar in degree to that produced by the hypnotic barbiturates—amylobarbitone, pentobarbitone and hexobarbitone—and also similar to the stabilization effects of the excitatory barbiturates 5-(2-ethyl hexyl)-5-ethylbarbituric acid and 5-(4-methyl heptyl)-5-ethylbarbituric acid. The thiobarbiturate thiopentone gave moderate protection against haemolysis, but no protection at all was detected with nealbarbitone, methyl phenobarbitone and secbutobarbitone.

Table 1

Drug	Concentration of drug which produced least haemolysis (M)	No. of observations	Haemolysis expressed as ratio control/test
Chlorpromazine	10^{-4}	9	0·17
Prenylamine	10^{-5}	9	0·29
Barbituric acid	10^{-7}	12	0·76
Amylbarbitone	10^{-6}	6	0·82
Pentobarbitone	10^{-6}	6	0·72
Hexobarbitone	10^{-6}	12	0·73
5-(2-Ethyl hexyl)-5-ethylbarbituric acid	10^{-6}	9	0·72
5-(4-Methyl heptyl)-5-ethylbarbituric acid	10^{-6}	6	0·84
Thiobarbituric acid	10^{-6}	9	0·88
Thiopentone	10^{-6}	9	0·58
Bemegride	10^{-6}	6	0·73
Adrenaline	10^{-6}	6	0·55
Noradrenaline	10^{-6}	6	0·63
Hyoscine	10^{-6}	9	0·84
Atropine	10^{-6}	6	0·79
Amphetamine sulphate	10^{-6}	9	0·61
Physostigmine	10^{-6}	6	0·54
Acetylcholine	10^{-6}	6	0·45

Although iproniazid, nialamid and acetazolamide, which are thought to act by the inhibition of an enzyme, did not stabilize the red cell membrane, the anticholinesterase physostigmine did produce moderate stabilization. There is a similar lack of correlation between membrane stabilization by drugs which are receptor blockers; propranalol, for example, does not exhibit a membrane protecting effect, while atropine and hyoscine do. Acetylcholine, adrenaline and noradrenaline, which can alter the physiological properties of the membranes of excitable cells, all produce a much smaller stabilizing influence of the red cell membrane than prenylamine and chlorpromazine. The stimulant drug bemegride also produces erythrocyte stabilization. In concentrations of 10^{-3}–10^{-4} M all the above drugs produced haemolysis; sucrose, however, did not cause either haemolysis or red cell membrane stabilization. These results indicate a general lack of correlation between inhibition of neuronal activity and red cell stabilization, particularly in the case of the pharmacologically active and inert barbiturates and in the stabilization produced by the central stimulant bemegride. The changes produced by a drug are, however, not likely to be the same in all types of membrane, and the effects on osmotic resistance may be different from the effects on resistance to chemical disruption[7]. Also some drugs, such as chlorpromazine, may act on a wide range of sites on the surface of membranes and in this way produce a marked change in the net physical properties of the membrane. Conversely, monoamine oxidase inhibitors, which react with only a limited number of membrane bound enzyme proteins, result in no detectable overall change in membrane resistance.

W. Mikikits
A. Mortara
R. G. Spector

Department of Pharmacology,
Guy's Hospital Medical School,
London.

Received December 11, 1969.

[1] Weatherall, M., *Recent Advances in Pharmacology*, fourth ed. (edit. by Robson, J. M., and Stacey, R. S.), 1 (Churchill, London, 1968).
[2] Spirtes, M. A., and Guth, P. S., *Biochem. Pharmacol.*, **12**, 37 (1963).
[3] Seeman, P., and Weinstein, J., *Biochem. Pharmacol.*, **15**, 1737 (1966).
[4] Tanaka, K., and Tizuka, Y., *Biochem. Pharmacol.*, **17**, 2023 (1968).
[5] Curtis, D. R., *Brit. Med. Bull.*, **21**, 5 (1965).
[6] Von Euler, U. S., and Lishajko, F., *Biochimica Applicata*, **14**, 17 (1968).
[7] Shepherd, H., Tsien, W. H., and Burghardt, C., *Biochem. Pharmacol.*, **18**, 2215 (1969).

Effects of General Anaesthetics on Na^+ Transport in Human Red Cells

Theories of general anaesthesia have frequently been based on the suggestion that anaesthetic agents perturb the transport of ions through cell membranes[1,2]. In some theories, inhibition of transport has been invoked[3,4], whereas in others[5], facilitated transport in the presence of anaesthetics is proposed, but experimental evidence for either of these views is often contradictory. Anaesthetics have been observed to inhibit cation fluxes across nerve cell membranes during the transmission of impulses[6], and recently work on inverted frog skin preparations[7] and on the isolated toad bladder[8] has indicated that active cation transport may be affected by general anaesthetics. We have investigated the effect of ether and chloroform on active cation transport in human red cells.

The method is based on the well established observation that cold-stored erythrocytes lose K^+ and gain Na^+. On incubation in the appropriate medium the cells re-establish normal cation levels by active transport[9]. Cold-stored red cells were suspended in an active medium[10] and incubated at 37° C for 4 h, a period that gave rise to a conveniently measured change in cation concentrations. Subsequently the cells were quenched in ice, centrifuged, washed and after haemolysis analysed for haemoglobin, Na^+ and K^+. Experiments were carried out in the presence of various quantities of anaesthetics. The active and passive components of cation transport were resolved with the cardiac glycoside, ouabain, which in concentrations of 10^{-5} M is known effectively to eliminate active transport[11]. The results for one experiment with ether are illustrated in Fig. 1; the internal Na^+ concentration after 4 h incubation is plotted for various concentrations of ether in both the presence and absence of ouabain. The rise in intracellular Na^+ concentration and the degree of haemolysis of ouabain treated cells as a function of ether concentration are illustrated in Fig. 2; this indicates that both processes become significant at similar anaesthetic concentrations. The fall of internal K^+ concentration showed a similar pattern of behaviour. Experiments with chloroform exhibited the same trends that had been observed with ether. The concentration required to produce a 10 μequiv. ml.$^{-1}$ change in Na^+ concentration within red cells was 0·14 M for ether and 0·018 M for chloroform. Thus the concentrations of anaesthetics required to alter the rate of active cation transport significantly, to alter the rate of passive leakage of cations and also to produce significant haemolysis were considerably higher than the mean arterial blood concentrations occurring during clinical—stage 3—anaesthesia (0·013 M for ether[12] and 0·0016 M for chloroform[13]). These results indicate that no marked changes in the cation fluxes, active or passive, are induced by anaesthetics until a concentration is reached such that disruption of the membrane occurs, sufficiently gross to allow haemolysis though at lower concentrations there is some evidence of increased cationic transport. This, of course, does not conflict with the evidence that anaesthetics may influence the changes of permeability to Na^+ in the membranes of excitable tissues which accompany the action potential[1,6], or that anaesthetics modify the behaviour of erythrocytes in hypotonic conditions[14]. The results, however, must throw doubt on those theories of general anaesthesia which propose that anaesthetics act by their perturbation of the transport of cations through biological membranes in general[3-5,15-17], and suggest that the effects of general anaesthetics are more specific than have frequently been assumed.

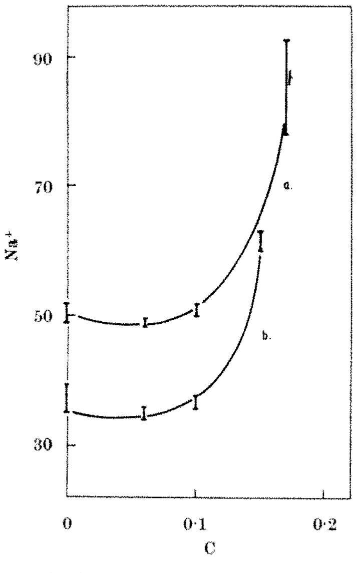


Fig. 1. Na^+ concentration in red cells (μequiv. ml.$^{-1}$) after 4 h incubation as a function of ether concentration (M). *a*, In the presence of 10^{-5} M ouabain; *b*, without ouabain.

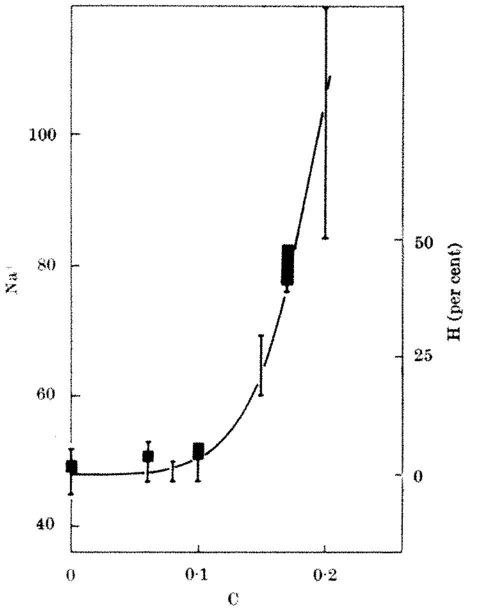


Fig. 2. Na^+ concentration in red cells (μequiv. ml.$^{-1}$) after 4 h incubation (narrow bars) and percentage haemolysis (wide bars), in the presence of 10^{-5} M ouabain, as a function of ether concentration (M).

We thank the US Office of Naval Research for financial

support and Professor W. D. M. Paton and Dr E. Gill for helpful advice.

M. J. Halsey
E. B. Smith
T. E. Wood

Physical Chemistry Laboratory,
South Parks Road,
Oxford.

Received November 13, 1969.

[1] Cherkin, A., *Ann. Rev. Pharmacol.*, **9**, 259 (1969).
[2] Smith, E. B., *Physiology and Medicine of Diving* (edit. by Bennett and Elliot), 8 (Baillière, Tindall and Cassell, London, 1969).
[3] Mullins, L. J., *Chem. Rev.*, **54**, 289 (1954).
[4] Miller, S. L., *Proc. US Nat. Acad. Sci.*, **47**, 1515 (1961).
[5] Bangham, A. D., Standish, M. M., and Miller, N., *Nature*, **208**, 1295 (1965).
[6] Mullins, L. J., *Fed. Proc.*, **27**, 898 (1968).
[7] Gottlieb, S. F., and Savran, S. V., *Anesthesiology*, **28**, 324 (1967).
[8] Anderson, N. B., *Anesthesiology*, **28**, 438 (1967).
[9] Harris, J. E., *J. Biol. Chem.*, **141**, 579 (1941).
[10] Whittam, R., *J. Physiol.*, **154**, 608 (1960); Pakhle, Y. S., thesis, Univ. Oxford (1962).
[11] Kahn, J. B., and Acheson, G. H., *J. Pharmacol. Exp. Therap.*, **115**, 305 (1955).
[12] Faulconer, A., *Anesthesiology*, **13**, 361 (1952).
[13] Thomas, D. M., MacKrell, T. N., and Conner, E. H., *Anesthesiology*, **22**, 542 (1961).
[14] Seeman, P., Sanks, T., Argent, W., and Kwaut, W. O., *Biochim. Biophys. Acta*, **183**, 476 (1969).
[15] Latner, A. L., *Proc. Roy. Soc. Med.*, **58**, 895 (1965).
[16] McIlwain, H., *Enzymes and Drug Action* (edit. by Mongar and de Reuck), 170 (Ciba Foundation Symposium, 1962).
[17] Bennett, P. B., and Hayward, A. J., *Nature*, **213**, 938 (1967).

Possible Mechanism of Action of Morphine on Brain

We have sought to extend previous studies[1-3] on the possible relationship between the effects of morphine on behaviour and alterations of biogenic amine metabolism. We propose that if morphine alters the metabolism of serotonin and catecholamines in brain, changing the relative amount of their free forms available for synaptic transmission and degradation, then depletion of the amines before administration of morphine should modify the effects of the narcotic on behaviour. The recent report by Way *et al.*[2] that depletion of brain serotonin inhibits the development of morphine tolerance in mice supports this proposal.

We chose the "spontaneous" motor activity of adult albino rats (Holtzmann strain) as the behavioural variable measured after a morphine challenge. A steel box 45 × 35 × 23 cm was constructed with a spring-loaded floor mounted over a piezoelectric transducer. The voltages from the latter were amplified, rectified and integrated at 10 min intervals. The score for each animal was read directly from the integrator. Daily activity runs were made to minimize emotional stress during final testing. Each group had equal numbers of males and females with average weights of 308 g and 205 g respectively. The drugs used in the pre-treatment are known to interfere with the metabolism of serotonin and/or catecholamines. We assumed that other possible effects were not critically involved, and this was partially borne out by some of the experiments. Two series of animals were used: rats which had not been previously exposed to morphine and which were therefore non-tolerant ("naive" series), and rats which had received 20 mg/kg/day of morphine sulphate for six days. This schedule, although not adequate to ensure tolerance to massive doses of the narcotic, was sufficient to eliminate the usual depressant action of a 10 mg/kg single dose of morphine in naive animals and replace it by a definite increase in motor activity. The experimental data are expressed in the tables as the average ratio of the post-injection activity (20 min after) to that estimated 10 min before the challenge dose of the narcotic for each treatment group. Thus a ratio above 1·0 represents an increase and below 1·0 a decrease in motor activity. All drugs were dissolved in 0·9 per cent saline—except α-methyl-*p*-tyrosine (AMPT) and *p*-chlorophenylalanine (PCPA) which were given in a steroid suspension vehicle[4]—and injected intraperitoneally. The control groups received saline or the suspension vehicle alone.

Table 1. EFFECT OF MORPHINE CHALLENGE (20 mg/kg) ON SPONTANEOUS MOTOR ACTIVITY AFTER VARIOUS PRE-TREATMENTS: NAIVE RATS

Pre-treatment	Dose (mg/kg/day)	No.	Mean ratio: post/pre-injection	P†
Controls*		16	0·18	—
PCPA	300	8	3·14	< 0·01
PCPA + 5-hydroxytryptophan	300 100	4	0·35	> 0·05
5-Hydroxytryptophan	50–100	11	0·73	> 0·05
Iproniazid	50	4	0·81	< 0·03

* No difference in ratios was found between the saline and solvent pre-treated controls in both series. These groups were therefore combined.

† All P values refer to comparisons with the control group, according to the two tailed Mann-Whitney U test.

Table 2. EFFECT OF MORPHINE CHALLENGE (10 mg/kg) ON SPONTANEOUS MOTOR ACTIVITY AFTER VARIOUS PRE-TREATMENTS: TOLERANT RATS

Pre-treatment	Dose (mg/kg/day)	No.	Mean ratio: post/pre-injection	P
Controls		8	4·20	—
AMPT	80	8	1·40	< 0·03
AMPT + L-DOPA	80 100	4	10·00 +	< 0·01
Diethyldithiocarbamate	500	8	1·81	< 0·04
Imipramine	25	4	0·44	< 0·01

PCPA, which inhibits the hydroxylation of tryptophan—the rate-limiting reaction in the synthesis of serotonin[5]—was administered to "naive" rats to determine whether previous depletion of serotonin from brain would modify the depression induced by morphine. Rats were injected three times per day with PCPA (300 mg/kg/day) and then challenged with a single dose of morphine (20 mg/kg) on the sixth day after the first dose of PCPA. Controls which received solvent were severely depressed by morphine; the rats which had received PCPA were clearly excited by the narcotic (Table 1). The specificity of this effect of PCPA was shown by administering 5-hydroxytryptophan[5] concurrently with the PCPA in another group of animals. These were depressed by morphine. 5-Hydroxytryptophan alone (either 50 or 100 mg/kg in different rats) as well as a monoamine oxidase inhibitor (iproniazid), injected daily for five days before challenge with morphine, reduced slightly the depressant effect of the morphine compared with parallel saline control groups. The last injection of both 5-hydroxytryptophan and iproniazid preceded the morphine challenge by at least 24 h.

The "tolerant" series (Table 2) was used chiefly to study the effects of manipulation of catecholamine levels on the response to morphine. The control tolerant animals for this series were prepared by injection of 20 mg/kg/day of morphine for six days. These rats were markedly excited by a 10 mg/kg dose of morphine whereas non-tolerant animals were depressed by the same challenge. A priming injection of 20 mg/kg of morphine was given to all the animals in this series 4 h before the final test, to minimize the possibility that abstinence phenomena might interfere with our results. AMPT is known to inhibit the hydroxylation of tyrosine into L-3,4-dihydroxyphenylalanine (L-DOPA), the rate limiting reaction in the synthesis of dopamine and norepinephrine. Its effect is therefore the depletion of these amines from brain and other tissues[6]. Two injections of AMPT within the last 24 h of the experiment, 12 h apart, prevented the excitation induced in tolerant rats by the morphine challenge. Administration of L-DOPA 1 h before the challenge, in an otherwise identical AMPT-treated group, reversed the effect of AMPT, so that clear-cut excitement was produced by the narcotic. Diethyldithiocarbamate has been shown to block the β-hydroxylation of dopamine into norepinephrine[7]. Injection of this agent 4 h before a challenge with morphine, also in tolerant rats, reduced the excitation induced by the challenge. Finally, imipramine—which apparently exerts some of its effects on behaviour by interfering with re-uptake of norepinephrine into its storage form[8]—given 2 h before testing, also prevented the motor hyperactivity induced by morphine.

We conclude that at least some of the behavioural effects of morphine administration can be reversed by pre-treatment with drugs which affect biogenic amine metabolism. The specificity of some of these effects was demonstrated by their elimination when amine precursors (5-hydroxytryptophan and L-DOPA) were administered in conjunction with PCPA and AMPT, respectively. While no gross alterations of total biogenic amine levels have been found to result from single or multiple doses of morphine[9,10], the evidence for morphine-induced alterations in amine turnover rates is rather definite. Thus Gunne[1] and Haubrich and Blake[11] have shown that the brain levels and the urinary excretion of 5-hydroxyindoleacetic acid and of catecholamine metabolites are increased substantially after administration of morphine, and Way *et al.* have shown increased accumulation of serotonin in morphinized animals treated with a monoamine oxidase inhibitor, compared with non-morphine treated controls[2]. If increases in rate of turnover—rather than increases in the total (free and stored) levels—of the amines are the consequence of morphine treatment, two possible conclusions are suggested. Either the activities of rate-limiting enzymes concerned in the synthesis of the amines increase following morphine or, perhaps more likely, amine re-uptake into storage forms[12,13] is impeded by morphine, thus increasing the amount of free amines available for synaptic transmission and metabolic degradation. Recent reports on the antagonism between reserpine and morphine[14,15] and the interference we have found between imipramine and morphine support the second alternative, which also requires fewer assumptions. We now plan to test this hypothesis directly. If morphine is indeed found to interfere significantly with the uptake of serotonin and norepinephrine, our behavioural results would lead us to expect differences in the degree and duration of this interference, to account for the well known differences in the effects of morphine in tolerant and non-tolerant animals.

E. EIDELBERG
A. S. SCHWARTZ

Division of Neurobiology,
Barrow Neurological Institute,
St Joseph's Hospital,
Phoenix, Arizona 85013.

Received September 19; revised December 11, 1969.

[1] Gunne, L. M., *Acta Physiol. Scand.*, **62**, Suppl. 204 (1963).
[2] Way, E. L., Loh, H. H., and Shen, F. H., *Science*, **162**, 1290 (1968).
[3] Takagi, H., and Kuriki, H., *Intern. J. Neuropharm.*, **8**, 195 (1969)
[4] Koella, W. P., Feldstein, A., and Czicman, J. S., *Electroenceph. Clin. Neurophysiol.*, **25**, 481 (1968).
[5] Koe, K. B., and Weissman, A., *J. Pharmacol. Exp. Therap.*, **154**, 499 (1966).
[6] Spector, S., Sjoerdsma, A., and Udenfriend, S., *J. Pharmacol. Exp. Therap.*, **147**, 86 (1965).
[7] Carlsson, A., Lindqvist, M., Fuxe, K., and Hökfelt, T., *J. Pharm. Pharmacol.*, **18**, 60 (1966).
[8] Glowinski, J., and Axelrod, J., *Nature*, **204**, 1318 (1964).
[9] Maynert, E. W., and Klingman, G. I., *J. Pharmacol. Exp. Therap.*, **135**, 285 (1962).
[10] Cochin, J., and Axelrod, J., *J. Pharmacol. Exp. Therap.*, **125**, 105 (1959).
[11] Haubrich, D. R., and Blake, D. E., *Fed. Proc.*, **28**, 2985 (1969).
[12] von Euler, U. S., and Hillarp, N. A., *Nature*, **177**, 44 (1956).
[13] Carlsson, A., Hillarp, N. A., and Waldeck, B., *Acta Physiol. Scand.*, **59**, Supp. 215 (1963).
[14] Smith, A., Lisper, K., and Hayashida, K., *Fed. Proc.*, **28**, 15 (1969).
[15] Shen, F. S., Loh, H. H., and Way, E. L., *Fed. Proc.*, **28**, 2986 (1969).

Schizophrenia as a Genetic Morphism

THE incidence of schizophrenia in most communities is of the order of 1 per cent. Yet the fertility of schizophrenic males is low, as noted by Huxley *et al.*[1]; and it has since been established that the fertility of schizophrenic females is low, too[2]. The second observation serves to strengthen the suggestion of Huxley *et al.* that the fertility of schizophrenics seems too low for the disorder to be maintained at its present rate by mutation alone. In explanation they noted the resistance of schizophrenics to surgical and wound shock, to visceral perforation, to high doses of histamine, insulin and thyroxine, and to pain. They noted also the possibility that schizophrenics may be resistant to diseases such as smallpox and bubonic plague which have occurred in widespread epidemics.

Moran[3] suggested that the proposed robustness of the schizophrenic genotype might be manifested at an earlier stage of life: "I therefore suggest that the presence of a gene for schizophrenia has a protective effect for one or more of the genetic factors which cause the loss of fertilized ova".

It may be worth noting that the only two relevant studies known to me do not support Moran on this point. These suggested that, in contrast with control samples, there was a higher incidence of foetal loss among the sibs of schizophrenic propositi[4], and that there was a higher proportion of miscarriages among the pregnancies of psychotic women (64 per cent of whom had been diagnosed as schizophrenic)[5].

There are two problems about the incidence of schizophrenia: in the first place it seems very high, and in the second it seems not to be declining (as it should be if a schizophrenic gene protected its bearer against bubonic plague or smallpox). I can offer no fresh answer to the first. In regard to the second, it seems possible to me that either the level of schizophrenia is being maintained by an increasing degree of psychological stress to which we are said to be exposed, or that a true decline in schizophrenic rates is being masked by less extreme criteria of definition. Possibly one does not have to be so mad these days to be called "schizophrenic".

I am supported by a grant from the Population Council of New York.

WILLIAM H. JAMES

Department of Biometry,
Galton Laboratory,
University College, London.

Received July 25, 1969; revised January 23, 1970.

[1] Huxley, J., Mayr, E., Osmond, H., and Hoffer, A., *Nature*, **204**, 220 (1964).
[2] Stevens, B., *Brit. Med. J.*, **4**, 22 (1969).
[3] Moran, P. A. P., *Nature*, **206**, 1111 (1965).
[4] Terris, M., Lapouse, R., and Monk, M. A., *Amer. J. Psychiat.*, **121**, 476 (1964).
[5] Winokur, G., Guze, S. B., and Pfeiffer, E., *Amer. J. Psychiat.*, **115**, 1097 (1959).

Possible Relationships between Orienting and Diving Reflexes

THE diving reflex has been studied extensively in aquatic organisms that breathe air and submerge to obtain food or to escape from predators such as ducks, seals and alligators[1]. This reflex seems to have two phases: an initial phase which may be mediated by the vagus and trigeminal nerves[2], and a later one which follows prolonged holding of the breath during voluntary or forcible submersion[3]. The initial phase includes bradycardia[1,4], apnoea (specifically, an interruption of respiration)[1,4], peripheral vasoconstriction[4,5] and cephalic vasodilation[5]. Gaunt and Gans[6] have reported that bradycardia in *Caiman crocolilius* is minimal during spontaneous dives but great during the approach of an investigator or during handling. They suggest that early diving bradycardia is mainly "psychogenic" in origin and results from "threatening" stimuli. We have been studying (ref. 7 and unpublished results) the responses of the wholly aquatic salamander *Necturus maculosus* (mudpuppy) and have noted similarities to the early components of diving, specifically bradycardia and an interruption of respiration. (*Necturus* breathes through

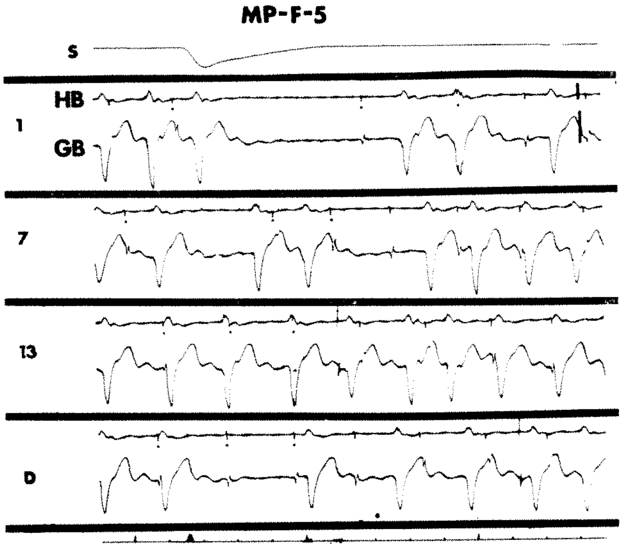


Fig. 1. The effect of repeated shadow stimulation on heart beat (HB) and gill beat (GB) in one animal. Passage of the shadow was recorded by a photocell on the aquarium (S). Initial shadow presentation (1) produced bradycardia and apnoea seen first as an interruption for about 4 s, followed by resumption at a slower and more variable rate. Repeated shadow presentation (at approximately 120 s intervals for this animal) led to a decrement in the degree of bradycardia and apnoea (trial 7) and finally to no effect (trial 13). The apnoea was reinstituted by changing the shadow direction (D). Heart beats immediately before, during and after the shadow are denoted by dots. Bandpass: HB, 15–75 Hz; GB, 1–75 Hz. Calibrations, 10 μV and 1 s.

large external gills beating back and forth at 0·5–1·0 beats/s.) Insofar as it does not dive, we suggest here that these responses of *Necturus* are components of the orienting reflex[8,9] rather than the initial diving reflex, and that both reflexes may reflect some common neural substrate involving adaptive responses to external stimulation.

Necturus maculosus obtained from a commercial dealer were kept and tested in water at 19° C. Sixty animals were tested singly in a 'Plexiglas' aquarium, 28 × 12 × 6 cm, housed in a refrigerator. Dim illumination (2·5 foot lamberts at the aquarium) was provided by a parallel array of six luminescent strips (Sylvania 'Tape-Lite' *PTL* 175) mounted 30 cm above the aquarium. The illusion of a moving shadow was created over the aquarium by sequentially interrupting current to each strip with silent solid state circuitry[10]. The shadow velocity was 0·11 m/s. Respiration (gill beating) and heart beat were recorded, without affixing leads to the animal, by stainless steel studs (5 mm diameter) embedded in the sides of the aquarium below the surface. Voltages generated by water waves after gill beating, and EKGs recorded by volume conduction, were recorded via high gain differential amplifiers on a polygraph. We shall give a complete validation of the recording technique elsewhere (see also ref. 7). It has the advantage of working without sensors or restraint of the subject. One limitation is that gill and heart beats may be obscured during large movements, although the movements are revealed by their own characteristic voltages following water turbulence. Our data came from quiescent animals.

The animals equilibrated for at least 24 h before the shadow was first presented. After the first stimulus, respiration was interrupted, as could be seen from the cessation of gill beat in all animals, and bradycardia in 83 per cent, seen at first as one or more "skipped" beats. Periodic presentation (30–400 s) of the stimulus evoked the same responses but with less vigour, until neither apnoea nor bradycardia occurred (Fig. 1). We interpreted the waning response as habituation, because apnoea and bradycardia can be brought back by a fresh stimulus such as reversing the direction of the shadow or applying vibration rather than shadow. Extensive investigations of other parameters, such as the interval between trials, confirmed the interpretation of habituation[7,11]. Response habituation is characteristic of the orienting reflex[8,9]. Interestingly, the orienting reflex includes the same characteristics as the early phase of the diving reflex: bradycardia[12], apnoea[13], peripheral vasoconstriction[9] and cephalic vasodilation[9,14]. These and other similarities between the early phase of diving and the orienting reflex are summarized in Table 1, in which the duck and mudpuppy are compared. While some data are lacking, the close similarity of the reflexes seems obvious. We do not suggest that the initial phase of the diving reflex is identical to the orienting reflex, although it would be interesting to know whether any of the early components of "spontaneous" diving exhibits habituation.

Table 1. A COMPARISON OF COMPONENTS OF THE DIVING REFLEX IN THE DUCK AND ORIENTING REFLEX IN THE MUDPUPPY

	Early phase of "diving reflex": (*Anas* sp.), duck	"Orienting reflex"; (*Necturus maculosus*), mudpuppy
1. Apnoea present ?	+ + + (ref. 1)	+ + + (ref. 7 and unpublished results)
2. Apnoea after end-expiration ?	+ + (ref. 1)	+ + (ref. 7)
3. Bradycardia ?	+ + + (ref. 1)	+ + (ref. 7 and unpublished results)
4. Bradycardia or standstill at end diastole ?	+ + (ref. 1)	+ + (ref. 7)
5. Peripheral vasoconstriction	+ + + (refs. 5, 20)	+ (ref. 23)
6. Cephalic vasodilation	+ + + (refs. 5, 12, 20)	(?)
7. Vasodilation also in masseter muscles and oesophagus	+ + + (ref. 5)	(?)
8. Decrease in somatic myographic activity	+ (ref. 21)	+ (ref. 7)
9. Potentiated by struggle ?	+ + (ref. 22)	+ + + (ref. 7)
10. Primary vagal mediation ?	+ + (ref. 22)	+ (?) (ref. 23)
11. Blocked by atropine ?	+ (ref. 21)	+ (?) (ref. 23)
12. Habituates ?	(?)	+ + +

+ + +, Always present.
+ +, Usually present.
+, Suggested but not conclusively demonstrated.
+ (?), Seen in "diving" frogs; no data on *Necturus*.
(?), No data.

Belkin[15] has suggested that components of the diving reflex may have evolved as specializations from physiological responses to more general situations of external stimulation of an animal. *Necturus* occupies an evolutionary position in which components of the orienting response might include such primitive physiological responses. If the diving and orienting reflexes are so related they may be expected to have closely related neural substrates. We note that stimulating the midbrain produces components of the initial diving reflex and the orienting reflex[16,17]; components of both reflexes have been elicited also from the amygdaloid nucleus[16,18,19]. It would seem that the diving and orienting reflexes have enough in common to justify further thought on their interrelation.

This work was supported by a grant to N. M. W. from the US Public Health Service.

DAVID A. GOODMAN*
NORMAN M. WEINBERGER

Department of Psychobiology,
University of California,
Irvine,
California 92664.

Received November 26, 1969.

* Present address: Psychobiology Research Laboratory, VA Hospital, Sepulveda, California.

[1] Andersen, H. T., *Physiol. Rev.*, **46**, 212 (1966).
[2] Andersen, H. T., *Acta Physiol. Scand.*, **58**, 263 (1963).
[3] Jones, D. R., and Shelton, G., *J. Exp. Biol.*, **41**, 417 (1964).
[4] Fergl, E., and Folkow, B., *Acta Physiol. Scand.*, **37**, 99 (1963).
[5] Johansen, K., *Acta Physiol. Scand.*, **62**, 1 (1964).
[6] Gaunt, A., and Gans, C., *Nature*, **223**, 207 (1969).
[7] Goodman, D. A., thesis, University of California, Irvine (1969).

[8] Pavlov, I., *Conditioned Reflexes* (Oxford University Press, New York, 1927).

[9] Sokolov, E., *Perception and the Conditioned Reflex* (Macmillan, New York, 1963).

[10] Weinberger, N. M., and Goodman, D. A., *Behav. Res. Meth. and Instr.*, **1**, 192 (1969).

[11] Thompson, R. F., and Spencer, W. A., *Psychol. Rev.*, **173**, 16 (1966).

[12] Graham, F., and Clifton, R., *Psychol. Bull.*, **65**, 306 (1966).

[13] Davis, R. C., Buchwald, A. M., and Frankmann, R., *Psychol. Mon.*, 405 (1955).

[14] Lynn, R., *Attention, Arousal and the Orienting Reaction* (Pergamon, New York, 1966).

[15] Belkin, D. A., *Amer. Zool.*, **8**, 775 (1968).

[16] Ursin, H., Wester, K., and Ursin, R., *Electroenceph. Clin. Neurophysiol.*, **23**, 41 (1967).

[17] Jasper, H. H., in *Reticular Formation of the Brain* (edit. by Jasper, H. H., *et al.*), 319 (Churchill, London, 1958).

[18] Reiz, D., and McHugh, P., *Amer. J. Physiol.*, **214**, 601 (1968).

[19] Ursin, H., and Kaada, B. R., *Exp. Neurol.*, **2**, 109 (1960).

[20] Folkow, B., Buxe, K., and Sonnenschein, R., *Acta Physiol. Scand.*, **67**, 327 (1966).

[21] Folkow, B., Nilsson, N., and Yonce, L., *Acta Physiol. Scand.*, **70**, 347 (1967).

[22] Andersen, H. T., *Acta Physiol. Scand.*, **53**, 23 (1961).

[23] Lund, G. F., and Dingle, H. J., *J. Exp. Biol.*, **48**, 265 (1968).

Reproductive Cycle of a Neotropical Insectivorous Bat, *Myotis nigricans*

REPRODUCTIVE patterns in bats fall into three main categories. A synchronous breeding season and monoestry is common in all holarctic bats[1]. Continual asynchronous breeding is found in some tropical bats of the families Desmodontidae[2], Phyllostomatidae[3], Pteropodidae, Emballonuridae and Molossidae[4]. In some palaeotropical bats seasonal polyoestry is the rule, with two synchronized breeding seasons each year[4,5].

Because of the rigorous environment of the temperate zone, parturition in holarctic bats is largely limited to May, June and July, when temperatures are mild and food is abundant. Most of these temperate zone bats belong to the insectivorous family Vespertilionidae. Neotropical bats which breed continually are either sanguivorous or frugivorous and are probably never exposed to a shortage of food[6].

Myotis nigricans, the first neotropical Vespertilionid whose reproduction has been studied, has a fourth type of reproductive cycle. These bats breed continually during most of the year but become reproductively quiescent for about 3 months during the latter part of the rainy season (Fig. 1).

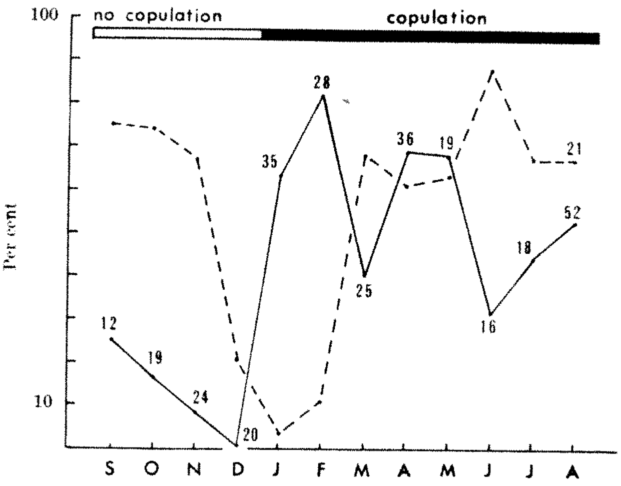


Fig. 1. Percentage of pregnant (———) and lactating (- - -) females in one year. The numbers are sample sizes.

We examined reproductive tracts of more than five hundred *M. nigricans* from September 1968 to September 1969. Animals were collected at weekly intervals from roosts on Barro Colorado Island and the nearby town of Gamboa, Canal Zone. Pregnant females were taken in every month of the year except December. In November only a few pregnancies were noted, and all of those were near term in the first week of the month. After a 7 week period with no pregnancies, 63 per cent of the females collected during January were found to be pregnant. The first pregnancies were noted on January 3 and parturition began in the last week of February. This and subsequent observations lead us to believe that the gestation period for *M. nigricans* is near the 50–60 days reported for other members of the genus[7]. The three pregnancy peaks are about 3 months apart and our observations show that this is about the time needed for gestation and lactation of one litter. The animals tend to become less synchronized as the year proceeds, as shown by the fact that the peaks become progressively shorter, and there is always a certain number of pregnancies at any given time.

The curve for lactation is similar to that for pregnancy, but, of course, shows a time lag of one to two months. The total of the two percentages is sometimes more than one hundred, indicating animals that are both pregnant and lactating. Evidence for polyoestry consists of numerous individuals that were both pregnant and lactating, as well as continuous observations of banded individuals which produced at least two young during the year. This indicates post-partum oestrus at least occasionally, and we feel from other observations that it is the rule. This is the first conclusively demonstrated instance of polyoestry in the family Vespertilionidae, although there is suggestive evidence of its occurrence in a few Old World species of the genus *Pipistrellus*[5,7].

If the gestation period is less than 60 days, it is hypothetically possible for a given female to produce four young in a year. We feel that because of individual variation in times of copulation, ovulation, fertilization, gestation period and post-partum oestrus, three is a more usual number.

The fact that no young are produced from December to March may relate to seasonality of the food supply. During the dry season (January–March), insect populations are probably lower than at other times of the year[8]. There is usually an insect bloom in April when the rains begin. It seems that the strategy of the bats is to wean the young at this time of year and to avoid weaning young at times of lowered food supply. Copulation must therefore cease around the middle of September, and the last young of the year are born in November and weaned in December. Copulation begins again in January (we observed it many times). The first young are born in March and weaned in April. The cues for this cycle are as yet unknown, but the critical times are September and December, so that temperature and rainfall would seem to be extremely unlikely. Photoperiod is a possibility, but changes at this latitude are slight (1 h and 3 min from summer solstice to winter solstice).

We thank the Smithsonian Tropical Research Institute. The US National Science Foundation provided J. S. F. with a grant.

DON E. WILSON
JAMES S. FINDLEY

Department of Biology,
University of New Mexico,
Albuquerque,
New Mexico 87106.

Received January 9, 1970.

[1] Cockrum, E. L., *Trans. Kansas Acad. Sci.*, **58**, 48 (1955).

[2] Wimsatt, W. A., and Trapido, H., *Amer. J. Anat.*, **91**, 415 (1952).

[3] Tamsitt, J. R., and Valdivieso, D., *Amer. Mid. Nat.*, **73**, 150 (1965).

[4] Mutere, F. A., *Acta Trop.*, **25**, 97 (1968).

[5] Brosset, A., *Mammalia*, **26**, 176 (1962).

[6] Tamsitt, J. R., and Valdivieso, D., *Nature*, **198**, 104 (1963).

[7] Asdell, S. A., *Patterns of Mammalian Reproduction*, 93 (Comstock, Ithaca, 1964).

[8] Wilson, D. E., *Mammalia* (in the press).

Acoustic Basis for Individual Recognition by Voice in the Gannet

A RECENT article by W. H. T.[1] reviewed the general question of the perceptual basis for group organization by birds and other vertebrates. It pointed out that successful social organization of colonially nesting sea birds must in many cases depend on a capacity for mutual recognition at some distance, at least between mates and also between parents and young, perhaps extending to other members of the group. While many species of birds (such as gulls and ducks) have visual powers so acute that they are able to recognize individuals at distances of 50 m or more, it was nevertheless shown that auditory recognition of individuals by subtle voice characteristics also plays a part—sometimes major—in identification. Indeed, in some examples of gulls, penguins, auks and terns, the evidence suggests that audio-vocal recognition is likely to be more effective in the difficult conditions of life in a dense breeding colony than is visual recognition. As an example, work on the sandwich tern (*Sterna sandvicensis*)[2] has shown in some detail that the individual vocal patterns are constant and can be distinctively categorized in terms of a pattern of energy distribution expressed as frequency against time, which is so well shown by the spectrograph. This kind of pattern is, of course, what we ourselves hear as organized sounds with their characteristic temporal arrangement and tonal quality, as in speech or music.

Our object here is briefly to summarize some interesting new findings with gannets (*Sula bassana*); a full account is in the press[3]. The interesting features are that the usual frequency-time parameters were of little use in analysing individual vocal features; instead, changes in the amplitude envelope were used. Further, the first part of each call was sufficient to give individual information.

Until recently, the most extensive work on the individuality of sounds has dealt with humans. A great many experiments suggest that amplitude fluctuations of speech are relatively unimportant and, indeed, may be removed electronically without seriously affecting intelligibility[4]. Computer recognition of individual speech shows that frequency-time parameters are more useful than amplitude characteristics[5]. In the gannet, amplitude changes are highly individual, though whether the birds themselves use this characteristic has not yet been tested. (Playback experiments, to be described in a subsequent paper, were made using only the whole calls—to which the birds do respond.) We have other observations, however, which would suggest that amplitude changes may be important. Thus sandwich tern calls, which have been shown to be individual from other measures, have been analysed for amplitude characteristics and show marked individuality (unpublished observation). Whether this phenomenon is general among birds remains to be seen.

We made recordings for a month from a small colony of known individual gannets on the Bass Rock (Firth of Forth, East Lothian, Scotland). The calls were subsequently analysed on a sound spectrograph with an amplitude display attachment. The typical landing calls of the gannet are shown as standard sonograms in Fig. 1. Both gross timing analysis (wide band sonograms, Fig. 1*a*) and gross frequency analysis (narrow band sonograms, Fig. 1*b*) show considerable overlap between individuals and are obviously not useful in making any individual identifications. The measure which did show consistent individual features is shown in Fig. 2, which depicts the amplitude of the sound envelope as a function of time. It should be obvious from this figure that the consistency resides in the first few peaks and troughs. The observations were sufficiently encouraging to warrant a more precise method of measurement.

One of the drawbacks of the amplitude display device is a slow response time, so that slowing down the sound gives a better picture of any fine detail, and in the final analysis we used the calls at half speed. With a curve tracer, readings of the log amplitude were obtained from each call at millimetre intervals, taking the sonogram baseline as 0. Each millimetre interval corresponded to 0·005 s in real time, and the number of intervals involved will be referred to here as N. By aligning the calls at the lowest point of the first major trough, all component calls in a sequence were averaged to give a single numerical "profile" of a complete call; each ordinate of the final profile was therefore an average of the log amplitudes at corresponding millimetre intervals. The profiles obtained were checked by replotting them on to graph tracing paper for comparison with the original calls; the fit was usually very good. At half speed, the length of the calls varied from $N = 55$ to $N = 100$. To reduce the amount of information and expedite subsequent analysis, the profiles were cut to the length of the shortest ($N = 55$).

Matching was subsequently done by a computer using linear correlation[5] (Pearson's correlation coefficient, r_p) as a measure of similarity. In the manner described above, we obtained three profiles for each of nine different males, and two from another, each representing calls uttered on separate occasions. (Female calls were not investigated at this time.) One profile of each male was chosen, at random, as a reference sound. These ten references (Fig. 3) were stored in the computer; each of the remaining profiles (sample sounds) was then compared with the list to see with which reference it matched best. Matching consisted of calculating r_p for the sample against each of the references. The sample was said to match with the reference that gave the highest r_p, regardless of

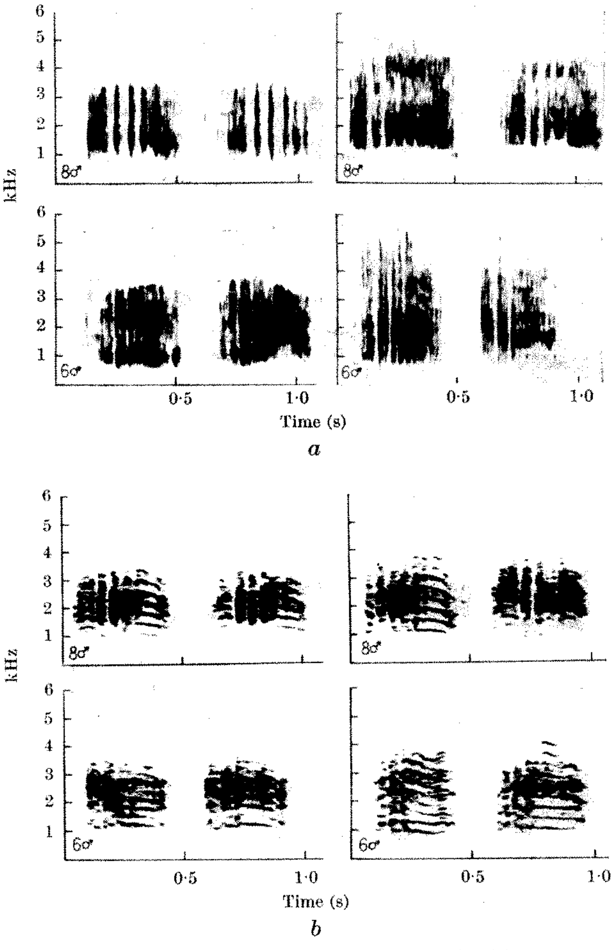


Fig. 1. Landing calls of two male gannets uttered on two separate occasions. (A full landing sequence may contain from six to more than twelve calls.) *a*, Wide band sonograms—normal speed (7·5 inches/s); *b*, narrow band sonograms—normal speed.

May we introduce you to an interesting Polish periodical entitled

ARCHIVUM IMMUNOLOGIAE ET THERAPIAE EXPERIMENTALIS

Published by the Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences. It publishes the works of Polish and Foreign scientists undertaking experimental research in the fields of immunology, microbiology, virology, hematology, experimental oncology, chemical therapy and biochemistry. Published bimonthly. Articles in English. Annual subscription £12.10.0.
Subscription orders are received by local foreign press distributors or
Ruch Export and Import Enterprise, Warszawa 1, P.O. BOX 154, Wronia 23, Poland.
Subscriptions may be paid into the account of Ruch Export and Import Enterprise Warszawa, at the Bank Handlowy in Warszawa.

Thermal Neutron Diffraction

Edited by B. T. M. Willis

This book is based on papers presented at an international summer school held at Harwell in July 1968 on Accuracy in Neutron Diffraction. Recent research on the magnetic and nuclear elastic scattering of thermal neutrons is reviewed by leading workers in the field. This work has led to new information on the electronic and nuclear charge distributions in solids, and the book should therefore be of interest to crystallographers, solid-state physicists, and chemists. 130 text figures paper covers 65/-

Mind, Brain and Adaptation in the Nineteenth Century

Cerebral Localization and its Biological Context from Gall to Ferrier

Robert M. Young

The author examines ideas of the nature and localization of the functions of the brain in the light of the philosophical constraints at work in the sciences of mind and brain in the nineteenth century. He pays particular attention to the phrenology, sensory-motor physiology, associationist psychology, and the theory of evolution as applied to the study of psychology. 1 text figure 65/-

The Collected Papers of G. H. Hardy

Including Joint Papers with
J. E. Littlewood and others

VOLUME IV

Edited by a Committee appointed by the London Mathematical Society

The main object of this publication is to render more accessible the papers of the great mathematician, which in their original form appeared in many journals over a period of almost sixty years. Frontispiece £6

The Biology of Pseudoscorpions

Peter Weygoldt

This is the first comprehensive treatment of the biology of pseudoscorpions. Published in Germany in 1966, it has been translated by the author and expanded to include his observations from a recent trip to the United States.
Mr. Weygoldt shows that, although pseudoscorpions seem to be a uniform group of average arachnids, closer study reveals the diversity of their reproductive and developmental biology, their ecology and their behaviour
114 text figures 58/- *Harvard University Press*

Oxford University Press

MATHEMATICS...

Two New Texts from W. B. SAUNDERS Co. Ltd.

Epstein:

LINEAR FUNCTIONAL ANALYSIS

This new book provides a first look at linear functional analysis in which the abstract concepts are preceded by the study of non-trivial concrete problems. Once abstract methods are developed they are used to solve some classical problems. Some topics receiving special attention are: measure and integration; integral equations; estimation of eigenvalues of Hermitian kernels; L^2 theory of Fourier transforms. Accessible, as a text or reference, to university students in mathematics, applied mathematics, physics and mathematical engineering.

CONTENTS:
Metric Spaces—Lebesgue Measure and Integration—The L^p and l^p Spaces—Normed Linear Space—Linear Functionals—Linear Transformations and Operators—Operators on Finite-Dimensional Spaces—Elements of Spectral Theory in Infinite-Dimensional Hilbert Spaces.

229 pages. Illustrated. February 1970 £4 1s. 0d.

By: Bernard Epstein, *Professor of Mathematics, University of New Mexico.*

Ballard:

GEOMETRY

A mathematically substantial textbook in geometry which rests on some metric concepts. This assumption of some metric axioms provides relative unity with other mathematics familiar to beginning university and advanced secondary school students.

CONTENTS:
Points, Lines, and Planes—Distance and Betweenness—Convexity and Separation—Degree Measure—Congruence of Triangles—More about Triangles; Some Inequalities—Parallels and Similarity—Circles—Space Figures—Area and Volume—Measurement—Co-ordinate Geometry of the Plane—Spheres—Euclid's Parallel Postulate—The Theorem of Desargues—Axiom Systems for Euclidean Geometry—Finite Geometries—Pythagorean Triples—Cardinal Numbers—The Jordan Curve Theorem.

By: William R. Ballard, *Department of Mathematics, University of Montana*

238 pages. 178 illustrations. February 1970 £3 16s. 6d.

W. B. SAUNDERS COMPANY Ltd
12 Dyott Street London WC1

BACK VOLUMES OF SCIENTIFIC PERIODICALS

We are always interested in buying your collections of duplicates of Scientific, Technical and Medical Journals at the highest prevailing prices. Please send us your lists of surplus journals, which will receive our immediate attention.

We are Distributors of Back Issues of Journals including Pergamon Press Journals and many other Publishers. We invite you to write in for our regularly issued Bulletins and Catalogues and should be pleased to be of assistance in your acquisitions.

ROBERT MAXWELL & CO. LTD.
Back Issues Department
4, Midland Road
OLNEY, Bucks, England.
Tel. Olney 529

Periodicals Wanted

Highest prices paid for sets, runs, and volumes of Technical, Scientific, Medical, and General Publications in all languages.

Please submit your lists of items available, for our prompt attention.

Established 1889
ABRAHAMS MAGAZINE SERVICE, INC.
Dept. A, 56 E. 13th Street, New York, N.Y. 10003

MATRIX ALGEBRA FOR BUSINESS AND ECONOMICS
by **S. R. Searle** *and* **Warren H. Hausman,** *both at Cornell University*
Deals with the application of matrix algebra to problems of economics and business administration.
due April 1970 384 pages 40s

Published by
John Wiley
& Sons Ltd
Baffins Lane
Chichester
Sussex

Advertisement Department for
Nature
T. G. SCOTT AND SON LIMITED
1 Clement's Inn, London WC2A 2ED
01-242 6264

its absolute value. This identifying procedure was replicated using progressively shorter sounds (by reducing N) to test the suggestion arrived at when matching the displays by eye: that the most characteristic parts were the first few peaks and troughs. A matching "session" was ended when a sample sound no longer matched its own reference.

The results of these analyses are shown diagrammatically in Fig. 4, where intervals along the horizontal axis represent reference sounds and intervals on the vertical axis represent the sample sounds; corresponding intervals on the two axes are associated with the same male. If the achieved match is shown by a blackened triangle, then, with perfect matching, one would expect a diagonal of black squares (except in the case of male 10, which had only one sample sound). The figure shows that as N decreases, there are very few errors in matching until $N = 30$, by which time there are three mis-matches. At $N = 25$ and below, identification becomes more difficult. (Compare this with Fig. 3, which shows, for each reference, the smallest amount of that profile which was necessary to maintain identity with one of its own sample sounds.) Because the technique is essentially one of pattern matching, it is not surprising that where confusions did occur (for example, males 3 and 4), they were between shapes that were subjectively the most similar. (In this sense linear correlation, which gives as much weight to similarities as to differences, may not be the most sensitive statistical test to use. Moreover, the eye does not operate that way.) These results do, however, confirm our suggestion that gannets have individual calls and, further, that there is enough information in the beginning parts of each call to distinguish one individual from another.

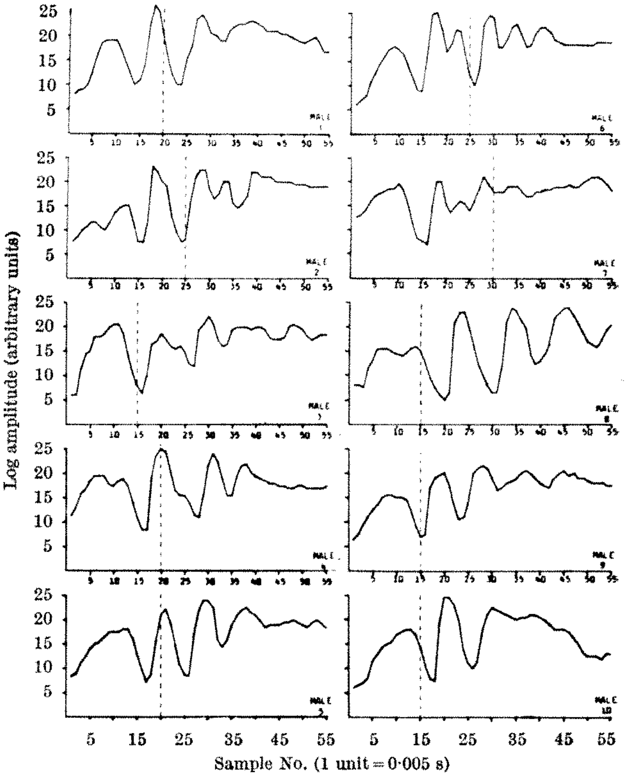


Fig. 3. The reference profiles with which the sample profiles were correlated. Dotted lines show the smallest amount of that sound which was necessary to match with one of its own sample sounds.

If there is enough information in the first part of the call to identify the caller, what is the function of the end part? It is probably worth noting that we are restricting our questions by looking merely for individuality in a call; the call of an incoming bird could well carry much more information than its identity. But it makes intuitive sense that the identifying part of a call should be at the beginning (after comparatively long periods of silence) so that sitting individuals, who probably pay low level attention to all sounds coming from the proper direction, can receive individual information efficiently. The differences we have found do, however, depend to some extent on the method of analysis we have chosen (amplitude detection), and it might be that the sounds are individually different in a number of ways. This is not surprising, for one might expect that any sound selected to be individually recognizable in noisy conditions would show its uniqueness in a number of ways. The envelope amplitude was the most obvious feature to us and then, primarily, in the first parts of the call. It may also be that information is coded differently in different parts of the sound. So, for example, the lack of consistent detail in the amplitude tracings above $N = 30$, which is also shown by the lack of improvement in matching above that N, might occur because a different kind of information is present. If differences in frequency were important here, our analysis method would not find them. A few sounds examined on an oscillograph, which has a fast response time, did suggest some consistent detail in the end regions of the call as well, but this was not as regular a feature as the beginning parts (see Fig. 2).

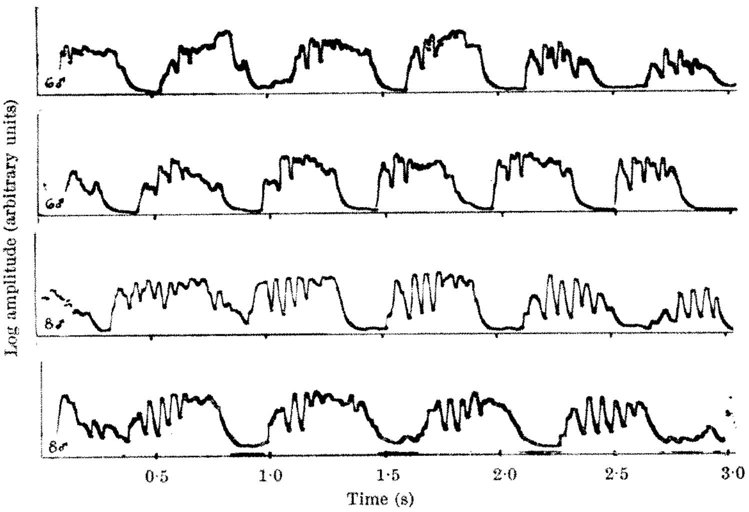


Fig. 2. Amplitude displays of landing calls of two male gannets; each call was uttered on a separate occasion. Normal speed (7·5 inches/s).

Our work tells us very little about how gannets themselves perceive sound. They would presumably make use of as much information as possible. Sea birds present interesting acoustical problems in that their environment is extremely noisy and they usually call while in flight. In conditions of movement and noise, what would be the most effective signal? One possibility is that intermittent signals, such as those given by the gannets, might be very easy to localize. Sitting birds do not appear to be searching for their absent mates. Birds tend to approach their colony up-wind, however, diverging towards their particular nests only when they are some yards away. So an incoming bird might well be identified by its line of flight as well as by any individuality of voice. The impulsive nature of the beginning of each call probably makes accurate localization easier, and by tracking approaching sources of sound, a sitting bird might quickly detect one which was approaching its nest and, at the same time, gain further information from the individual structure at the beginning of each call. Birds have an acute temporal sense[6] and individuality might reside less

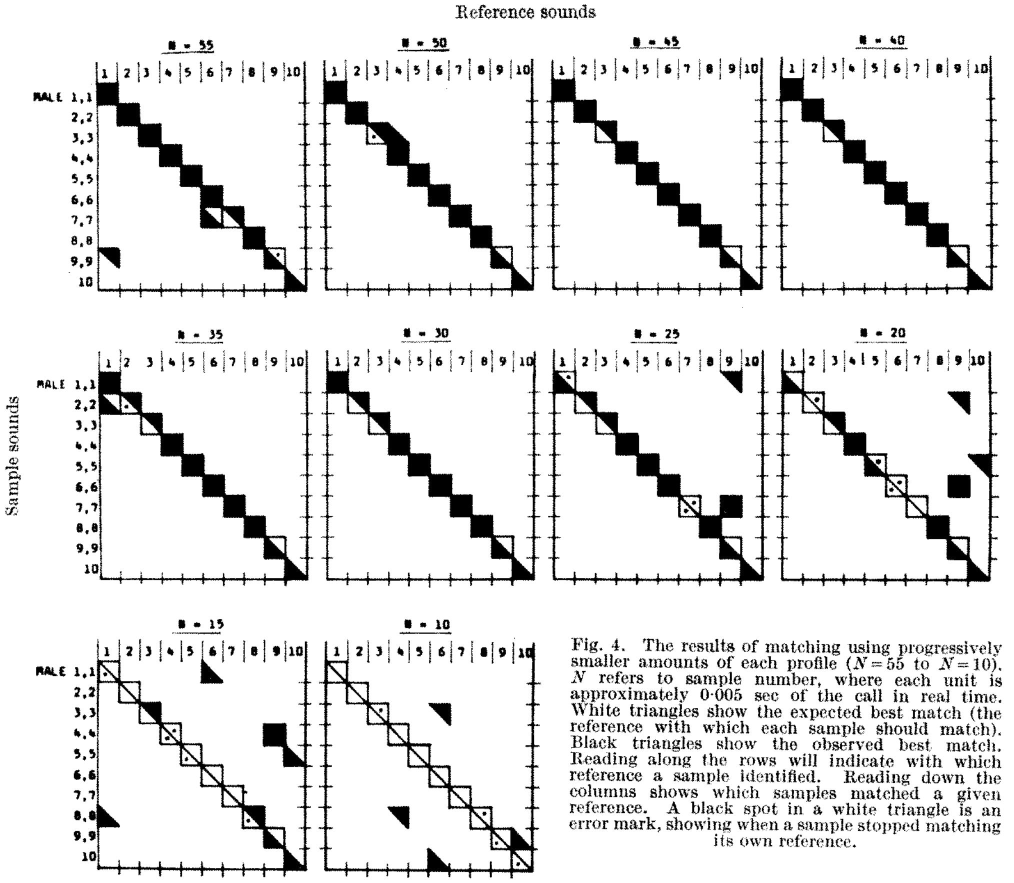


Fig. 4. The results of matching using progressively smaller amounts of each profile ($N = 55$ to $N = 10$). N refers to sample number, where each unit is approximately 0·005 sec of the call in real time. White triangles show the expected best match (the reference with which each sample should match). Black triangles show the observed best match. Reading along the rows will indicate with which reference a sample identified. Reading down the columns shows which samples matched a given reference. A black spot in a white triangle is an error mark, showing when a sample stopped matching its own reference.

in the amplitude *per se* than in its temporal patterning as a series of impulses. Further, because the amplitude envelope of the calls can be both averaged and cross-correlated, it would follow that they vary regularly in time.

Birds discriminate frequency as well as humans[6] and have a finer temporal sense. How well they perceive amplitude is not known. If amplitude were perceived, what possible function could it have? One speculation could be that it provides a way of accommodating for changes resulting from movement. There are changes in the temporal structure of sound in proportion to the rate of movement of its source (the Doppler effect). It follows that all sound frequencies received are also changed. This would mean that the frequency and time structure of an approaching bird's calls would be heard with a more or less constant shift from their true values, the shift depending on its speed of approach. Amplitude would, however, change only gradually as the bird came nearer. Whether birds are capable of discriminating these changes has yet to be tested.

We wish to make two further points about the use of such techniques. First, not only is the question of individuality of voice production interesting in itself, but it could be used as a fruitful approach to the question of bird perception. That is to say, by analysing those features which make calls distinctive, one goes a step towards effective testing of what the animal is capable of hearing. Sound manipulation and playback would, of course, be the next step. Second, developing techniques for readily identifying individuals on the basis of their sounds would make certain types of field work possible. For example, in conditions where physical marking of individual animals may be difficult or disruptive, or where it is not always possible to see the animals easily, voice-tagging might be most helpful. Further, if reliable voice identification were possible, it might make possible "blind" experiments in the field, with subsequent identification of individuals in the laboratory.

SHEILA J. WHITE
R. E. C. WHITE
W. H. THORPE

Sub-Department of Animal Behaviour
(Department of Zoology),
University of Cambridge,
High Street, Madingley, Cambridge.

Received January 19, 1970.

[1] Thorpe, W. H., *Nature*, **220**, 124 (1968).
[2] Hutchison, R. E., Stevenson, J. G., and Thorpe, W. H., *Behaviour*, **32**, 150 (1968).
[3] White, Sheila J., and White, R. E. C., *Behaviour* (in the press).
[4] Licklider, J. C. R., and Miller, G. A., in *Handbook of Experimental Psychology* (edit. by Stevens, S. S.), 1040 (Wiley, New York, 1951).
[5] Pruzansky, Sandra, *J. Acoust. Soc. Amer.*, **58**, 354 (1963).
[6] Schwartzkopff, J., *Fortschr. Zool.*, **12**, 206 (1960).

Faunal Provinces in the Ordovician of North Atlantic Areas

WILLIAMS[1] has recently pointed out a number of quantitatively significant relationships between Ordovician brachiopod faunas on both sides of the Atlantic. In the Early Ordovician and the early stages of the Middle Ordovician three brachiopod faunal provinces persisted in the area of the British Isles. The provinces occurred as belts in the NE–SW strike direction of the Caledonian geosyncline. Along the northern margin of the geosyncline, extending through NW Ireland and Scotland, there was a province with mainly North American faunal affinities (American Province). Williams suggests that the migration of the brachiopods in this province took place by a current transporting the brachiopod spat towards the NE. The central part of the geosyncline was occupied by anaerobic bottoms with graptolitic mud, devoid of significant brachiopod fauna. South of this belt there was a strip extending through SE Ireland and NW Wales (Anglesey), in which the Lower–Middle Ordovician brachiopod faunas are most closely related to stratigraphically equivalent faunas of the Baltic area (Baltic Province). Williams shows that these faunas probably spread with a current system flowing towards the SW. The same current system was responsible for the migration of the Anglo-Welsh fauna occupying the southern marginal area of the geosyncline.

The fairly precise model sketched above was stated by its author to be based exclusively on brachiopods. This limitation is not as serious as it might seem at first, for brachiopods are in many cases the dominant faunal elements. They are greatly diversified and have been subjected to intensive study by many authors in different countries. Within the well aerated, current-swept realms of sedimentation to which they must have been restricted even in the Ordovician, they seem to have been relatively free in their choice of sedimentary substrate.

It was thus a reasonable starting hypothesis that brachiopod faunal provinces were separated by geographic barriers such as different current systems, rather than by purely ecological control. Evidence from the distribution of genera of graptolites and trilobites does not seem to offer any essential contradiction to this model.

The concept developed by Williams has been sketched in some detail because it will be shown how the conodonts fit into the picture. Our knowledge of conodont distribution in the Ordovician on both sides of the Atlantic is too fragmentary for detailed reconstructions of the palaeogeography to be founded on conodonts alone. They might, however, help in the interpretation of distributions of other faunal elements. One serious limitation is that the mode of life of the conodonts is as unknown as their precise place in the animal kingdom. What we do know is that they were marine animals with an unusual spreading ability. Further, they were independent of substrate to a greater extent than any other well-known group of fossil animals.

In the Arenigian, the first of four stratigraphic intervals discussed by Williams, a Baltic conodont fauna was found by Lamont and Lindström[2] in the southern uplands of Scotland—that is, within or adjacent to the American Province of Williams. Twenty-two species were listed. Because present knowledge about conodont apparatuses consists of several different-looking elements, the number of species has to be reduced to sixteen. Four elements were identified with some doubt. The rest of the fauna is beautifully preserved, and conspecific with forms that occur in the Billingen substage (BI_{ss}) of the Lower Arenigian Latorp stage all over the Baltic Shield. Until now this fauna has not been found in North America. American faunas that are roughly contemporary seem to be characterized by a number of panderodonts (in the widest sense, including *Acanthodus*) that have not been found in Europe. The easternmost recorded occurrence of *Acanthodus* is near Chambersburg in southern Pennsylvania[3].

The same fauna contains *Loxodus*, which is highly characteristic and is another North American element that has not been found in Europe in spite of intensive and extensive collecting. The genus *Cordylodus* found in the same section was cosmopolitan in the Tremadocian but is absent in most Arenigian faunas in Europe.

In the Llandeilian to basal Caradocian, which is close to another interval discussed by Williams, the belt from southern Scandinavia through Scotland to the Appalachians has a characteristic conodont fauna consisting of *Periodon*, *Pygodus*, *Cordylodus spinatus* Hadding, and coarse, costate forms related to *Drepanodus* s.str.[2,4-6]. Elements of this fauna only rarely transgressed into the American Midcontinent[7]. Conversely, Llandeilian conodont faunas recovered from Wales have strong affinities to certain Midcontinent faunas[8]. During the Caradocian the provincial boundaries tended to become less sharp.

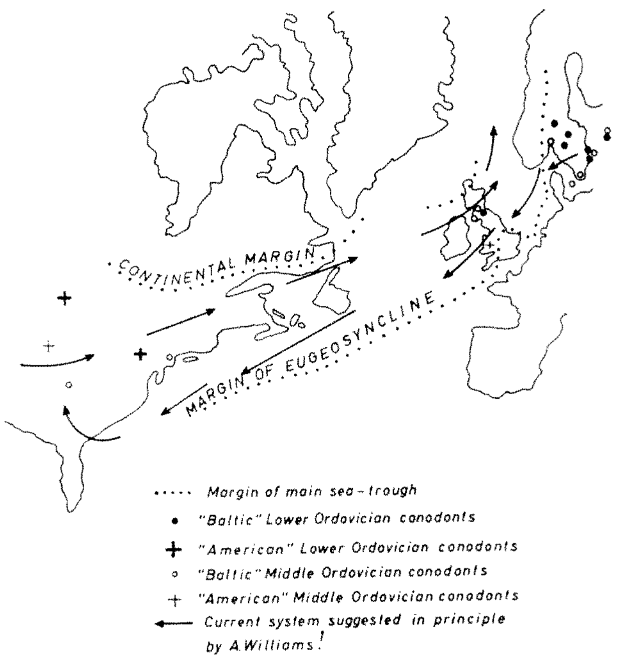

CONTINENTAL MARGIN
MARGIN OF EUGEOSYNCLINE
Margin of main sea-trough
"Baltic" Lower Ordovician conodonts
"American" Lower Ordovician conodonts
"Baltic" Middle Ordovician conodonts
"American" Middle Ordovician conodonts
Current system suggested in principle by A.Williams.[1]

Fig. 1. The American and European continents in an arbitrary relative position suggesting some continental drift.

It would clearly be impossible to derive a simple and consistent current system from the distribution of brachiopods and conodonts. Using the conodonts alone might turn out to be more misleading than using the brachiopods. But the conodont evidence, scant though it is, proves that the current pattern explaining the distribution of brachiopods in the Ordovician has to be modified to fit the real case. Ecological control of faunal distribution should perhaps also be considered more seriously.

M. LINDSTRÖM

Geologisches Institut der
Philipps-Universität,
355 Marburg, Germany.

Received December 17, 1969.

[1] Williams, A., in *The Pre-Cambrian and Lower Palaeozoic Rocks of Wales*, 117 (Univ. Wales Press, 1969).

[2] Lamont, A., and Lindström, M., *Trans. Edin. Geol. Soc.*, **17**, 60 (1957).

[3] Sando, W. J., *Geol. Soc. Amer. Bull.*, **13**, 1 (1958).

[4] Ethington, R. L., Furnish, W. M., and Markewicz, F. J., *J. Paleontol.*, **32**, 763 (1958).

[5] Sweet, W. C., and Bergström, S. M., *J. Paleontol.*, **36**, 1214 (1962).

[6] Lindström, M., *Conodonts* (Elsevier, Amsterdam, 1964).

[7] Bergström, S. M., and Sweet, W. C., *Bull. Amer. Paleontol.*, **50**, No. 229 (1966).

[8] Bergström, S. M., *Acta Univ. Lundensis*, Sect. 2, No. 3 (1964).

Hybrid Vigour in Seedlings of Re-created London Plane

THE London plane (*Platanus* × *acerifolia* (Ait.) Willd. = *P.* × *hispanica* Muenchh.) has long been regarded as an accidental hybrid between the oriental plane (*P. orientalis* L.) and the American plane (*P. occidentalis* L.). The segregation of characters in populations of seedlings derived from open pollination of London planes is evidence of this hybrid origin. Whether the tree originated at the Oxford Botanic Garden about 1670 (ref. 1) or developed earlier may never be known. Regardless of its origin, the London plane has proved extremely valuable as a tree that will endure the difficult environments of modern cities.

Both the common and scientific names of the London plane refer to an assemblage of trees that are generally intermediate between the two putative parents. It is doubtful whether any living, mature tree can be verified today as a true first generation hybrid. The variability among London planes is especially notable in the eastern United States, where trees of seedling origin have been planted along city streets. In addition to variation in morphology, the London planes in America exhibit a wide range of susceptibility to damage by sycamore anthracnose caused by the fungus *Gnomonia platani* Kleb.

There have been no previous reports of attempts at the controlled repetition of the cross, which I have now accomplished. In the spring of 1968, I made reciprocal crosses between trees of oriental and American planes growing at the US National Arboretum. This hybridization was part of an extensive breeding programme designed to develop better trees for city planting. The oriental planes were of authentic Turkish origin and were fruiting heavily at 14 yr old. The American planes were mature, native trees growing in the Arboretum.

Seed from these crosses was collected in December 1968, and kept at 5° C. Seeds were sown in flats in the greenhouse on April 28, 1969. Seedlings from hybrid and non-hybrid seedlots were handled alike during all cultural treatments. No fertilizer was applied to any of the plants while they were in the flats or pots throughout the growing season. Final heights were measured on October 22, 1969.

Unusual vigour was apparent in the hybrids even in the cotyledon stage. Cotyledons of hybrid seedlings were longer and broader than non-hybrids from the same mother tree. Average heights of fifty comparable 1 yr old seedlings per seedlot were for *occidentalis*, 14·8 cm; for *occidentalis* × *orientalis*, 26·3 cm; for *orientalis* × *occidentalis*, 29·1 cm; and for *orientalis*, 13·8 cm.

Height differences between the reciprocal hybrid populations were not significant by the *t* test, but the average height of the hybrids was nearly twice that of the parent species. Future measurements on a statistically designed field planting will determine whether this juvenile superiority of the hybrids is maintained in later life.

FRANK S. SANTAMOUR, jun.

US National Arboretum,
Crops Research Division,
Agricultural Research Service,
US Department of Agriculture,
Washington DC 20002.

Received December 12, 1969.

[1] Henry, A., and Flood, M. C., *Proc. Roy. Irish Acad.*, 35, 9 (1919).

Transition Probabilities for a Forest

THE vegetation on a tract changes as a field or felled forest becomes a forest again, and a quantitative statement of the changes is needed for comparing one period of changes with another and for logical extrapolation to the equilibrium or steady state. Enumeration of the stems in four mixed hardwood forests during 40 yr has enabled us to test "transition probabilities" as a means of stating, analysing and making use of the observed changes.

If parts of a tract of land are dominated by one of several classes or states of vegetation, the changes from one state to another during a given interval, such as a decade, can be described by transition probabilities. A matrix of the transition frequencies for all states is a convenient summary of the changes. If the transitions do not depend on how the forest reached its present state but only on the present state, and if the frequencies are constant from decade to decade, the matrix is powerful as well as convenient. It is a stationary Markov chain[1], which has two useful properties.

First, the probabilities for an interval of several decades can be calculated by multiplying the matrix by itself. The second useful property involves calculating the steady state.

These properties are obviously desirable, and the only problem is whether the changes in a forest behave as a stationary Markov chain. Observations on a sufficient number of plots during a sufficiently long time to test the behaviour are rare, but fortunately we are heirs to an enumeration of all stems larger than 12 mm diameter, breast high, in about 5 hectares of mixed hardwoods in four central Connecticut forests. The first enumeration was made in 1927 when the trees were mostly 25 to 40 yr old[2]. It was repeated 10, 30 (ref. 3) and 40 (ref. 4) yr later. The enumerations have included 30,000 stems, but the population has decreased to half its original size. When the transects of the surveys are divided into 0·01 hectare tracts 20 m long and 5 m wide, and disturbed, dry and wet tracts are discarded, 327 fairly uniform plots remain for testing the behaviour of transition probabilities.

Sets of states can be conceived and the plots classified accordingly, but those that are easily understood and that produce constant probabilities independent of preceding states are most useful. Several classifications have been made and will be fully described elsewhere[4]. For this brief communication, however, the tracts were classified as maple, oak, birch, other major species or minor species according to which of the five classes had the most stems on the tract.

The stationary nature of the transitions among these classes in the forest is seen by comparing probabilities from period to period, but their Markovian nature is less easily demonstrated. The customary test for this requires that the classes be subdivided according to their antecedents and then observing whether the antecedents affect the transitions. In our case of five classes, subdivision would create twenty-five classes, and our 327 plots are too few to permit this treatment. Therefore another course has been taken.

Table 1. TRANSITION PROBABILITIES

	1927				
	Maple	Oak	Birch	Other	Minor
Observed 1937					
Maple	82	16	13	7	7
Oak	7	72	2	3	7
Birch	2	8	83	7	7
Other	0	0	2	69	7
Minor	9	4	0	14	72
Anticipated, 40 yr					
Maple	53	35	32	22	23
Oak	16	33	8	11	15
Birch	11	19	50	18	18
Other	2	2	4	26	11
Minor	18	11	6	23	33
Observed 1967					
Maple	62	32	22	45	20
Oak	0	15	0	0	4
Birch	16	23	54	10	21
Other	2	7	2	17	5
Minor	20	33	22	28	50

These transition probabilities are those observed in 1927–1937 (upper matrix); those for 40 yr obtained by multiplying the 1927–1937 matrix of probabilities by itself three times (middle matrix), and those observed in 1927–1967 (lower matrix).

The two properties of the stationary Markov chain which we described earlier were used; they produce much the same answers regardless of the decade of transitions used to obtain the probabilities. This has been taken as evidence of the stationary Markovian nature of the transitions in the forest. In the first demonstration (middle of Table 1) the transition probabilities for 40 yr are calculated by multiplying the matrix for the first decade (top of Table 1) by itself three times. These 40 yr probabilities are compared with the probabilities actually observed between 1927 and 1967. The principal difference between the transitions anticipated by multiplying the 1927–1937 matrix and the transitions observed concerns oak, which has persisted less than anticipated. On the other hand, several important features were anticipated. The persistence of maple, birch and minor; the transitions from oak to maple and birch; the transitions from birch to maple; and the transitions from other and minor to maple and birch could have been rather well predicted in 1937 by manipulating the matrix of probabilities for the first decade, as is done in the middle of Table 1.

Although the plots were not numerous enough to divide into twenty-five classes, they were divided into older and younger forests. They were also divided into moister and drier groups. The probabilities were reassuringly similar in different groups.

Table 2. PERCENTAGES OF FOREST PLOTS IN FIVE CLASSES AT STEADY STATE ANTICIPATED FROM TRANSITIONS OBSERVED IN TWO DECADES

Observations	Maple	Oak	Birch	Other	Minor
1927–1937	39	17	22	5	17
1957–1967	45	1	32	6	17

The final comparison is between the steady states anticipated from the transitions of the first and last decades. If the changes in the forest were an ideal stationary Markov chain, the two steady states would be the same. Whether the transitions of the thickets of 1927–37 or forests of 1957–1967 were extrapolated, the prediction was about the same: many plots of maple or birch, some minor and few oak or other (Table 2). The last decade of drought and defoliation did not change the direction of trends evident in the first decade. Thus the foresight of our predecessors who established the plots has permitted a demonstration that some transition probabilities for the forest can be treated as if they came from a stationary Markov chain, providing both an orderly way of comparing forest changes during periods of different length and of extrapolating into the future.

Paul E. Waggoner
George R. Stephens

Connecticut Agricultural
Experiment Station,
New Haven,
Connecticut 06504.

Received November 3, 1969.

[1] Feller, W., *An Introduction to Probability Theory and its Application*, **1** (Wiley, New York, 1957).

[2] Hicock, H. W., Morgan, M. F., Lutz, H. J., Bull, H., and Lunt, H. A., *Conn. Agric. Exp. Sta. Bull.*, 330 (1931).

[3] Olson, A. R., *Conn. Agric. Sta. Bull.*, 669 (1965).

[4] Stephens, G. R., and Waggoner, P. E., *Conn. Agric. Sta. Bull.*, 707 (in the press).

Possible Side Effects of Fungicides on Banana and Coffee Diseases

Experience suggests that excessive application of fungicidal sprays to crops has not had as many undesirable biological side effects as the excessive application of insecticides. Nor has there been much evidence, at least at the level of field application, of acquired resistance to fungicides[1].

There are various ways in which fungicide sprays may have an effect other than that intended, and in this respect recent publications on the non-parasitic microflora of leaves are interesting. Hislop and Cox[2], for example, discussed the implication of fungicide usage in relation to the balance of saprophytic and parasitic microorganisms of the phylloplane. And Furtado[3] emphasized that copper sprays bring about an increase in the population of virulent *Colletotrichum coffeanum* Noack in coffee bark. He quoted how coffee berry disease increased in Kenya as a result of copper spraying. Wallis and I[4] produced experimental evidence for this in 1967.

This apparent favouring of a virulent pathogen at the expense of a related saprophytic or less virulent form is a most interesting fungicidal side effect. The recent replacement of *Mycosphaerella musicola* Leach, the cause of Sigatoka disease of bananas, by *Mycosphaerella* sp., the cause of the much more serious black leaf streak disease, in Fiji and other Pacific territories could be viewed in this light. Details of this disease and its geographic distribution and spread have been described several times[5–8]. The black leaf streak fungus produces conidiophores sparsely and ascospores abundantly, in contrast to *M. musicola* which produces dense fascicles of conidiophores and relatively few ascospores. When he first observed this disease Rhodes[9] noted it on plantings where Sigatoka disease had previously been controlled effectively by oil spraying, which was not controlling the new disease satisfactorily. Stover and Dickson[10] noted that one of the effects of oil on *M. musicola* was to reduce the number of sporodochia and thus the number of conidiophores. It is conceivable that oil favours a form which already has the habit of sparse conidiophore production and relies chiefly on ascospore infection.

It can be argued that black leaf streak disease has so far not been reported in the principal banana producing countries where oil spraying is common. But coffee berry disease is not present in the principal coffee producing countries although *Colletotrichum coffeanum* is ubiquitous. Only in parts of Africa has there been replacement by a virulent strain. The mechanism by which these two virulent fungi have become prevalent is not known, and no similarity is now suggested. But the situation provides a warning that we should perhaps examine the effects of fungicide as carefully as those of other chemicals used for plant protection.

I. D. Firman

Department of Agriculture,
Suva, Fiji.

Received January 6, 1970.

[1] Sisler, H. D., and Cox, C. E., *Plant Pathology*, **2** (Academic Press, 1960).

[2] Hislop, E. C., and Cox, T. W., *Trans. Brit. Mycol. Soc.*, **52** (2), 223 (1969).

[3] Furtado, I., *Trans. Brit. Mycol. Soc.*, **53** (2), 325 (1969).

[4] Wallis, J. A. N., and Firman, I. D., *Ann. Appl. Biol.*, **59**, 111 (1967).

[5] Leach, R., *Coun. Pap. Fiji*, No. 38 (1964).

[6] Graham, K. M., *Dept. Agric. Fiji, Bull.*, **49** (1969).

[7] Meredith, D. S., and Lawrence, J. S., *Trans. Brit. Mycol. Soc.*, **52** (3), 459 (1969).

[8] Meredith, D. S., and Firman, I. D., *Trop. Agric. Trinidad*, **47** (in the press).

[9] Rhodes, P. L., *Commonwealth Phytopathol. News*, **10**, 38 (1964).

[10] Stover, R. H., and Dickson, J. D., *Canad. J. Bot.*, **46**, 1495 (1968).

Hemiparasitic Flowering Plants: Phenotypic Canalization by Hosts

The idea of combining the desired qualities of two distinct genotypes is old and universally appreciated. The genetic information can be brought together in two ways: within the same zygote through hybridization; or, for example, in higher plants, by grafting the stem of a superior fruit onto a particularly hardy root system. It is increasingly clear that the phenomenon of grafting (like hybridization) is a common natural process in which the root systems (and stems) of genetically distinct individuals become functionally interconnected.

Such interconnexions are formed naturally in at least three ways: by mycorrhizal fungi[1,2], by natural root grafting among forest trees[3] and through the haustorial connexions of holoparasitic and hemiparasitic vascular plants (see, for example, ref. 4). Although the mechanisms of coupling and transfer differ markedly in these bridge-forming plants, they have in common the capacity to share the biochemical products of other genetically distinct individuals.

The hemiparasitic flowering plants that live in annual grasslands are of special interest because each year they come into random contact with a wide range of potential

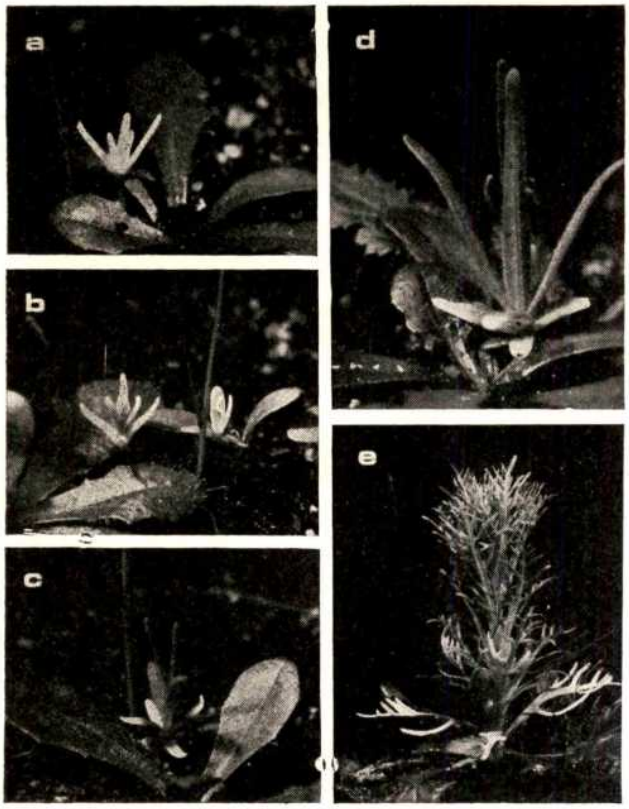


Fig. 1. *a–d*, Five week old albino *Orthocarpus* seedlings with host plants, *Hypochoeris glabra* and *Festuca myuros*. *a*, 1 cm seedling with 3 pairs of white leaves; *b*, seedling on left (1 cm) shows third pair of leaves beginning to turn green; *c*, 1·5 cm, *d*, 2·2 cm, initiation of rapid heterotrophic growth following production of green leaves. *e*, Eleven week old individual (12 cm) showing initial white leaves plus temporary chlorosis of new leaves as a result of low light intensity.

host species. Annual hemiparasites characteristically form haustorial connexions (root grafts) with most of the plants that surround them, including members of their own population. Single individuals may form root grafts with two or three different host species at the same time, and there may be as many as fifty potential host species available to the population as a whole[5].

Individuals of many hemiparasitic species are also capable of independent autotrophic growth. Populations of the annual grassland hemiparasite *Orthocarpus purpurascens* (Scrophulariaceae) contain a full range of autotrophic abilities, from individuals that can grow and reproduce without a host, to a variable number of plants that must have a host to mature and reproduce. When individuals are provided with host plants, mortality is often decreased and reproductive potential is usually markedly increased[6].

Because individuals with host plants survive better, normally non-viable individuals may be chemically buffered or canalized. Studies on the translocation of water, minerals, carbohydrates[7–11] and amino-acids[12] show that many, if not most, materials travelling in the host vascular system are received by grafted hemiparasites. To demonstrate the buffering capacity of host plants, one must be able to identify a lethal or semilethal phenotype at an early stage in development, before it is associated with the host plant. I now report an experiment with albino *Orthocarpus* which conclusively demonstrates deficiency correction through physiological buffering by host plants.

The albino seedlings were naturally produced after two generations of inbreeding between genetically self-incompatible *O. purpurascens* siblings. One progeny sample yielded about 10 per cent achlorophyllous seedlings; the remaining 90 per cent were entirely normal. At first, all seedlings had green cotyledons and the albino seedlings could not be identified until the first or second pair of true leaves emerged, about 10–14 days after germination. At the first sign of chlorosis, one day old host seedlings (*Hypochoeris glabra*, *Erodium botrys* and *Festuca myuros*) were introduced in various combinations around some of the albino seedlings and all other normal *Orthocarpus* seedlings removed. From a total of seventy albino seedlings, forty received host plants, ten were left surrounded by varying numbers of normal *O. purpurascens* seedlings and twenty were grown as single isolated individuals. The pots were randomized and placed in a controlled environment chamber with a 12 h photoperiod and diurnal and nocturnal temperatures of 21° and 11° C respectively. They were watered daily with deionized water and given liquid fertilizer every two weeks.

All thirty albino seedlings grown singly or with normal *Orthocarpus* siblings remained white and eventually died. The initiation of chlorosis varied with each individual, but two patterns were apparent: either the first pairs of true leaves were white as they emerged, or they were green or yellow and later turned white. All seedlings showing any signs of chlorosis continued to lighten until they were white, and thereafter produced only white leaves. Most of these individuals died within the first 6–8 weeks, but a few produced up to six pairs of very small white leaves and maintained themselves for up to 12 weeks.

In marked contrast to the minus-host treatment, ten of the forty albinos supplied with host plants began to turn green and grow rapidly during 12 weeks after the introduction of the three host species (Fig. 1). In general,

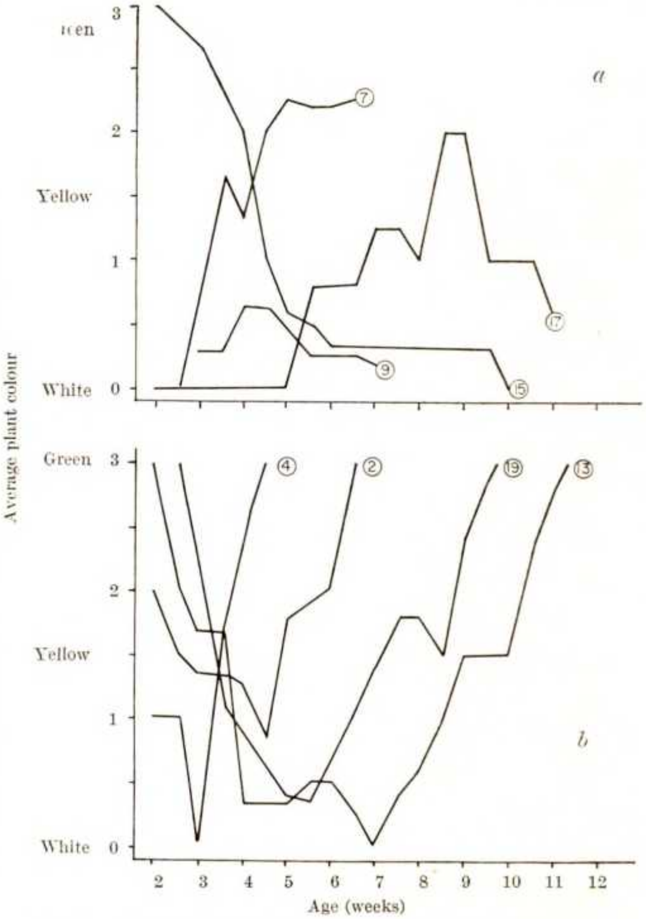


Fig. 2. *a*, *b*, Fluctuations in average chlorophyll content of 8 albino *Orthocarpus* seedlings grown with host plants. Leaf pairs were assigned values of 0 (white), 1 (light yellow), 2 (light green) or 3 (green), and averaged for each plant. Numbers in circles identify the experimental plants (see text).

once an albino seedling began to produce green leaves it also began to grow at the rapid rate characteristic of normally grafted *Orthocarpus* plants. The response of the albino seedlings to these host plants varied both temporally and with respect to colour, growth and survival (Fig. 2).

Seedlings that first produced a pair of white leaves (numbers 7, 9, 17, Fig. 2*a*) showed variable greening responses to their hosts but finally died. Plant number 15 (in Fig. 2*a*) exemplifies the many individuals that did not respond to their host plants, either through inadequate haustoria formation or because the host plants could not provide what the hemiparasitic individual lacked.

Seedlings that first produced green or yellow leaves had the highest incidence of survival (Fig. 2*b*). The short photosynthetic period during early seedling development may thus have increased the energy available for haustoria formation. But the different degrees of chlorosis first shown by the total seedling sample undoubtedly reflect a range of genetic differences. It is more likely that successful deficiency correction was possible only in those individuals with the least complex blocks in chlorophyll synthesis.

Five of the ten grafted seedlings survived and flowered normally. All individuals were fertile, but all sibling crosses failed, evidently because each individual was homozygous for identical incompatibility alleles. Reciprocal crosses between the albino individuals and normal green population members produced abundant seed.

This experiment provides an extreme but recognizable example of the kind of random chemical buffering that necessarily results from interconnexions between genetically distinct individuals. Viewed positively, developmental buffering by a wide range of host plants may be an important mechanism that occasionally canalizes genetically sub-optimal recombinants and mutants towards an optimal phenotype. A negative result of biochemical buffering is the probable accumulation of genes deleterious to autotrophism, leading to increased dependence on heterotrophic nutrition. In *Orthocarpus* populations, such relaxation of selection for autotrophism by chemical buffering is clearly counterbalanced by continual selection for autotrophism (1) in the seedling stage before host contact, (2) after haustoria formation if the host contribution is unsuitable, and (3) when host plants are not available.

This work was supported by the US National Science Foundation.

P. R. ATSATT

Department of Population and Environmental Biology,
University of California,
Irvine, California 92664.

Received November 24, 1969.

[1] Bjorkman, E., *Phys. Plant.*, **13**, 308 (1960).
[2] Campbell, E. O., *Trans. Roy. Soc. NZ*, **2**, 73 (1963).
[3] Graham, jun., B. F., and Bormann, F. H., *Bot. Rev.*, **32**, 255 (1966).
[4] Fineran, B. A., *Phytomorphology*, **13**, 249 (1963).
[5] Thurman, L. D., thesis, Univ. California at Berkeley (1965).
[6] Atsatt, P. R., thesis, Univ. California at Los Angeles (1966).
[7] Rogers, W. E., and Nelson, R. R., *Phytopathology*, **52**, 1064 (1962).
[8] Cantlon, J. E., Curtis, E. J. C., and Malcolm, W. M., *Ecology*, **44**, 466 (1963).
[9] Govier, R. N., and Harper, J. L., *Nature*, **205**, 722 (1965).
[10] Malcolm, W. M., *Ecology*, **47**, 179 (1966).
[11] Okonkwo, S. N. C., *Amer. J. Bot.*, **53**, 142 (1966).
[12] Govier, R. N., and Pate, J. S., *New Phytol.*, **66**, 285 (1967).

Gliding in a Blue–Green Alga: a Possible Mechanism

GLIDING motility has been studied for many years with limited success[1-3]. The gliding movements displayed by several species of blue-green algae and bacteria have not as yet been satisfactorily explained. Earlier observations of gross behaviour and morphology have suggested several mechanisms, but we believe that none of the previous explanations encompass all of the motility characteristics observed in the blue-green alga, *Oscillatoria princeps* Vaucher (Fig. 1).

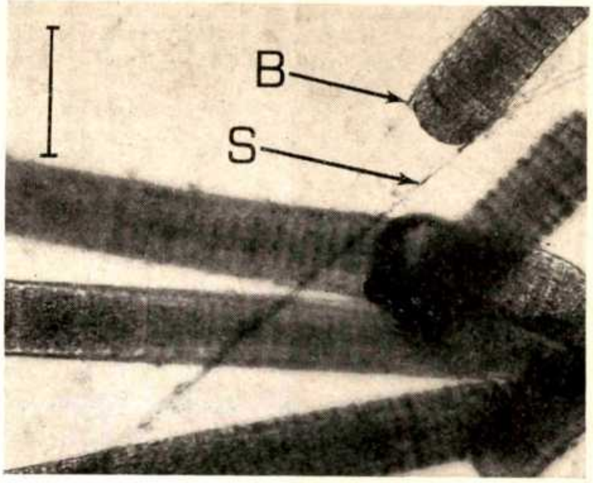


Fig. 1. Gliding trichomes of *O. princeps* are pictured using bright-field illumination. The sheath (S) is being shed by one trichome. A burr (B) of a broken cell is visible. The marker indicates 50 μm.

Our observations suggest that gliding motility is the result of unidirectional travelling waves on the cell surface acting against a solid substrate or a closely associated elastic sheath. These waves may be produced by a lateral deformation[4] of elements of a parallel array of fibrils (50 to 80 Å wide) which we have found to be continuous over the surface of the 30 μm wide trichome. This fibrillar array is wrapped around the trichome in a right-handed helix with a pitch of 60 degrees. The constant rotation of the trichome during gliding seems to be directly related to the dimensions of this helix.

Gliding motility, as noted by Jarosch[5], requires that the organism should be in contact with a solid substrate serving as a structural base on which the motility mechanism of the trichome acts. A thin elastic sheath is generally present and facilitates the purchase of the motility mechanism on the substrate by assuring a close contact on both sides. When contact is not established (as with trichomes suspended in liquid medium) the sheath material is displaced helically in bands over the surface of the trichome. Lacking contact with a solid substrate, the trichome itself does not move. The motility mechanism is thus acting between the cell wall and the sheath. Microscopic examination of the gliding trichomes confirms that the location of the shear zone is between the cell wall and the sheath envelope.

The cell wall of *O. princeps* is approximately 0·2 μm thick, and rigid. The burr of the cell wall left extended when a trichome is broken is not deformed by the elastic sheath casing (Fig. 1). Instead, the sheath is distorted by the burr as it is shed behind the moving trichome. The cell wall does not undergo any alterations in shape observable by light microscopy during the gliding process. Consequently, we have concluded that the motility system is probably located on the superficial portion of the cell wall.

Cells were fragmented by ultrasonic disintegration and the walls isolated by centrifugation. This process produced a large number of small fragments which were tightly coiled. They were mainly cylindrical wall casings which originally encompassed single cells. Ultrastructural examinination revealed that the fragments were turned inside-out. We interpret this to mean that there is a layer on the outer surface of the cell wall which is exerting an elastic force that causes the fragments to coil in this manner. The gross structure of the wall is, however, apparently unchanged by the isolation procedure. These observations also show that the surface of the cell wall may be the site of the motility system.

Ultrastructurally, the cell wall is composed mainly of a thick (2000 Å) structural layer immediately outside the plasma membrane. A 160 Å thick layer is present on the outer surface of the structural layer. The structural layer is penetrated by a series of 700 Å wide pores which terminate at the 160 Å layer. The cell wall is completed by a 90 Å thick membrane-like layer loosely wrapped around the other layers. The 160 Å layer and the outer "membrane" are continuous over the trichome surface and do not participate in cross-wall formation.

Surface sections of chemically fixed *O. princeps* were prepared as well as freeze-etch replicas of the trichome surface. A parallel array of 50 to 80 Å thick fibrils (Figs. 2 and 3) was found on the outer surface of the structural layer, apparently as part of the 160 Å layer. The fibrils seem to be continuous over the cross-wall junctions. If it is assumed that each fibril rapidly propagates waves of bending along its length, it may also be reasoned that the amplitude will be somewhat dampened (as compared with bacterial flagellar waves[7]) by the outer membrane-like layer or by the matrix in which the fibrils may be located. A solid substrate would be required for this type of mechanism to produce movement. As mentioned earlier, we have found this to be true.

The fibrillar array is aligned obliquely (Fig. 2) around the trichome, creating a helix with a pitch of about 60 degrees (that is, 30 degrees from the longitudinal axis). We have observed that gliding movements in *O. princeps* are always associated with a right-handed rotation relative to the direction of movement. It is likely that rotation is a necessary characteristic of gliding in this species. Attempts to impede rotations stop linear translation as well. The helix traced by any surface point on a gliding trichome also has a pitch of approximately 60 degrees. The same helical pattern is traced regardless of the velocity of forward movement. *O. princeps* glides at rates approaching 4 cm h^{-1} within 1 per cent agar. The velocity is greatly reduced by using higher concentrations of agar (up to 4 per cent) to harden the surrounding medium. Even here, the same pitch of the helical pattern is retained. This finding is consistent with the hypothesis that distortions of the fibrils actually constitute the propelling force. The reduced velocity in the more solid medium may result from an increase of slippage between the propelling waves and the substrate. Only a propulsion mechanism aligned helically in the manner of the fibrils would allow the perfectly matched helical movement patterns to be retained in these restrictive conditions. No other structural features (including pores) of *O. princeps* were found with a helical pattern.

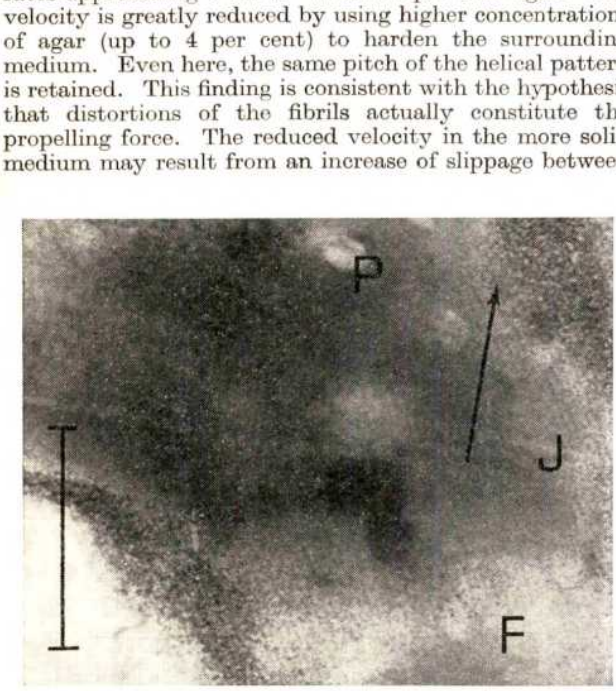


Fig. 2. Surface section of a chemically fixed trichome of *O. princeps*, revealing 50–80 Å wide fibrils (F) which are continuous across the cross-wall junction (J). A row of small junctional pores is seen on either side of the cross-wall. 700 Å wide pores are seen in the structural layer of the cell wall. The arrow indicates the longitudinal axis of the trichome. The marker indicates 500 nm. Image is reversed; Siemens Elmiskop 1A.

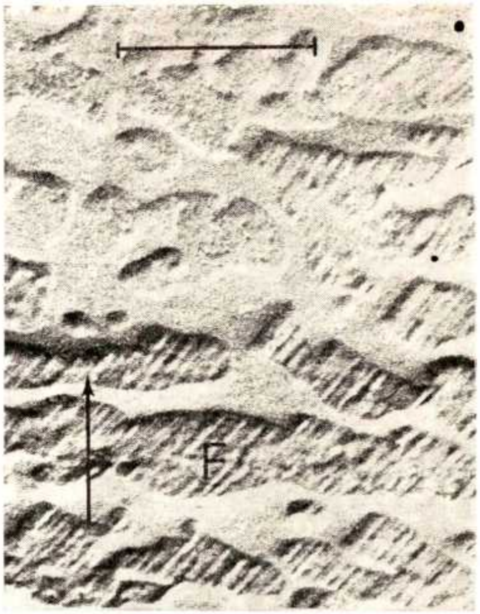


Fig. 3. Freeze-etch replica of a trichome surface revealing a parallel array of 50–80 Å wide fibrils (F). The arrow indicates the direction of platinum shadowing. The marker represents 500 nm.

We have observed that trichomes are able to reverse the direction of movement quickly with no apparent difference in speed[8]. The helix displayed by the surface point on a trichome which has reversed remains unchanged (that is, the direction in which the point travels to trace the helix is simply reversed).

It could be argued that the rotations observed during gliding movements are produced by the helical topography of the cell wall fibrils while some other mechanism such as mucilage extrusion[9] provides the force needed to move the trichome. No evidence has been produced from either light or electron microscopy to support this argument[10–12]. We believe that the undeformed fibrils themselves are too small to act as screw threads that simply guide the rotations. Further, because the fibrillar layer is covered by the outer "membrane" the process would be even less likely.

We prefer to believe that the fibrils actually propel the trichome. If the waves propagated by the fibrils are coordinated only to the extent that they are all travelling in the same direction, trichome movement with associated rotation should be the result when the sheath is attached to a solid substrate. Trichomes not against a solid substrate would displace the sheath in a helical fashion, as noted earlier. Reversals are explained if there is a rapid shift in trichome polarity with a subsequent change in the direction of wave propagation.

R. W. C. was supported by the US National Science Foundation.

LAWRENCE N. HALFEN
RICHARD W. CASTENHOLZ

Department of Biology,
University of Oregon,
Eugene, Oregon 97403.

Received January 6, 1970.

[1] Burkholder, P. R., *Quart. Rev. Biol.*, **9**, 438 (1934).
[2] Schmid, G., *Flora*, **111**, 327 (1918).
[3] Schulz, G., *Arch. Mikrobiol.*, **21**, 335 (1955).
[4] Jarosch, R., *Osterreich. Bot. Zeit.*, **111**, 143 (1964).

[5] Jarosch, R., in *Physiology and Biochemistry of Algae* (edit. by Lewin, R.), 573 (Academic Press, New York, 1962).

[6] Salton, M. R. J., *The Bacterial Cell Wall*, 42 (Elsevier, 1964).

[7] Holwill, M. E. J., *Physiol. Rev.*, **46**, 696 (1966).

[8] Fritsch, F. E., *The Structure and Reproduction of the Algae*, **2**, 800 (Cambridge Univ. Press, 1945).

[9] Prell, H., *Arch. Protist.*, **42**, 99 (1921).

[10] Jost, M., *Arch. Mikrobiol.*, **50**, 211 (1965).

[11] Pankratz, H. S., and Bowen, C. C., *Amer. J. Bot.*, **50**, 387 (1963).

[12] Ris, H., and Singh, R. N., *J. Biophys. Biochem. Cytol.*, **9**, 63 (1961).

Isolation of a Virus infecting a Strain of *Mycoplasma laidlawii*

MYCOPLASMAS are a group of microorganisms which can grow on cell-free medium and which are characterized by pleomorphism, for they are bound by a triple layered unit membrane instead of a rigid cell wall. The smallest reproductive forms are about 100 to 150 nm in size. They are the smallest known free-living organisms comparable in size with the myxoviruses, to which they show some morphological resemblance. The largest mycoplasma cell can exceed 1,000 nm in size.

In view of the heterogeneity and wide host range of viruses, it seemed most unlikely that the biological characteristics of the mycoplasma cell would make the whole group resistant to infection with these agents. It was decided to investigate the susceptibility of mycoplasmas isolated from bovine nasal passages to virus attack. The bovine nasal passage was thought to be a favourable site for the multiplication and interaction of various mycoplasma species and strains. Virus screening was carried out by adding filtrates from other mycoplasma isolates to test mycoplasma. This report describes the isolation of a virus which infects a strain of *Mycoplasma laidlawii*.

Broth cultures of different isolates of mycoplasmas were prepared. These were not necessarily pure cultures, for many were recent isolates and not yet cloned. Aliquots of these were filtered through 'Millipore' 200 nm membranes. The filtrates were tested by adding a drop to solid medium plates seeded with each of the other mycoplasma cultures. The plates were examined after incubation for 24–48 h at 37° C, and showed a confluent lawn of host mycoplasmas; there was one exception where a distinct, clear zone of inhibition or lysis occurred at the site of application of the drop of filtrate. This zone, which covered the same area as the filtrate drop, was made up of a central partially clear area surrounded by a clear periphery with a partially clear halo on the outside.

The filtrate containing the inhibitory or lytic agent was completely inactivated on exposure to a temperature of 100° C (but not 60° C) for 30 min and ultraviolet light. It was sensitive to 5 per cent chloroform for 10 min but insensitive to 20 per cent ethylether at 4° C for 18 h. The agent, harvested from lawns of the host mycoplasma and diluted in phosphate buffered saline (PBS), *p*H 7·3, containing 10 per cent foetal calf serum, was serially filtrated through 'Millipore' membrane filters of decreasing pore size. It passed readily through filters of 10 nm pore size.

The agent in the initial filtrate was titrated in PBS in ten-fold dilutions and produced single isolated areas or plaques about 2–3 mm in diameter at high dilutions (titre of filtrate $2\cdot5\times10^9$ plaque forming units/ml.) on a lawn of the host mycoplasma. At lower dilutions these discrete areas coalesced to form one large zone (Fig. 1). Addition of a high dilution of the agent to broth cultures of growing mycoplasmas resulted in a 4–5 log increase in titre of the agent. Furthermore, a culture containing the agent in which the mycoplasmas had been inactivated by heating at 60° C for 30 min was subcultured on the host mycoplasma on solid medium 8 times. Each time a plaque was subinoculated into PBS and heated at 60° C for 30 min; the final subculture was equivalent to at least a 10^{-33} dilution of the original mycoplasma culture. There was no evidence of replication of the agent in the absence of actively growing cells of the specific mycoplasma. It therefore seems that this agent possesses properties consistent with those of a virus.

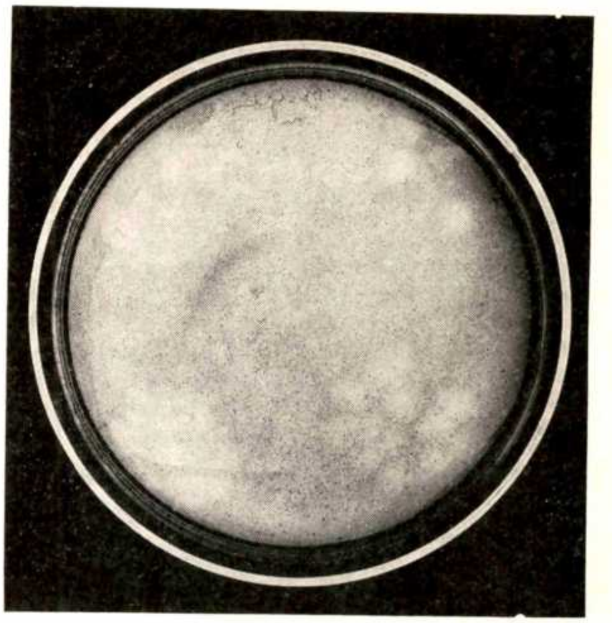


Fig. 1. Plaques formed by serial ten-fold dilutions of virus on *Mycoplasma laidlawii* strain BN1 culture plates. Stained neutral red.

The culture responsible for the host mycoplasma lawn was inoculated on to solid medium. It was purified by picking a single colony three times. The cloned culture (strain BN1) continued to receive the virus. This was apparent on a lawn of the cloned strain from the formation of plaques at the site of virus application. The cloned culture was able to grow at 22° C in serum-free medium and in the growth inhibition test[1] it was inhibited by antiserum to *M. laidlawii*.

This is the first report of the isolation of a virus which infects mycoplasmas. There has been a previous report[2], however, of small electron-dense bodies about 25–30 nm resembling intracellular bacteriophage in electron micrographs of the EAY strain of human mycoplasma, but, as far as is known, no virus was isolated then or subsequently. Evidence from the filtration experiments indicates that a viable unit of this virus can be exceedingly small.

I thank Mr I. Jebbett for the photograph and Miss Sara Wyld for technical assistance.

R. N. GOURLAY

ARC Institute for Research on Animal Diseases,
Compton, Newbury,
Berkshire.

Received December 22, 1969.

[1] Clyde, W. A., *J. Immunol.*, **92**, 958 (1964).

[2] Swartzendruber, D. C., Clark, J., and Murphy, W. H., *Bact. Proc.*, 151 (1967).

Entropy of Microbial Growth

A MAJOR problem in the thermodynamics of growth has been to account for the increase in information and structural entropy occurring during synthesis of new cellular material[1,2]. Thermodynamic measurements of the growth process in microorganisms now show that high fluxes of thermodynamic entropy occur, and are more than sufficient to account for the concurrent increase in information.

Table 1. THERMODYNAMIC DATA FOR ANAEROBIC GROWTH OF MICROORGANISMS WITH GLUCOSE AS ENERGY SOURCE

Organism	ATP Moles/ mole glucose	ΔH_c Observed kcalories/mole glucose	ΔF_c Calculated kcalories/mole glucose	Y_G Observed g cells/mole glucose	$T\Delta S_c$ kcalories/ mole	$\frac{\Delta H_c}{T}$ Calories/degree/ mole	ΔS_e Calories/ degree/ g cells	References
Zymomonas mobilis	1	−32	−56	8·4	24	106	25	7, 10
Streptococcus lactis	2	−26	−50	19·5	24	84	8	3, 10
Streptococcus faecalis	2	−31	−55	17	24	100	12	10, 24
Saccharomyces cereviseae	2	−31	−62	23	31	102	8	7, 10

Microcalorimetric studies with growing organisms show that while a large output of energy accompanies the process of catabolism, the amount of this energy incorporated into chemical bonds during growth is very small. The observed enthalpy change during growth agrees, within limits of experimental error, with the predicted value calculated from the difference between the heats of formation of the energy source and the degraded products of catabolism[3-7]. Generally, the enthalpy change from the coupled endergonic reactions of synthesis would be expected to subtract from ΔH_c, the enthalpy of catabolism[8], so that the enthalpy of growth, ΔH_s, is too small to be detected. Theoretical calculations confirm the experimental findings; in a rich complex medium containing the essential monomers for cellular synthesis, ΔH_s approaches zero[9]; that is, the energy incorporated into chemical bonds is not significant compared with the energy change from catabolism. Calculations of free energy change support the same view; the free energy of catabolism, ΔF_c, required to produce one mole of adenosine triphosphate (ATP) by the Embden–Meyerhof pathway of anaerobic glycolysis is at least -20 kcalories[10], but the coupled anabolic processes give rise to 10 g of cells with a free energy of synthesis, ΔF_s, of less than 100 calories/g[11] as calculated from the chemical composition of the cells. At least forty consistent determinations are available, showing that with many different microorganisms, about 10 g (dry weight) of new cellular material is synthesized for each mole of ATP produced by the catabolism of an energy source by a known metabolic pathway[12]. Calculations based on the known cost of energy for biosynthesis of cellular components indicate that up to ten times as much cellular material could be synthesized if all the ATP available from catabolic processes were used strictly for chemical syntheses[13,14]. These differences in energy between anabolic and catabolic processes cannot be attributed to a large requirement of energy for maintenance of the cells. Indirect estimations in growing cells suggest a small maintenance requirement[15], and, in non-growing cells, the use of the approximation that the entropy production of an organism can be determined by microcalorimetry[16] gives very rough estimates of the order of 10^{-3} calories/degree/g/h for cells of *Streptococcus faecalis* at 37° C (ref. 17), quite insignificant on the level of the growth process.

Thermodynamically, balanced exponential growth is a well defined steady state[18]. Each unit mass of growing cells may be considered as an open system which generates entropy internally at a constant rate, $\mathrm{d}\,S_i/\mathrm{d}t$, but, because a steady state exists, the internal entropy of the cells is maintained at a constant level by means of an equivalent outflow of entropy to the surroundings at a rate $\mathrm{d}\,S_e/\mathrm{d}t$ (ref. 19); each unit mass of existing cells is also synthesizing a new cellular material at a constant rate. Thus the entropically unfavourable process of growth becomes possible by coupling to the entropy production of catabolism[9,16].

In the microcalorimetric experiments conducted at constant temperature listed in Table 1, the outflow of entropy is due almost entirely to the degradation of the energy source. The thermodynamic entropy, ΔS_s, of the cellular material synthesized, calculated from ΔH_s and ΔF_s, is about 0·3 calories/degree/g. Independent calculations by Morowitz[9] give values of the same order; this is small compared with $\mathrm{d}\,S_i/\mathrm{d}t$. The outflow of entropy then has two major components, one ($T\Delta S$) due to catabolism and rejection of degraded products to the environment and the other $(\Delta H_c)/T$ due to the heat of the reaction; this heat is generated within the cells and then flows out to the environment[9].

Table 1 shows data reported for several organisms; these allow the calculation of the outflow of thermodynamic entropy during the production of 1 g of cells. The value of ΔS_e so calculated is greater than that necessary to accord with estimates of the amount of information contained in a bacterial cell. Such estimates, made by a variety of methods[20-22], range from 10^{11}–10^{13} bits per cell; ΔS_e can be compared with these by calculating the number of cells per g and applying the factor R ln 2 (ref. 20); all the organisms listed then give calculated values above 10^{13} bits per cell.

The data clearly demonstrate that only a small portion of the available energy is actually incorporated into cellular material; the major thermodynamic process is a large outflow of entropy from the growing cells. There is evidence for efficient energetic coupling during growth[23], so that the energy is not wasted. It follows that the major consumption of energy is that required for the correct placement of molecules within the cells into organized groups or structures.

W. W. FORREST

CSIRO,
Division of Nutritional Biochemistry,
Kintore Avenue,
Adelaide, South Australia.

Received October 7; revised December 8, 1969.

[1] Woolhouse, H. W., *Nature*, **213**, 952 (1967).
[2] Popper, K. R., *Nature*, **214**, 322 (1967).
[3] Boivinet, P., thesis (Université d'Aix-Marseille, 1964).
[4] Belaich, J.-P., and Senez, J. C., *Colloq. Int. Cent. Nat. Rech. Sci.*, **156**, 381 (1967).
[5] Senez, J. C., and Belaich, J.-P., *Colloq. Int. Cent. Nat. Rech. Sci.*, **124**, 357 (1965).
[6] Grangetto, A., PhD thesis (Université d'Aix-Marseille, 1963).
[7] Belaich, J.-P., Senez, J. C., and Murgier, M., *J. Bacteriol.*, **95**, 1750 (1968).
[8] Wilkie, D. R., *Prog. Biophys. Mol. Biol.*, **10**, 259 (1960).
[9] Morowitz, H. J., *Biochim. Biophys. Acta*, **40**, 340 (1960).
[10] Senez, J. C., *Bacteriol. Rev.*, **26**, 95 (1962).
[11] Morowitz, H. J., *Energy Flow in Biology* (Academic Press, New York, 1968).
[12] Stouthamer, A. H., *Molar Growth Yields in Microbiological Methods* (edit. by Ribbons, D. W., and Norris, J. R.), **1** (Academic Press, London, 1969).
[13] Gunsalus, I. C., and Shuster, C. W., in *The Bacteria* (edit. by Gunsalus, I. C., and Stanier, R. V.), **11**, 1 (Academic Press, New York, 1961).
[14] Fukiu, S., and Hirata, A., *J. Gen. Appl. Microbiol. Japan*, **14**, 47 (1968).
[15] Pirt, S. J., *Proc. Roy. Soc.*, B, **163**, 224 (1965).
[16] Prigogine, I., *Thermodynamics of Irreversible Processes* (Interscience, New York, 1961).
[17] Forrest, W. W., and Walker, D. J., *Biochem. Biophys. Res. Commun.*, **13**, 217 (1963).
[18] Forrest, W. W., and Walker, D. J., *Nature*, **201**, 49 (1964).
[19] Denbigh, K. G., *The Thermodynamics of the Steady State* (Methuen, London, 1951).
[20] Linschitz, H., *Information Theory in Biology* (edit. by Quastler, H.) (University of Illinois Press, Urbana, 1953).
[21] Quastler, H., *Information Theory in Biology* (edit. by Quastler, H.) (University of Illinois Press, Urbana, 1953).
[22] Morowitz, H. J., *Bull. Math. Biophys.*, **85**, 17 (1955).
[23] Cole, H. A., Wimpenny, J. W. T., and Hughes, D. E., *Biochim. Biophys. Acta*, **143**, 445 (1967).
[24] Forrest, W. W., Walker, D. J., and Hopgood, M. F., *J. Bacteriol.*, **82**, 648 (1961).

Book Reviews

STRUGGLE FOR INITIATIVE

Atomic Shield 1947/52

(A History of the United States Atomic Energy Commission, Vol. 2.) Pp. xviii + 718. (Pennsylvania State University Press: University Park and London, November 1969.) $11.95.

THE possibility of war between the United States and the Soviet Union—real or imagined—was the background against which the atomic energy programme developed in the United States during the period covered by this volume. It seems inevitable therefore that, once the attempt to set up under the United Nations a system of control acceptable to both sides had failed, there was little chance of success for those who hoped that the newly formed Atomic Energy Commission would be able to concentrate on the peaceful uses of atomic energy, free from military pressures. The main theme of the book is the response of the commission to the continuous demands for increased weapons production.

In this context, the appointment of Lilienthal as the first chairman of the AEC was surely a mistake. He was an idealist who had achieved great success as chairman of the Tennessee Valley Authority for fifteen years—but the circumstances were quite different, and he seems always to be struggling against the tide. He was succeeded by Dean, who was more realistic and provides an interesting contrast in personality.

An account is given in considerable detail of the relations between the commissioners and the general manager (at first appointed by the President, later by the commissioners), and between the commission in Washington and their staff and contractors in the field. As in volume one, the authors present the detailed statements and attitudes of the principal characters, leaving the reader to form his own picture of each one, and to judge the correctness or otherwise of the decisions taken, political or administrative. Unfortunately, very few technical details can be given: most of them are still classified, as the authors explain in their preface. The interest must therefore chiefly lie in the interplay of personalities. One chapter deals with unclassified research in radiation biology, high-energy physics, and the transplutonium elements; but these and some references to radioisotopes and research reactors are not in the main theme.

The Atomic Energy Act, 1946, set up the commission and defined its powers, duties and organization in considerable detail. It also established a Congressional Joint Committee to make continuing studies of the commission's activities. Throughout the period they increased the scope of their interaction with the commission and the degree to which they took the initiative in directing and pressing the commission on matters of policy and sometimes of detail. This direct interference by a congressional committee in the affairs of a branch of the executive is unusual and makes most interesting reading.

The Atomic Energy Act also provided for a General Advisory Committee. Among its first members were people who had been long in atomic energy work, such as Conant, Fermi, Oppenheimer and Rabi. Though the committee had no executive powers it is very clear that the great scientific prestige of their members made them a powerful influence in determining the course to be followed by the commission.

With each successive demonstration of Soviet nuclear capability, the work of the commission increased to provide the new levels of materials and weapons requested by the military. On the naval side, Rickover's drive to develop nuclear powered submarines was already showing results.

Towards the end there is increasing emphasis on the activities of two men who are making special contributions: McMahon as the dedicated chairman of the JCAE, convinced that only the thermonuclear weapon can save the peace of the world; and Teller, resigning from Los Alamos in frustration at what he considered to be a totally inadequate scale of effort on the thermonuclear project, and getting support from McMahon and from Lawrence for setting up a new laboratory at Livermore.

The chapters on British proposals for re-establishing an effective interchange of information are interesting, but the wording of the Atomic Energy Act would have made it very difficult even without the strong opposition that existed in many places, including the commission itself. It seemed that on each occasion when a formula had been found which promised some relaxation, there would be some incident that confirmed the views of those who held that it would be against US interests.

J. F. JACKSON

THE BERNOULLI SAGA

Die Werke von Jakob Bernoulli

Herausgegeben von der Naturforschenden Gesellschaft in Basel. Band 1. Pp. xii + 541. (Birkhäuser Verlag: Basel, 1969.) Sfr 76.

Hydrodynamics

By Daniel Bernoulli. And Hydraulics. By Johann Bernoulli. Translated from the Latin by Thomas Carmody and Helmut Kobus. Pp. xv + 456. (Dover: New York; Constable: London, November 1969.) 142*s* 6*d*; $15.

THE history of the Bernoulli family is well known; starting with a burst of genius in the two brothers John and James, it included two of nearly equal talent in the next generation (Nicolas and Daniel) and continued a tradition of excellence down to this day. To these first two generations goes much of the credit for the creation of the modern science of rational mechanics. The story has been well told by Truesdell: taking physical ideas from Newton and Huygens, and mathematical tools from Leibniz, they achieved effective methods for the solution of many deep problems. Their student and successor, Euler, then brought the science near to the classic form finally achieved in the nineteenth century. It should not be thought that theirs was a happy family endeavour; John and James quarrelled over priority, and late in life John tried to steal credit for achievements in hydrodynamics from his son, Daniel.

These two volumes which have recently appeared provide material on very different periods in the history of the family. The first volume of James Bernoulli's *Works* is devoted to "Juvenalia": material dating from the period before his discovery of Newton's mechanics and Leibniz's calculus. The editor's modest title for this work may be misleading: Bernoulli was long past his schooldays by this time, and the pieces (most of them published) show full mastery of the mathematics and natural philosophy (speculative and experimental) of his time. In metaphysics and logic, his work shows a mixture of the remnants of a scholastic type of philosophy (which flourished in German universities through the seventeenth century, following its renovation in the sixteenth) and Cartesianism. The shock of the complementary achievements of Newton and Leibniz must have been great. We know that by 1690 Huygens was too old to adjust to them, but the young Bernoullis rose to the challenge and developed the elements of a new synthesis.

The second volume will be of greater interest to a reader trained in mathematical physics. For here in English translation are two classics, in which the science of hydrodynamics was founded. Putting the two works together has greatly enhanced their value; for in this pair, there is a scientific and a personal drama. Daniel Bernoulli's book (of 1738) explores a variety of problems of practical and theoretical interest, including the motion of waters inside and on leaving vessels, hydraulic machines, and "elastic fluids". His basic conceptual tool was not Newtonian mechanics, but what can be called the pendulum principle, derived from Galileo through Huygens. (The reader who wants to browse this work should first study the brief section at the beginning of chapter three where the fundamental ideas are developed; almost any subsequent argument can then be followed easily.) Application of this principle enabled him to make a first investigation on the problem of the pressure of a moving fluid on the walls of the pipe; and he did direct experiments to test his results, as was his practice.

Daniel's aged father John immediately attacked this last problem; and by improving its conception (and using Newtonian mechanics) he was able to derive its solution in the classic form of "Bernoulli's Theorem". On this special topic he produced a masterpiece; but, not content with the credit for improvement, he tried, by various devices, to arrogate full credit to himself. Euler was a friend of both father and son; and whether he avoided compromising himself in this nasty business is a question of historical interpretation. The preface by Hunter Rouse tells the story well; he could have pointed out that the first paragraph of Euler's letter to John, printed with the book, did give basic priority to Daniel.

In fact, the two books give a good sample of the distinctive styles of father and son: Daniel was a man of wide interests (he taught medicine at Basle !), concerned with experiment and with technical applications, but lacking the deep mastery of physical concept and mathematical technique that brought immortality to his father and uncle. In the translation, notation is slightly modernized but the words stay close to the original; this is fortunate, because a modernization of Daniel's mechanics would have made it unintelligible. It is a pleasure to see that Dover Books, who in the past have served the history of science so well by providing inexpensive editions of classics in translation, are back in the field, even if at a "library" price.

J. R. RAVETZ

COMPUTER ECONOMICS

The Economics of Computers

By William F. Sharpe. Pp. x + 571. (Columbia University Press: New York and London, October 1969.) \$10; 90*s*.

THIS is a report of a study made by the Rand Corporation. It is in two main sections: the first, of 180 pages, is on economic theory generally while the second deals specifically with the computer world and has chapters on the computer industry generally, on the marketing and pricing of computers and associated services, and on the costs and effectiveness of complete computer systems and of major components such as central processors and stores ("memories" here).

The first section gives a lot of useful background material which it is handy to have by one, but the real interest is in the second. The chapter on "The Computer Industry" gives a fascinating account of the history, development and present (meaning 1968) structure of this remarkable enterprise, whose growth from zero in 1950 to its present status of a truly major industry is one of the phenomena of our time.

The author emphasizes the importance of the part played by government in this development; on p. 186 he says "... it can plausibly be argued that without government (and particularly military) backing there might be no computer industry today". The whole book is concerned almost exclusively with the American scene and the author make no bones about it—IBM dominates, and has done since 1956, just as it dominated the punched card and tabulating machine market in the pre-computer era. The rest of the world gets two pages at the very end, in a section headed "Computers Abroad"—but, ironically enough, the author says (p. 204) that "perhaps the most analysed group of computer manufacturers is that in the United Kingdom". This chapter is chiefly a presentation of facts, as are those on sales and rental of computers, and also, though to a lesser extent, chapter twelve (the final chapter) on "Services, Markets and Costs".

The titles of two long chapters in the middle of the book hold promise of great interest—chapters nine and ten on "Cost and Effectiveness of Computer Systems" and of "Memory" respectively. Computers are expensive things to buy and to run—big systems are very expensive—and it is obviously very desirable to have some measure of cost-effectiveness to guide one's choice between rival manufacturers' offers. They are also very complicated things, so one can expect the problem of finding such a measure to be difficult. The main impact of these chapters is to show just how difficult it is. The author deals with all the types of performance measurements which are well known in the computing world; for example, simple criteria depending on the times for a very few basic operations, more complicated instruction-mixes, times for standard machine-code programs or "bench-mark" collections of programs in high-level languages and several less well known ones proposed by various individual investigators. Some of these latter seem rather odd, such as the formula on p. 313 which involves a number of constants and cook-factors whose values were determined, apparently, by a sort of public opinion poll (though among only forty-three "engineers, programmers and other knowledgeable people"). Many proposed methods involve weighting factors for various operating times, which again are very subjective. The author is quite honest about all this, and shows by quoting results of actual tests that different measures can give wildly inconsistent ratings of the relative performance of two systems. The main lesson to be learned is that one has to be very cautious in interpreting the results of any such tests. This is not altogether unknown.

The book is quite long—556 pages—and is packed with detailed factual information. Wherever possible the arguments are carried on quantitatively, so there is a fair amount of algebra but no very elaborate mathematics. It is well worth having as a stimulus to thought about the assessment of computer performance, for its surveys of the industry and for the very large number of references to relevant work, up to 1968, most of which would be very difficult for the unaided worker to track down.

J. HOWLETT

BUSINESS IS BUSINESS

Think

A Biography of the Watsons and IBM. By William Rodgers. Pp. 320 + 10 plates. (Weidenfeld and Nicolson: London, January 1970.) 50*s*.

How IBM with "the punched card that changed the world" is becoming the largest corporation on Earth is the subject of this new book by William Rodgers. Concentrating on the people rather than the computers, Mr Rodgers shows that these amazing machines are less fascinating than the managers who sell them, and his book has therefore apparently not gone down well with the company.

This book will be grist for many an amateur psychologist. What is to be made of Thomas J. Watson who turned

IBM into the world's largest non-union company and whose incredible presence loomed over IBM from three years after its founding until his death in 1956 ? From a beginning, peddling pianos and organs from the back of a wagon, Watson graduated by a tortuous route to become a salesman for the National Cash Register Company where he came under the influence of John Henry Patterson, the founder of "The Cash" as it was called. From Patterson, Watson learned how to work under a tartar who made a speciality of sales conferences where salesmen who missed their quota publicly confessed failure, as well as the ways and means of knocking out competition which later attracted attention in a lingering anti-trust case.

Under the threat of a prison sentence—a result of the anti-trust action—which simply died away in a welter of appeals, Watson left The Cash in 1914 to join an unpromising holding company called The Computer-Tabulating-Recording Company which Watson later rechristened International Business Machines, manufacturing as well as bookkeeping machines such equipment as scales and bacon slicers. Watson moved slowly at first, no doubt because deference had become deeply ingrained through his experiences with Patterson, but by 1924 he was visibly in charge and becoming a replica of Patterson. From then on he steered IBM up to the 1950s when his son Thomas J. Watson jun. took over.

IBM probably weathered the depression better than most as a result of government demands for improved bookkeeping that stimulated the sale of machines, just as the effects of the Marshall plan helped IBM in Europe after the Second World War, and during the depression Watson is said to have been the most highly paid citizen in the United States, a record which took some living down. He instituted a whole IBM ritual (Rodgers calls it worship) with THINK as the ubiquitous all-purpose symbol which had to appear on every executive's desk.

Watson also presided over IBM's big mistake—the failure to take on ENIAC, what might be called the first electronic computer, when it was handed to IBM on a plate by its builders, J. P. Eckert and J. W. Mauchley. IBM had been working on large electromechanical computers in cooperation with, among others, the astronomer Wallace Eckert (no relation of J. P. Eckert) who used IBM equipment to check the calculations in Brown's lunar tables, and Howard Aiken who was responsible for the electromechanical IBM Mark 1 machine at Harvard which made a sound "like a roomful of ladies knitting". Like many computers since, the Mark 1 was made obsolete almost as soon as it started work by ENIAC, which used valves. Discouraged, apparently by Watson, Eckert and Mauchley went to Remington-Rand where they produced the first commercial electronic computer, UNIVAC. Remington-Rand failed to press their advantage, however, and Rodgers quotes "snatched defeat from the jaws of victory".

IBM entered the computer industry proper in 1953 with the 701 machine and its 4,000 valves. The company has not been without its crises since. There was the failure of the STRETCH computer which left the way open to Control Data Corporation in the field of big machines. In the mid sixties there was the situation where taken together companies such as Honeywell, Control Data, Remington-Rand and Burroughs were ahead in computer technology, but not, as usual, in marketing. This stimulated the establishment of the IBM 360 series, announced in 1964, one of the first families of compatible computers.

Once again IBM is embroiled in anti-trust cases which could drag on until 1974, and Rodgers sees it as conceivable that the company could be broken up, although he is convinced that the pieces will continue to grow like the whole. Perhaps because company material was denied him, Rodgers's account becomes less complete as the time he describes approaches the present. Apart from the Eckert-Mauchley incident, for example, Rodgers gives few hints about why IBM was so slow in entering the electronic computer business. But Watson's background in selling built up in the years with The Cash and earlier developed in IBM the receptiveness to the wishes of customers, which was manifested in IBM's provision of software for computer users and the development of a powerful service organization. The company's performance in the military area, something less than might have been anticipated, encouraged the search for business markets which in the end are proving more lucrative. Rodgers's book, then, is not going to be a panacea for the companies eking out their market shares from the crumbs dropped by IBM, nor does it say how the world is going to live with IBM and its computers, but it is an extraordinary account of the people who fashioned the company.

EDWARD PHILLIPS

RUSSIAN PROBABILITY

Probability Theory

Basic Concepts, Limit Theorems, Random Processes. By Yu. V. Prohorov and Yu. A. Rozanov. Translated by K. Krickeberg and H. Urmitzer. (Die Grundlehren der mathematischen Wissenschaften in Einzeldarstellungen mit besonderer Berucksichtigung der Anwendungsgebiete, Band 157.) Pp. xi + 401. (Springer-Verlag: Berlin and New York, 1969.) 68 DM; $18.70.

THE Russian school of probabilists has, throughout this century, been in the forefront of advance both in the pure mathematical theory and in its application to practical problems. It is therefore particularly interesting to have available in English this standard text by two distinguished and influential authors. There are, of course, a number of texts available at this level (senior undergraduate and postgraduate) which cover roughly the same ground, and it is fascinating to compare both the content and the flavour of the different books.

Apart from such differences, the main point to remark is that the authors are to be taken at their word when they describe the book as "a survey of the most important results, methods and trends of modern probability theory". Almost all proofs are omitted, and it thus becomes possible to cover a remarkably wide field in some depth. Indeed, the book tempts one to wonder whether the insistence on detailed proof which informs most authors of advanced texts in pure mathematics is altogether a good thing.

As one would expect, there is a good deal about the basic measure-theoretic (and topological) structure underlying probabilistic problems, and random processes in particular, and a welcome account of the limit theorems for independent variables, both classical and more recent. The chapter on Markov processes is surprisingly earthy, and far in spirit from the work of Dynkin and his followers, but the interesting and detailed final chapter on stationary processes is more predictable. A number of topics usually absent from accounts of probability theory make at least brief appearances; information, coding, optimal control, for example.

Although this book is not likely to be widely used as a text, it will find a useful place as a work of reference on many bookshelves. The translation, though stilted, is readable, and the printing is excellent.

J. F. C. KINGMAN

MORE NMR

Nuclear Magnetic Resonance Spectroscopy

By Ruth M. Lynden-Bell and Robin K. Harris. (Studies in Modern Chemistry.) Pp. viii + 160. (Nelson: London, November 1969.) 70*s* boards; 35*s* paper.

THE justification for yet another book on the principles of nuclear magnetic resonance spectroscopy cannot but be a slender one. The authors believe that "there is a need for a short monograph dealing with the physico-chemical basis of the subject". This is an arguable point. Most undergraduates will not be able, with their overcrowded syllabus, to devote the time and effort to NMR that this book requires; and postgraduate students will want to go further than it can take them. The authors have been bred on NMR, and their grasp of the fundamentals clearly shows up in their style. The text is lucid and commendably up to date.

An attempt has been made, with some success, to present the physico-chemical basis of NMR spectroscopy without becoming too immersed in the mathematics. The direct consequences of the use of SI units are briefly summarized in an introduction.

The first chapter deals with basic principles and presents a standard treatment of the NMR phenomenon in both classical and quantum mechanical terms. A clear discussion of equivalence and some experimental aspects are included. Chapter two describes the quantum mechanics involved in spectrum analysis, including the composite particle method for equivalent nuclei. Chapter three covers the various factors which may affect the NMR parameters. The contributions to nuclear screening of electric dipoles, bond anisotropies, ring currents, solvent effects and similar factors are detailed along with the theories of spin coupling in a concise but clear fashion. In line with current practice, all discussion of shielding and de-shielding of nuclei is in terms of shifts in frequency rather than field.

The final chapter covers relaxation and related phenomena and certain aspects of double resonance. Problems are given at the end of each chapter, together with some of the answers. The book is attractively produced and looks remarkably free of errors. The only three that I have noticed are quite trivial.

The contents are familiar stuff; indeed, the authors do not pretend that they are anything else. But the book is attractively written, and for this reason worthy of study by those giving NMR courses to undergraduates and postgraduates. The paperback is a little expensive.

D. E. WEBSTER

RADIATION HAZARDS

Induced Radioactivity

By Marcel Barbier. Pp. xiv + 424. (North-Holland: Amsterdam, 1969.) 152*s*.

THE increasing number of nuclear reactors and high-energy particle accelerators in operation has caused the study and control of radiation hazards to assume a growing importance. A significant part of the radiation dangers associated with nuclear installations arises from the artificial radioactivity induced in materials exposed to the primary radiations present and, because the induced activity may persist for a considerable time after the primary flux has been turned off, it is particularly important to be able to estimate its intensity in advance. This book is a survey of induced radioactivity as a radiation hazard, with special emphasis on the problems of predicting radiation fluxes caused by activities induced by particle accelerators, as well as by reactors.

The subject is a complex one, for the radiation danger to be expected from a sample of irradiated material is a function of many variables in addition to the nuclear cross-sections involved. Dr Barbier has attempted to make a systematic treatment of the problem, and has put into an orderly and useful form a large amount of data from theoretical calculations and experimental measurements.

The work may be described roughly as a textbook on induced radioactivity, coupled with a reference handbook of nuclear data relevant to the subject. It begins with an elementary introduction to the physical phenomena involved, and there are general accounts of growth and decay of activity, absorption of radiations and biological effects. Parameters of use for practical calculations are introduced (such as the "danger parameter" which takes into account activation cross-sections in a material, self-absorption, and biological effectiveness of the radiations emitted). Separate chapters then deal in detail with activation involving spallation reactions and compound nucleus reactions; and activation by neutrons, by electrons and photons, and by heavy ions. Finally, activation in human tissues is discussed. Graphs and tables are provided for each topic, including, in many cases, results obtained by Barbier and his colleagues at CERN. Each chapter is followed by a number of numerical problems, with solutions given at the end of the book. This is an unusual feature in a book of this nature, but such problems can be useful for somebody approaching the subject for the first time.

There are a series of appendices containing reference data which will probably be as useful for those who have to make activation calculations as the text itself. As well as standard data, including a reprint of the "G.E. Chart of the Nuclides", there are special tables and graphs for use in calculations of activation by charged particles, neutrons and photons. There are some fifty graphs in this part of the book alone.

Altogether, this is a comprehensive book which brings data from many sources into a convenient form. It should form a very useful reference work for anybody engaged in assessing hazards of induced radioactivity around accelerators or reactors.

R. W. P. DREVER

SOLUTION BEHAVIOUR

Solute–Solvent Interactions

Edited by J. F. Coetzee and Calvin D. Ritchie. Pp. xiv + 653. (Dekker: New York and London, July 1969.) $29.50; 280*s*.

To bring together within one volume the whole spectrum of knowledge on solute–solvent interactions is an impossible task, and the editors are to be complimented in making a selection of topics covering several representative areas of the subject. Each of the eight chapters is written by an expert in the particular field and care has been taken to relate ideas between the various contributions wherever this is possible.

The first chapter, by J. W. Larson and L. G. Hepler, is concerned primarily with the thermodynamics of ionization of acids in aqueous solution. Extensive tables of thermodynamic data are included for both organic and inorganic materials. The second chaper, by R. G. Bates, is devoted to effects of the medium and *p*H in non-aqueous solvents. This is followed by a discussion of acidity functions by R. H. Boyd. The Hammett function is first considered, but the survey then broadens to acidity functions based on the use of tertiary aromatic amine indicators, amide indicators, azulenes and cyanocarbon anions. A brief description of experimental methods is included.

The fourth article, by C. D. Ritchie, deals with interactions in dipolar aprotic solvents. As comprehensive coverage of this topic is beyond the scope of a single chapter, four principal aspects have been presented—equilibrium acidities, proton transfer rates, nucleophilic substitutions and the dipolar complexing of non-electrolytes. It is possible that investigation of such fields by physical techniques may aid a better understanding of organic synthesis.

A contribution from H. Schneider concerns the selective solvation of ions in mixed solvents. The classical techniques of conductance and transference are first discussed and then follow more modern spectroscopic methods.

In chapter six, E. M. Arnett and D. R. McKelvey deal with the solvent isotope effect on solute behaviour. Almost all of the thermodynamic data refer to the H_2O/D_2O

system. The following contribution, by P. M. Laughton and R. E. Robertson, is closely related and considers the effect on equilibria and reactions. Once again the data refer to H_2O and D_2O.

J. F. Garst, in his chapter, deals with the variations in rate and equilibria of organo-alkali compounds in protic and aprotic solvents. The ethers are singled out for particular detail. The volume is concluded by a very brief appendix by the editors, listing the recommended methods of purifying selected solvents.

Taken overall, the book seems to have particular appeal to the specialist interested in the application of the methods of physical chemistry to the problem of solution behaviour. Perhaps its specialist interest is reflected in its high price. J. A. SALTHOUSE

FLUORIDE POISONING

Toxic Properties of Inorganic Fluorine Compounds

By R. Y. Eagers. Pp. x + 152. (Elsevier: Amsterdam, London and New York, 1969.) 55*s*.

SINCE the publication of Largent's excellent work (*Fluorosis*, Ohio State University Press, 1961) on the metabolism and toxicology of inorganic fluorine compounds, much information has been published on the subject which, with earlier works, is condensed in the 118 pages of this monograph. This book is best where it is more condensed, as in the sections on effects on insects, plants and bacteria or when discussing endemic fluoride poisoning in humans and in animals. The text is vague, repetitive and disorganized when the author tries to express his own ideas as in the major part of the chapter on human intoxications. His definitions are often inaccurate or ambiguous. Thus on page 8, the words suggest that acute fluoride poisoning from inhalation is not an industrial hazard although it may result from atmospheric pollution. In the chapter on acute poisoning (page 25) it is stated that "Acute fluoride poisoning by inhalation is essentially an industrial hazard", yet he goes on to say, "Normally, fluoride poisoning by inhalation may be described as chronic rather than acute. . . ". Sometimes even the subtitles are misleading, as under the heading of "Absorbed Fluorides". Here the author discusses the effect of exposure by the dermal route, but, hypnotized by the drive to marry title and material, he opens this section with the surprising statement that "Acute fluoride poisoning by absorption usually implies that the fluoride is absorbed through the skin as a result of local contact", but immediately proceeds, "Strictly speaking, all acute fluoride poisoning is the result of absorption", and then explains that ingested fluoride is absorbed through the gastrointestinal tract and inhaled fluoride is absorbed through the respiratory tract.

This disorganized compilation includes much repetition and the separation of logically inseparable parts. Thus fluorides are characterized and their threshold limits are given on pages 4–7 and again on pages 76–80. Acute, subacute and chronic animal experiments are reviewed in the second chapter entitled "Effects on Animals" under the subtitle of "Acute Poisoning" and in the third chapter on "Other Effects" under the subtitle of "Physiological Effects". This latter section is inserted between "Effects on Plants" and "Effects on Bacteria". The characterization of some of the fluoride compounds is in many cases too imprecise, and because preventive measures are proposed for some but not the other compounds, the reader might believe that these latter may be handled without risk.

The monograph may serve a useful purpose as a collection of 730 references, but the reader is advised to use only those pages and paragraphs in which the density of reference numbers is above a certain threshold limit. L. MAGOS

FUNGAL TOXIN

Aflatoxin

Scientific Background, Control and Implications. Edited by Leo A. Goldblatt. (Food Science and Technology : a Series of Monographs.) Pp. xii + 472. (Academic Press: New York and London, October 1969.) 201*s*.

ALTHOUGH the effects of mycotoxins have been observed for many years, comparatively little was known about their cause. From 1960 onwards this state of affairs has been dramatically altered as a result of the large-scale outbreak of a mycotoxicosis among turkey poults, which was known as "Turkey X" disease. The cause of "Turkey X" disease was eventually traced to the presence of a group of mycotoxins, in a groundnut-based feed contaminated with a strain of the mould *Aspergillus flavus*. The group name given to these mycotoxins was "aflatoxin". In addition to their toxic properties the aflatoxins were shown to be highly carcinogenic to many species. Since that time many research workers have expended a tremendous amount of effort in solving the problems associated with these compounds, and the net result of this work, which comprised more than five hundred papers by 1968, has been presented in this book under the editorship of Dr L. A. Goldblatt.

It is understandable that in attempting to produce a work on such a diversified subject, there must be a number of authors involved, and it is unavoidable that in a number of the contributions there is a tendency for repetition of the information.

The content of the book reflects the wide range of disciplines involved in solving the aflatoxin problem. The topics covered are dealt with in a total of fifteen chapters and they include the chemistry, production, assay, effects, metabolism and detoxification of the aflatoxins. The first chapter is a general introduction by the editor, and the subsequent ones contain a large amount of evidence and information, which in many cases is presented in a very useful tabular form. Another contribution is a list of precautionary measures to be taken when handling aflatoxins. These precautions should be made mandatory for all persons engaged in aflatoxin work, for it now seems extremely likely that aflatoxins are carcinogenic for man. In this latter connexion it is reassuring to read that a great deal of thought has been given to the protection of consumers from the aflatoxins. With this point in mind an appendix is included which contains a voluntary code of practice, currently in operation in the United States, for marketing and distribution of groundnuts.

Although the book is ostensibly about the aflatoxins, there are several chapters which contain information concerning fungal spoilage of food, and mycotoxins in general. These generalizations help to place the aflatoxin problem in perspective, for, after all, the aflatoxins constitute only a few of the known mycotoxins. In fact, it is claimed that some of these other mycotoxins have at least equal importance to that of the aflatoxins themselves.

There is no doubt that, because of the authoritative and intensive manner in which this book has been compiled, it will remain a standard text on the subject of aflatoxins for many years to come. M. F. DUTTON

YEAST BIOLOGY

The Yeasts

Vol. 1: Biology of Yeasts. Edited by Anthony H. Rose and J. S. Harrison. Pp. xiii + 508. (Academic Press: London and New York, November 1969.) 140*s*; $19.50.

YEASTS are constantly impinging on our daily life and they do so probably to a greater degree than other microorganisms. Not only are these organisms the exploited industrial slaves of baking, brewing, wine and cider making and distilling, but they are also sources of human and animal food. To the biochemist, yeast is an extremely adaptable organism, eminently convenient to use in a wide range of studies such as in current intensive investigations on mitochondrial structure and function. Eleven years have elapsed since Academic Press printed as a single volume *The Chemistry and Biology of Yeasts*, edited by Dr A. H. Cook, and it is perhaps a reflexion of the rapid flow of yeast research that the substantial volume under review is the first of three devoted to yeasts.

The contributions within volume one of *The Yeasts* fall broadly into four fields of study: (1) yeast taxonomy and systematics; (2) yeast ecology; (3) yeast life-cycles and genetics; and (4) yeast cytology. It is interesting that in the introduction the editors refer to the difficulty in providing a satisfactory definition for yeasts in spite of the fact that only about 350 species grouped into 39 genera are involved. A contribution by N. J. W. Kreger van Rij on "Taxonomy and Systematics of Yeast" is generously illustrated by photomicrographs and is comprehensive except for the traditional omission of black yeasts such as *Aureobasidium* (*Pullularia*) *pullulans*. (There is, incidentally, considerable reference to this yeast in chapter five but no entry in the index.) "Distribution of Yeasts in Nature" is splendidly surveyed by L. do Carmo-Sousa—she largely reviews data published after 1956 and up to 1967. The chapter by J. C. Gentles and C. J. La Touche on "Yeasts as Human and Animal Pathogens" has a title which seems to disclaim that man is an animal, but the distinction drawn is presumably one of convenience. Following the discovery in 1839 that thrush disease is caused by yeast, many examples of candiasis and other yeast infections have been described. The chapter describes them in detail along with serological and therapeutic aspects. To complete ecological considerations of yeasts, F. T. Last and D. Price provide a chapter on "Yeasts associated with Living Plants and their Environs" for which they have the unenviable task of separating the effects of yeasts from those of other fungi on plants. The authors attempt to answer such questions as where are fruit-inhabiting yeasts to be found in the winter, and do certain yeasts fix nitrogen in the soil?

The contribution on "Yeast Cytology" by P. Matile, H. Moor and C. F. Robinow is a substantial and valuable account of the structure and function of the various parts of the yeast cell. In particular, the extended consideration of Lindegren's various hypotheses on nuclear structure is particularly welcome. Like the chapter on "Yeast Genetics" by R. K. Mortimer and D. C. Hawthorne, that on "Yeast Cytology" brings together a great deal of research from a wide range of journals and provides an authoritative summary of present knowledge. "Sporulation and Hybridization of Yeasts" by R. R. Fowell is a comprehensive account of the methods used in yeast genetical work and fits in admirably with a second short chapter on "Life Cycles in Yeast" by the same author and the chapter on "Yeast Genetics" already mentioned.

The editors and authors are to be congratulated on this first volume. It is a valuable and well presented handbook for those carrying out yeast research, while biochemical and microbiological libraries will require it as a reference volume.

J. S. Hough

Obituaries

Dr A. Tindell Hopwood

Arthur Tindell Hopwood, a member of the scientific staff of the British Museum (Natural History) for thirty-three years, died in London on October 23, 1969.

Born at Macclesfield on July 15, 1897, Hopwood received his education at Manchester Grammar School and saw service during the 1914–18 war as a navigation officer in the Royal Naval Air Service. Afterwards he entered the University of Manchester and took the special honours course in geology and zoology devised by Professors O. T. Jones and S. J. Hickson. A born naturalist, Hopwood's enthusiasm for natural history—from conchology to human morphology—together with his attractive personality rendered him *persona grata* in the university departments and in the Manchester Museum, and he made full use of the facilities he was offered. His promise secured him the exceptional privilege of admission to the dissecting room to study limb musculature and osteology. This experience impressed upon him the dependence of form on function. That every bone is but the reflexion of its natural environment was a lesson well learnt, one which was to enhance the value of his later palaeontological work.

Hopwood graduated with second class honours in 1922 and proceeded to his doctorate in 1933. On graduation he was the holder of a Mark Stirrup scholarship in palaeontology, and he later served for a time on the Indian Geological Survey.

In 1924 he was appointed to the staff of the Department of Geology in the British Museum (Natural History) where he stayed until he retired (as principal scientific officer) in 1957. At the museum Hopwood was assigned to mammalian palaeontology, an arrangement admirably designed to make the best of his geological experience and his flair for the morphological, and justified by events: relatively soon there began to appear a series of original and joint papers on fossil mammals which earned him recognition in this field. The 1928 *Catalogue of Pontian Bovidae of Europe in the Department of Geology, British Museum (Natural History)*, in collaboration with Guy Pilgrim, was the forerunner of numerous authoritative publications contributing substantially to mammalian palaeontology. Their catholic range embraced equids, proboscideans, carnivores and various ungulates, but the most important score or so treated of fossil primates from the Miocene, Pliocene and Pleistocene of central and eastern Africa. By these, perhaps, Hopwood will be best remembered.

He immediately realized the rich significance of the Olduvai deposits, whose exploration he pioneered with Beck and Leakey and on whose mammalian fauna he pronounced perceptively. While his African palaeontological publications necessarily included the recognition and description of hitherto unknown mammalian genera and species—including the celebrated Miocene pongid, *Proconsul africanus* (erected 1933)—they also incorporated such significant considerations as the role of environmental phenomena (earth movements, ice ages, climate) in determining the physical characteristics of extinct faunas, and they showed unequivocally the contribution that palaeontology itself could make towards the elucidation of complicated geological sequences. Hopwood's knowledge of the adaptive plasticity of osseous tissue, whether in the recent or in the extinct mammal, is recognizable in his writings and it enabled him to assess any particular fossil with an acumen transcending the purely geological. With Halleyfield he epitomized much of his African work in the *Annotated Bibliography of Fossil Mammals of Africa* (1954).

Hopwood's consideration of environmental and functional factors in his approach to the palaeontological was reflected in his sustained interest in recent mammalian osteology. To this subject he gave much detailed attention, publishing a series of studies of the craniology or limb skeleton of African rhinoceroses, carnivores and antelopes. In so doing he stimulated interest in a somewhat neglected aspect of zoology, and his example has proved profitable to subsequent workers.

Preoccupation with mammalian palaeontology, however, by no means diminished his lifelong interest in the Mollusca: he was the recognized authority on the genus *Conus* and between 1930 and 1945 contributed considerably to malacological literature.

Recognized at home and overseas as an authoritative palaeontologist, taxonomist and bibliographer, Hopwood was much consulted by individuals and scientific societies. He served the Zoological Society of London on its publications committee (1944–61), as co-editor of the *Nomenclatur Zoologicus*, vol. 6 (1966), and as delegate to the zoological nomenclature colloquium held under the auspices of the Fifteenth International Congress of Zoology (1958). His long service to the Linnean Society of London saw him council member (1946–56), zoological secretary (1948–52) and twice vice-president (1947–48, 1955–56). His interest in the Mollusca gave the Malacological Society his services as council member (1930–49), secretary (1933–36) and president (1943–45). After retirement from the museum his faculty for attractive and lucid exposition found a decade's fruitful exercise as *professeur* at the Lycée Français.

Hopwood's natural endowment included a gentle, considerate disposition, a well developed artistic sense and a markedly philosophical turn of mind, much attracted to Teilhard de Chardin's concepts of natural phenomena. If occasionally, and wholly superficially, a trifle portentous, Hopwood was fundamentally a modest, merry-hearted man, perennially fascinated by the wonder of living things. An accommodating colleague and a staunch friend, he was prodigal of time and effort in assisting those who sought his counsel or his expertise. Many therefore remember him with affectionate gratitude.

Correspondence

Attitudes to Conservation

Sir,—*Nature* is right, of course, and Eliot Slater (*Nature*, **225**, 773; 1970) is wrong. All scientists in biology and medicine know, or should know, that there *is* a creed. It was stated by Pasteur as follows,

"You bring me the deepest joy that can be felt by a man whose invincible belief is that Science and Peace will triumph over Ignorance and War, that nations will unite, not to destroy, but to build, and that the future will belong to those who will have done most for suffering humanity.

Young men, have confidence in those powerful and safe methods, of which we do not yet know all the secrets. And, whatever your career may be, do not let yourselves become tainted by a deprecating and barren scepticism, do not let yourselves be discouraged by the sadness of certain hours which pass over nations. Live in the serene peace of laboratories and libraries. Say to yourselves first: 'What have I done for my instruction?' and, as you gradually advance, 'What have I done for my country?', until the time comes when you may have the immense happiness of thinking that you have contributed in some way to the progress and to the good of humanity. But, whether our efforts are or not favoured by life, let us be able to say, when we come near the great goal, 'I have done what I could'."

Since there is a creed, it is appropriate for scientists to accuse of heresies those who would thwart the needs of humanity for food and the prevention of disease. It is true that these needs both fall in the category of "Short-term expediency" in the terminology of Slater.

Slater asks, "What is the present rate of genocide, in terms of species per century?" But genocide refers to the extermination of racial, political, or cultural groups of *human beings*! Is Slater trying to extend the term to, let us say, *Salmonella typhi* and *Treponema pallidum*?

Certainly, a biologist could write, "human beings . . . may be more like ants and bees than laboratory rats"! In terms of social behavior, this is quite true. The *Hymenoptera*, and human beings, unlike rats, seldom devour their newborn young. However, perhaps human beings are changing. Slater, who has four children, says, "Right now, every new child born is an entry for the debit page, acceptable only if required for replacement".

Yours faithfully,

Thomas H. Jukes

Division of Medical Physics,
Donner Laboratory,
Berkeley, California, 94720.

Information Explosion

Sir,—It is refreshing to read Mr Bruce Sanford's letter (*Nature*, **225**, 979; 1970) which departs from the usual attitude of burying one's head in the sand in the hope that computers will solve the information explosion problem. Far too often the use of computers is voiced in the context of information retrieval but this is only part of the problem and the easiest part at that. The real problem is, as Mr Sanford implies, in the assimilation of information and the means of its conveyance to the storage media.

In this area, publishers could be of immense assistance by including in their journals informative summaries, compiled with the assistance of authors, which would enable the documentation expert to make a quicker assessment of the importance and application of an article without having, in the first instance, to read it in detail.

A genuine attempt to do this may be seen in the Ministry of Technology's TECHLINK Service. This relates to government research reports and enables the recipient to make a quick assessment of the value of a report before actually acquiring it.

No one can dispute the efficacy of computers for information storage but if they are to be used to advantage it is vital that greater attention is paid to the input stage which is still a manual operation.

Yours faithfully,

A. Johnson

"Sandown",
The Serpentine North,
Blundellsands,
Liverpool 23.

Light Carrying Electrons

Sir,—In connexion with our observation (*Phys. Rev. Letters*, **15**, 349; 1969) of the modulation of an electron wave by light (see *Nature*, **225**, 15; 1970), I would like to point out that Harris and Smith (*Nature*, **225**, 502; 1970) propose an explanation of electron bunching which, among others, we also mentioned in our paper as a possible mechanism and which was even briefly reported by your Solid State Physics Correspondent in his earlier note. In the meantime, however, even when disregarding the fact that the numbers do not check, we have come to the conclusion that the theory cannot be so simple because, if bunching were the explanation, the effect should also be observed without the presence of the thin crystalline film. However, this is not the case. There is also a continuous decrease in intensity of the light spots on the nonluminescent target with increasing distance between the thin film and the nonluminescent target.

With further experimental and theoretical studies under way, it appears that only a quantum mechanical theory can explain the effect.

Yours faithfully,

Helmut Schwarz

Rensselaer Polytechnic Institute,
East Windsor Hill,
Connecticut, 06028.

Anonymous Bounty

SIR,—Under the heading "Anonymous Bounty" (*Nature*, **225**, 783; 1970) you refer to the endowment fund of £250,000 given to the World Wildlife Fund, with the comment that the donor "gave no stipulation as to how the money should be spent". I would like to stress that the £250,000 itself cannot be spent at all—only the much more modest income from it.

Nevertheless the high priority projects for which we hope to find funds in 1970 include the following: the work of the Legislation Commission of the International Union for Conservation of Nature and Natural Resources in preparing an "International Convention on Export, Import and Transit of Certain Species of Wild Animals and Plants", the work of the International Wildfowl Research Bureau in preparing an "International Wetland Convention" designed to preserve marshes and their plant and animal communities; the work of the Advisory Committee on Oil Pollution, and of the International Council for Bird Preservation towards prevention of oil pollution; the work of the Charles Darwin Foundation for the Galapagos Islands in averting the extinction of the endemic flora and fauna, so much of which is of spectacular interest; the work of the Survival Service Commission of IUCN for threatened species, especially at the moment for apes, big cats, polar bears, wild cattle, rhinoceroses, sirenians, whales, the monkey-eating eagle, the white-winged wood duck, the Hawaiian goose, the cranes and the bustards, marine turtles, crocodiles and threatened plant species; a major conservation programme for Madagascar's unique fauna and flora; support for the 4,800 square miles of Manu National Park, an unspoiled valley in the Andes of Peru; support for the Cayapas Reserve in Ecuador; and the Awash National Park in Ethiopia; and the Shimba Hills in Kenya; the acquisition of a reserve area in the Papuan Gulf, New Guinea; the acquisition of marshlands at Marchauen in Austria; a concerted effort to save the tamaraw (the Philippine wild ox) and the kouprey (the Cambodian wild ox) from extinction; a management plan and survey of the Nile crocodile in Ethiopia and the marine turtles on the SE coast of Africa; a conservation programme for the great Indian bustard; a motor launch and equipment for the research station at Aldabra; a continuing scientific presence and programme for the Udjung Kulon Reserve in Java (where the last 25–28 Javan rhinos survive), and a comparable programme for the mountain gorilla in Rwanda and the Congo; support for the Marine National Park of Kenya; and for a bird observatory at Lake Nakuru, Kenya. Financial help for conservation is urgently needed in Indonesia, Sudan, India and Pakistan, and particularly for the College of African Wildlife Management at Mweka, Tanzania, and the School for the Training of Wildlife Specialists at Garoua, Cameroon. In Israel support is needed for a very large and imaginative programme of Field Study Centres for Conservation. Immediate help is also needed to save a number of oceanic islands from total degradation.

In the UK there are projects for land acquisition and reserve establishment in the following places: on the Ouse Washes in Norfolk and Cambridgeshire; at Martin Mere, Lancashire; on the Glen Washes, Lincolnshire; at Caerlaverock on the Solway Firth; at Predannack, Cornwall; at North Hill Tor, Glamorgan; near Hickling Broad, Norfolk; at Great Merrible Wood, Fox Covert and Poultney Wood in Leicestershire; at Colne Point, Essex; at Bix Bottom, Oxfordshire; and at Lower Whetmoor Woods, Gloucestershire. There is a project for the study and conservation of the large blue butterfly—probably Britain's most prominent species threatened with world extinction. Educational projects are required for Wicken Fen, Strangford Lough, and Fair Isle; and there is a research project on the disappearing sand lizard. There are some 16 nature reserve areas in Scotland which need support and this will be done through the Scottish Wildlife Trust.

I hope I may have made the point that a good deal more money is needed. Some of these projects may not seem very important in isolation, but if all can be undertaken they will add up to a significant amount of solid conservation achievement.

Yours faithfully,

PETER SCOTT

Slimbridge,
Gloucestershire.

University News

Dr T. F. Slater, University College Hospital Medical School, has been appointed to the newly established chair of biochemistry at **Brunel University.**

The title of **Professor of Chemical Pathology** has been conferred on **Dr F. V. Flynn** in respect of his post at University College Hospital Medical School, London, and that of **Reader in Mechanical Engineering** on **Dr R. M. Davies** in respect of his post at University College London.

Announcements

Five new members have been elected to the board of trustees of the **Salk Institute: Dr A. F. Brimmer,** US Federal Reserve Board; **Dr A. Hammer,** Occidental Petroleum Corporation; **Masaru Ibuka,** Sony Corporation; **Dr Seiji Kaya,** University of Tokyo; **Dr Norman H. Topping,** University of Southern California.

The fifth **Founders medal** of the US National Academy of Engineering has been awarded to **Professor Charles Stark Draper** of MIT.

The Council of the Royal Society has awarded the **John Murray travelling studentship** for 1970 to **Professor F. Oldfield,** New University of Ulster, to enable him to carry out research on recent productivity changes in eutrophic lakes.

ERRATUM. In the article "Growing Pains in British Universities" (*Nature*, **225**, 407; 1970), the second and third sentences in the third paragraph should read: "Fig. 1 shows that between 1959 and 1965, expenditure increased at an average annual rate of about 18 per cent, while student numbers rose by about 7 per cent a year. But after 1965, when Colleges of Advanced Technology received university status, the increase in expenditure seems to have been reduced to about 9 per cent a year, while student numbers continued to rise at an average yearly rate of about 8 per cent".

ERRATUM. In the article "Stimulation by Cyclic AMP of Intrinsic Protein Kinase Activity in Ox Brain Membrane Preparations", by M. Weller and R. Rodnight (*Nature*, **225**, 187; 1970), the ordinate of Fig. 1 should read: nmoles [^{32}P]-phosphorylserine/mg protein.

International Meetings

September 27–October 1, **Congress of the International Society for Fat Research,** Chicago (Dr F. Bradley, 136 Sharps Lane, Ruislip, Middlesex).

September 27–October 2, **Second Harden Conference,** Ashford, Kent (Dr A. P. Mathias, Department of Biochemistry, University College, Gower Street, London WC1).

September 28–October 2, **Gas Chromatography,** Dublin (The Conference Secretary, Institute of Petroleum, 61 New Cavendish Street, London WIM 2AR).

September 29–October 1, **Advances in Polymer Sciences and Technology,** London (R. H. Craven, Institution of the Rubber Industry, 4 Kensington Palace Gardens, London W8).

October 4–9, **Annual Meeting of the American Society for Information Science,** Philadelphia (ASIS, 2011 Eye Street, NW, Washington, DC, 20006, USA).

October 5–7, **Gallium Arsenide and Related Compounds,** Aachen (Conference Secretariat, Technische Hochschule Aachen, 5100 Aachen, Templergraben 55, Germany).

October 5–9, **Plutonium and Other Actinides,** Santa Fe (Dr A. E. Kay, Head of Research Metallurgy, Atomic Weapons Research Establishment, Aldermaston, Reading RG7 4PR).

October 6–10, **International Science Exhibition,** London (R. W. Boardman (Exhibitions), Ltd, 8 Leicester Street, Leicester Square, London WC2).

October 15–16, **Tuberculosis,** Bucharest (Secretariat, Union of Societies of Medical Sciences in the Socialist Republic of Romania, Progresului 8–10, Bucharest, Romania).

October 19–21, **Antimicrobial Agents and Chemotherapy,** Chicago (R. W. Sarber, American Society for Microbiology, 115 Huron View Boulevard, Ann Arbor, Michigan 48103, USA).

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

To Let: June and July 1970. 3–4 bedroom house near Epsom; only 32 minutes to Waterloo Station. Would exchange for similar near La Salpêtrière Hospital, Paris, if possible. Please contact Dr L. Herzberg, Neurology Department, St Thomas' Hospital, London SE1 (01-928 9292).

Vacant: Four bedroomed, centrally heated house, fully furnished in Harpenden, Hertfordshire (London 30 minutes). Available from September 1, 1970, for one year. Use of car may be arranged. Please contact: T. I. Kilenyi, Department of Geology, Sir John Cass College, Jewry Street, London EC3.

Wanted: Furnished 2-bedroom flat or house for American professor and family (two children, 18 and 20 years) within half-hour central London, rent no more than £50 per month, in exchange for 2-bedroom house (with 3rd spare room) in Laguna Beach, California, overlooking town and sea, from June 15 to September 1. No money involved. Please contact Dr Hannah Steinberg, Department of Pharmacology, University College London, Gower Street, London WC1.

Wanted: Furnished house or apartment in London area for university professor, wife and three daughters for 9–12 months beginning in the summer or autumn of 1970. Can offer a 4-double-bedroomed house in Denver, Colorado, in exchange for same or longer period. Please contact Dr M. Moskowitz, Clinical Research Centre, Mill Hill, London NW7.

British Diary

Monday, March 23

Digital Filters (6.30 p.m.) Mr R. C. V. Macario, Institution of Electronic and Radio Engineers; and the Institution of Electrical Engineers; at the University of Wales Institute of Science and Technology, Cardiff.

Electrical Aspects of Spacecraft (7.30 p.m.) Mr H. J. H. Sketch, Institution of Electrical Engineers, at the J. J. Thomson Laboratory, University of Reading, Whiteknights Park, Reading.

Integrated Management Systems in Manufacture (5.30 p.m.) Mr F. N. Stephenson, Institution of Electrical Engineers, jointly with the Institute of Management and Control and the I.Mech.E., at Savoy Place, London WC2.

Machinery Requirements for Expanded Plastics Production (6.30 p.m.) Plastics Institute, at the Coachmakers' Arms, 88 Marylebone Lane, London W1.

Memorial Service for Lord Jackson of Burnley, FRS (12 noon) at Holy Trinity Church, Prince Consort Road, London SW7.

Newer Polymers (10 a.m. symposium) Society of Chemical Industry, Plastics and Polymer Group, in Physics Lecture Theatre 1, Imperial College, London SW7.

Overhead-line Oscillations and the Effect of Aerodynamic Dampers (5.30 p.m.) Mr J. C. R. Hunt and Mr D. J. W. Richards, Institution of Electrical Engineers, at Savoy Place, London WC2.

Structural Dynamics (three-day symposium) Loughborough University of Technology, in association with the British Acoustical Society; the Institution of Civil Engineers; the Institution of Mechanical Engineers; and the Royal Aeronautical Society, at the University.

The Biochemistry of Steroid Hormone Action; Cerebral Enzymes and Isoenzymes; Membrane-bound Enzymes; Mitochondria and Calcium Transport, Professor A. L. Lehninger (Fifth Jubilee Lecture) (two-day meeting) Biochemical Society, at University College London, Gower Street, London WC1.

The Dielectric Properties of Heterogeneous Systems including Biological Materials (three-day meeting) Dielectrics Discussion Group, at the University College of Wales, Aberystwyth.

The Shape of Things: General Conceptions of the Nature of Things (7.45 p.m.) Mr Ian Ross, H. G. Wells Society, in the Common Room of the Electrical Engineering Department, Imperial College, Exhibition Road, London SW7.

Tuesday, March 24

Adhesion, Adhesives and Fibres (6 p.m.) Mr E. W. Garnish, Society of Chemical Industry, Paper and Textiles Group, at 14 Belgrave Square, London SW1.

ATLAS Language (5.30 p.m.) Mr J. A. Hill, Mr A. Hann and Mr A. H. Parker, Institution of Electrical Engineers, at Savoy Place, London WC2.

Automation in Air Traffic Control (6.30 p.m.) Mr A. Hartley-Smith, Institution of Electronic and Radio Engineers, at Havering Technical College, Hornchurch, Essex.

On Load Corrosion on No. 1 Boiler at Portabello Power Station (6 p.m. discussion) Institution of Mechanical Engineers, Steam Plant Group, at 1 Birdcage Walk, London SW1.

Wednesday, March 25

Computers and What They Can Do (1.10 p.m.) Professor Victor Price, City University, at the Graduate Business Centre, Gresham College, Basinghall Street, London EC2.

Extrusion Machinery (7.30 p.m.) Institution of the Rubber Industry; and the Plastics Institute, at the Queen's Hotel, Hull.

Integrated Iron and Steel Works as Responsible Producers and Converters of Energy (6 p.m.) Sir Henry Clay, Institution of Mechanical Engineers, at 1 Birdcage Walk, London SW1. (Sixth John Player Lecture.)

MCT-Margarine, a Tailormade Fat Composition with Respect to Physiology and Consistency (6.15 p.m.) Professor G. Billek, Society of Chemical Industry, Oils and Fats Group, at 14 Belgrave Square, London SW1.

Our Professional Duties. Problems in Establishing a Potash Industry in the North Yorkshire National Park (5.30 p.m.) Sir Frederick Warner, Society of Chemical Industry, at the University of Leeds. (9th Brotherton Memorial Lecture.)

Peripheral Development and Information Flows Inside Systems (2.30 p.m. colloquium) Institution of Electronic and Radio Engineers, Joint IERE/IEE Computer Group, at 9 Bedford Square, London WC1.

Recent Advances in Analytical Chemistry (10 a.m. symposium) Chemica Society; Royal Institute of Chemistry; Society of Chemical Industry; Society of Dyers and Colourists; and the Society for Analytical Chemistry, at University of Manchester Institute of Science and Technology, Sackville Street, Manchester 1.

Silicon Imaging Devices (2.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Victoria Line and its Successors (5.30 p.m.) Mr R. M. Robbins, Institution of Electrical Engineers, at Savoy Place, London WC2.

Vacuum Forming (7 p.m.) Mr G. W. Robertson, Plastics Institute, at St. Johns Hotel, Solihull.

Thursday, March 26

Adhesives and Adhesion (7 p.m.) Mr N. C. MacDonald, Oil and Colour Chemists' Association, at the Manor Hotel, Datchet, Bucks.

The Protection of Rubber and Plastics Against Degradation (7.30 p.m.) Mr G. E. Williams, Institution of the Rubber Industry, at the Scottish Building Centre, 425 Sauchiehall Street, Glasgow C2.

Monday, March 30

Rubber Blank Preparation (6.45 p.m.) Mr P. D. Lanham, Institution of the Rubber Industry, at the Waldorf Hotel, Cooper Street, Manchester 2.

Reports and Publications

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ministry of Technology. National Physical Laboratory—Report for 1968/1969. Pp. xviii + 269 + 20 plates. (London: HM Stationery Office, 1969.) 28*s* net. [2912

Ordnance Survey—Annual Report 1968/1969. Pp. v + 10 + 5 plates. (London: HM Stationery Office, 1969.) 13*s* (65*p*) net. [2912

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 256, No. 809 (23 December 1969): Structural Changes, Related to Reproduction, in the Hypothalamus and in the Pars Tuberalis of the Rhesus Monkey. Part 1: The Hypothalamus. Part 2: The Pars Tuberalis. By Sir Francis Knowles and T. C. Anand Kumar. Pp. 357–375 + plates 63–76. (London: The Royal Society, 1969.) 33*s* ; $4.30. [3012

International Means of Conservation of Natural Resources. Pp. 20. (London: The David Davies Memorial Institute of International Studies, 1969.) [11

Bulletin of the British Museum (Natural History). Vol. 19, No. 9: The Genus *Geonemertes*. By Carl Frederick Abel Pantin. Pp. 261–310. (London: British Museum (Natural History), 1969.) 36*s*. [11

Royal Observatory Bulletins. No. 155: Radial Velocities of Stars of Spectral Types A and F. By D. H. P. Jones and C. Margaret Haslam. Pp. 21–34. (London: Science Research Council, 1969.) 5*s* net. [21

Republic of Ireland. Sea and Inland Fisheries Report for 1968. Pp. 62. (Dublin: Stationery Office, 1969. Obtainable from Govt. Publications Sale Office, G.P.O. Arcade, Dublin.) 4*s*. [21

Countryside Commission Long Distance Footpath Guide No. 1: The Pennine Way. By Tom Stephenson. Pp. v + 110. (London: HM Stationery Office, 1969.) 3*s* net. [21

British Medical Bulletin, Vol. 26, No. 1, January 1970: Symposium on "Control of Human Fertility". Pp. 1–97. (London: The British Council, 1970.) 40*s*. [21

Strategies for Displaced Employees. By W. W. Daniel. (Broadsheet 517.) Pp. iv + 85. (London: Political and Economic Planning, 1970. Orders to Research Publications, 11 Nelson Road, London, SE10.) 16*s*. [51

Northern Ireland: Ministry of Agriculture. Leaflet No. 135: Careers in Agriculture and Horticulture. Pp. 12. (Belfast: Ministry of Agriculture, 1969.) [51

Republic of Ireland. National Science Council. Research and Development in Ireland 1967. By Diarmuid Murphy. Pp. 50. (Dublin: Stationery Office, 1969.) 3*s* 6*d*. [51

Department of Education and Science. Scales of Salaries for the Teaching Staff of Farm Institutes and for Teachers of Agricultural (including Horticultural) Subjects, England and Wales, 1969. Pp. v + 47. (London: HM Stationery Office, 1969.) 6*s* (30*p*) net. [61

Why Britain Needs a Population Policy: Statement by the Conservation Society. Pp. 22. (Potters Bar, Herts: Conservation Society, 21 Hanyards Lane, 1969.) [61

Department of Education and Science. Statistics of Education 1968. Vol. 5: Finance and Awards. Pp. xix + 56. (London: HM Stationery Office, 1969.) 18*s* (90*p*) net. [61

Loughborough University of Technology. Vice-Chancellor's Report to the University Court, 1968/1969. Pp. 69. (Loughborough: Loughborough University of Technology, 1970.) [61

Ministry of Technology—Aeronautical Quality Assurance Directorate. AQD Laboratories, Harefield—Descriptive Brochure. Pp. 21. (London: Ministry of Technology, 1970.) [61

Computer Software for Process Control. (Report of the Working Party on Real-time Computer Software, May–December 1968, Plant Engineering and Energy Division, BISRA.) Pp. 118. (London: BISRA, The Inter-Group Laboratories of the British Steel Corporation, 1969.) 60*s*. [71

Urea-Agronomic Applications. By Dr T. E. Tomlinson. Pp. 52. Proceedings No. 107: Modern Methods for Promoting the Sale of Fertilizers. By A. E. Cascino. Pp. 30. (London: The Fertilizer Society, 1969 and 1970.) [81

The Shortage of Mathematics and Science Teachers in Schools. Pp. 18. (London: Royal Society, 1969.) [91

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 265, No. 1167 (31 December 1969): The Fermi Surfaces of the Noble Metals. By M. R. Halse. Pp. 507–532. 14*s*; $1.80. Vol. 265, No. 1168 (31 December 1969): A Laboratory Model for the General Ocean Circulation. By D. J. Baker, jun., and A. R. Robinson. Pp. 533–566 26*s*; $3.40. Vol. 265, No. 1169 (31 December 1969): Studies of Sound Transmission Fluctuations in Shallow Coastal Waters. By D. E. Weston, A. A. Horrigan, S. J. L. Thomas and J. Revie. Pp. 567–608. 24*s*; $3.10. (London: Royal Society, 1969.) [91

The Health of the School Child: Report of the Chief Medical Officer of the Department of Education and Science for the years 1966–196[illegible]. Pp. iii + 136 + 9 plates. (London: HM Stationery Office, 1969.) 14*s* 6*d* (72·5*p*) net. [121

Royal Observatory Bulletins. No. 152: van der Held Curves of Growth. By A. L. T. Powell. Pp. E385–E408. (London: HM Stationery Office, 1969.) 4*s* net. [131

Other Countries

Anuario del Observatorio Astronomico de Madrid para 1970. Pp. 443. (Madrid: Observatorio Astronomico, 1969.) [1812

OECD. Review of National Science Policy—Canada. Pp. 453. (Paris: OECD; London: HM Stationery Office, 1969.) 54 francs; 82*s* ; $11.80. [1812

India: Council of Scientific and Industrial Research. Central Mining Research Station, Dhanbad. Research Paper No. 38: Convergence Measurement in a Bord and Pillar Working in a Coal Mine (Stowed Workings). By S. Samanta and B. Singh. Pp. 21. (Dhanbad, Bihar: Central Mining Research Station, 1967.) Rs. 2. [1812

Indian Council of Medical Research. Special Report Series, No. 60: Dietary Allowances for Indians. By C. Gopalan and B. S. Narasinga Rao. Pp. 90. (Hyderabad: Nutrition Research Laboratories, Indian Council of Medical Research, 1968.) [1812

Studies on the Blood Serum of the Euryhaline Cyprinodont Fish ***Fundulus heteroclitus***, Adapted to Fresh or to Salt Water. By Grace E. Pickford, F. Blake Grant and Bruce L. Umminger. Pp. 29–70. (Transactions of the Connecticut Academy of Arts and Sciences, Vol. 43, October 1969. Obtainable from Archon Books, Hamden, Conn.) [1812

Comptes Rendus des Travaux du Laboratoire Carlsberg. Vol. 37, No. 1: Hydrogen-Deuterium Exchange in Insulin. By David L. Coleman and Lars Willumsen. Pp. 1–20. 5 kr. Vol. 37, No. 2: Hydrogen Isotope Exchange in Ovalbumin with Use of Tritium as a Label. By Lars Willumsen. Pp. 21–48. 7 kr. Vol. 37, No. 3: The Fine Structure of ***Neobursaridium gigas*** (Balexh). By Jytte R. Nilsson. Pp. 49–76 + 17 plates. 24 kr. Vol. 37, No. 4: DNA-Polymerase Activity from Exponentially Multiplying and Dividing-Synchronized *Tetrahymena*. By R. Pearlman and O. E. Westergaard. Pp. 77–86. 3 kr. Vol. 37, No. 5: Some Spectral and Kinetic Properties of the Acyl Enzymes of Native and Chemically Modified Subtilisin Type Carlsberg and Type Novo. By Jack T. Johansen, Ronald W. A. Oliver and Ib Svendsen. Pp. 87–106. 5 kr. (Copenhague: Danish Science Press, Ltd., 1969.) [1812

A New Method for Measuring O_2 and CO_2 in Gas by Using a Glow Discharge. Edited by Masaji Mochizuki. (Monograph Series of the Research Institute of Applied Electricity, No. 17.) Pp. 65. (Sapporo, Japan: Research Institute of Applied Electricity, Hokkaido University, 1969.) [1912

Celestial Mechanics, Vol. 1, No. 1, June 1969. (An International Journal of Space Dynamics.) Pp. 1–140. Subscription price per volume of 4 issues Dfl. 100 (US $28). Postage will be added. Initially the journal will be published quarterly. Private persons (no institutes, laboratories, libraries, etc.) may subscribe at the reduced rate of Dfl. 35 (US $9.80) per volume. Postage will be added. (Dordrecht, Holland: D. Reidel Publishing Company, 1969.) [2212

FAO. The State of Food and Agriculture 1969: World Review; Review by Regions; Agricultural Marketing Improvement Programmes—Some Lessons from Recent Experience; Modernization of Institutions to Promote Development. Pp. ix + 196. (Rome: Food and Agriculture Organization of the United Nations; London: HM Stationery Office, 1969.) 48*s* ; $6. [2312

Geological Society of Australia, Special Publication No. 2: Proceedings of Specialists' Meeting held at Canberra, 25–31 May 1968. Pp. x + 389. (Canberra, ACT: Geological Society of Australia, c/o Department of Geology, Australian National University, 1969.) A$6.75. [2312

Annals of the New York Academy of Sciences. Vol. 164, Article 2: Second Conference on Psychophysiological Aspects of Cancer. By C. B. Bahnson and 48 other authors. Pp. 307–634. $20. Vol. 166, Article 2: The Phosphohydrolases—Their Biology, Biochemistry and Clinical Enzymology. By O. Bodansky and 53 other authors. Pp. 365–819. $24.50. (New York: New York Academy of Sciences, 1969.) [2312

Reactor Centrum Nederland. Summary of Activities, July 1, 1968–June 30, 1969. Pp. 16. (The Hague: Reactor Centrum Nederland, 1969.) [2312

University of Miami, Coral Gables. A Report from the President, July 1, 1968–June 30, 1969. Pp. 32. (Coral Gables, Florida: University of Miami, 1969.) [2312

Annals of the New York Academy of Sciences, Vol. 165, Article 1: Second Conference of the Problems of Colley's Anemia. By Harold Fink *et al.* Pp. 1–508. $20. Vol. 165, Article 2: Cyclitols and Phosphoinositides: Chemistry, Metabolism, and Function. By F. Eisenberg, Jr., G. Hauser *et al.* Pp. 509–819. $19.50. (New York: New York Academy of Sciences, 1969.) [2412

The Cleavage of the Void. By R. Robbins. Pp. 3. (Wellington, NZ: R. Robins, P.O. Box 1981, 1969.) [2412

Annals of the South African Museum, Vol. 56, Part 1: The Fauna of the *Tapinocephalus* Zone (Beaufort Beds of the Karoo). By L. D. Boonstra. Pp. 1–73. (Cape Town: South African Museum, 1969.) R5.60. [2412

British Honduras. Annual Report of the Forest Department for the year 1966. Pp. 9. (Belize: Forest Department, 1969.) [3012

HOW TO BUY NATURE

Volumes start in January, April, July and October but subscriptions may begin with any issue.

The direct postal price per subscription for one copy of NATURE each week is:

12 MONTHS (52 issues and 4 indexes)

Great Britain and Eire.............................. **£14.0.0 each**
United States of America........(by air freight)............**$48 each**
Canada..........................(by air freight)..........**$52 each**
Elsewhere overseas by surface mail....................**£14.0.0 each**
(Charge for delivery by air mail on application)
*Shorter periods *pro rata*. (Minimum three months.)

Editorial and Publishing Offices of "NATURE"

MACMILLAN (JOURNALS) LIMITED
4 LITTLE ESSEX STREET, LONDON WC2
Telephone Number: 01-836 6633. Telegrams: Phusis London WC2

Subscription Department
MACMILLAN (JOURNALS) LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 5431

Advertisements only should be addressed to
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone 01-242 6264/01-405 4743
Telegrams: Textualist London WC2

Registered as a newspaper at the Post Office

Copyright © Macmillan (Journals) Limited, March 21, 1970

Points you should know about the EM802

An electron microscope for universal application

The EM802 combines in one instrument all the facilities you need, whatever your field of research.

There is no loss of performance and no 'down-time' when changing from one mode of operation to another. The EM802 continues the long tradition of AEI for simplicity of operation.

To judge the 802 for yourself, come and use one at your leisure in our Consultant Laboratory. Ring Jay Bailey (Harlow 26761), or write, to fix a suitable time.

1. Goniometer stage allows 5 Å resolution even at the highest angle of tilt.
2. Push-button selected area diffraction at various camera lengths.
3. Push-button selection of bright or dark field images by all-electrical beam deflector with independent astigmatism correction.
4. Multi-purpose specimen chamber to meet a wide range of applications at high resolution.
5. Selected area diffraction facilities.
6. Anticontaminators.
7. Automated vacuum system.
8. Push-button controls.
9. Space for accessory control modules.

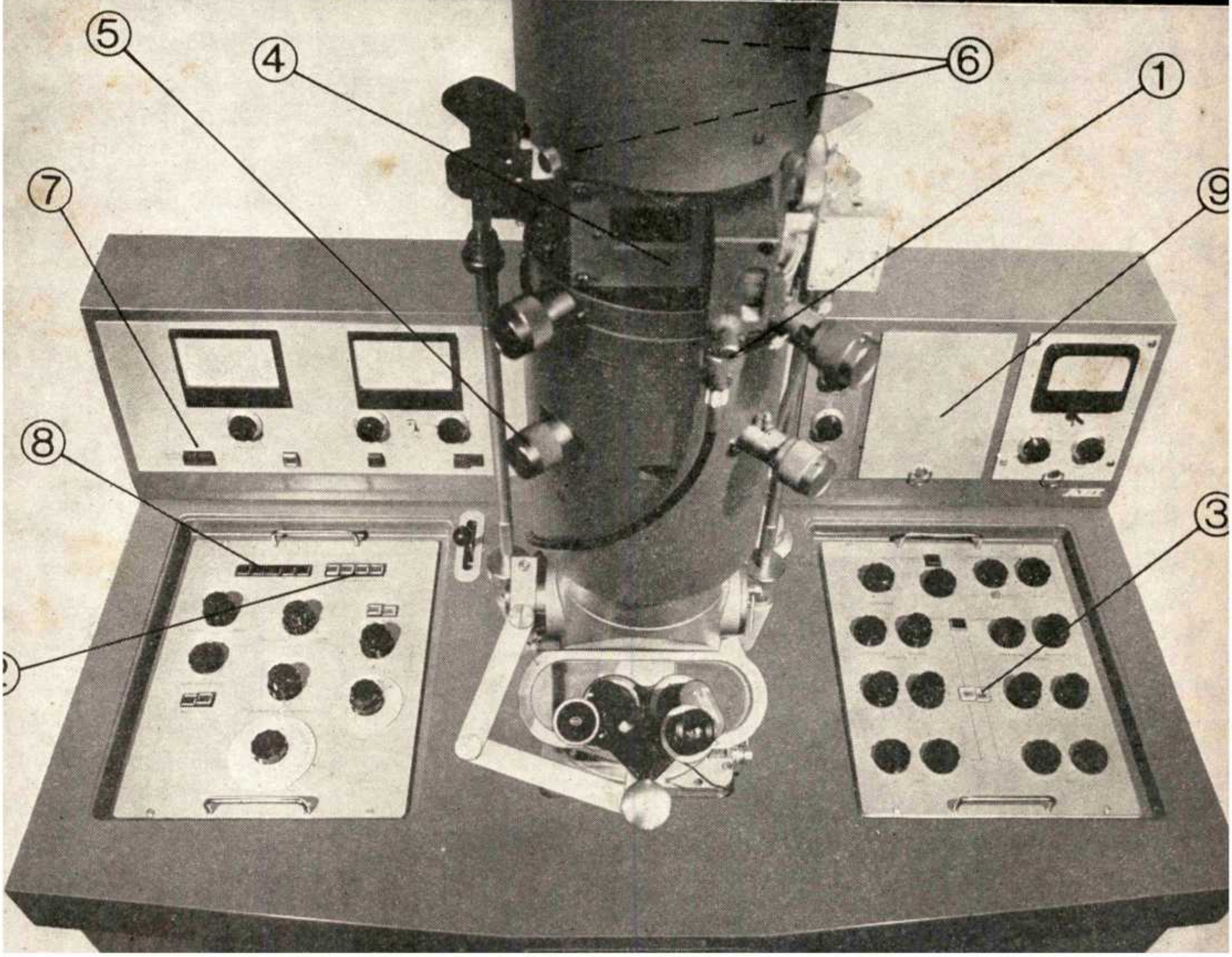
AEI SCIENTIFIC APPARATUS

1968

EUROPEAN LEADERS IN ELECTRON OPTICS

AEI Scientific Apparatus Limited (Dept 202)
PO Box 1, Harlow, Essex. Tel: Harlow 26761
And in the USA contact:
Picker Nuclear, 1275 Mamaroneck Avenue,
White Plains, New York 10605.

81113



Classified Advertisements

All copy is subject to the approval of the Publishers, who retain the right to refuse or withdraw advertisements at their discretion. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes. Semi-displayed £7 4s. per single column inch. Minimum £3 12s., each additional 1/12th of an inch 12s. Full page £190, Half page across £96. Colour (orange) £25 extra. 2/6 is charged for the re-direction of replies to advertisement with a box number.

ADVERTISEMENTS SHOULD BE ADDRESSED TO: T. G. Scott and Son, Limited, 1 Clement's Inn, London, WC2A 2ED. Telephone: 01-405 4743. Telegrams: Textualist, London, W.C.2.

APPOINTMENTS VACANT

FOOD RESEARCH

THE FRUIT AND VEGETABLE PRESERVATION R.A.

Vacancies exist at various levels for

TECHNOLOGISTS, MICROBIOLOGISTS, PHYSICISTS AND ENGINEERS

The work of the station is concerned with the range of fruit and vegetable processing and involves many disciplines.

Job specifications, salary scales, etc., available on application to the Director, Campden Research Station, Chipping Campden, Glos.

(1203)

NATIONAL INSTITUTE FOR RESEARCH IN DAIRYING

(UNIVERSITY OF READING)

BACTERIOLOGY DEPARTMENT

Immunologist or Biochemist required to study antibody production in ruminants; if necessary, training in the appropriate discipline will be arranged. The particular emphasis of the study will be on the protective role of secretory immunoglobulins in the alimentary tract of the young animal. Opportunities exist for the successful candidate to obtain a higher degree. The appointment will initially be for a period of three years, but future prospects are excellent for the right applicant. Salary scale, as Scientific Officer, £1,071 to £1,827; entry point dependent on age and experience. F.S.S.U.

Applications, on forms obtainable from the Secretary, N.I.R.D., Shinfield, Reading RG2 9AT, are invited from honours graduates. Quote: ref. 70/11. (1042)

UNIVERSITY OF OXFORD

RESEARCH OFFICER IN OPHTHALMIC BIOCHEMISTRY

A vacancy exists in the Nuffield Laboratory of Ophthalmology for a biochemist interested in study of biochemistry of the eye. Salary on the scale £1,885 to £2,725 per annum plus F.S.S.U. The appointment is for five years and is renewable. Topics currently being studied include enzymes and proteins of the normal and cataractous lens and the experimental production of lens opacities.

Applications (five copies, or one from overseas candidates), stating age, qualifications and experience, with a list of publications and names and addresses of two referees should reach the administrator, Nuffield Laboratory of Ophthalmology, Walton Street, Oxford, OX2 6AW (from whom further particulars may be obtained) by April 25, 1970. (1048)

Electricity Council Research Centre

PHYSICISTS

We are interested in a wide range of studies in interior domestic environments – (also offices, works, hospitals, etc.).

Specifically, we want to recruit Physicists for further studies in *Lighting* such environments, and further studies in the *Control* and *Instrumentation* of practical environments.

An interest in working in these topics coupled with a good physics or engineering degree is required. For one post, post-graduate experience of electronics is an additional requirement.

Salary on appointment, depending on ability, experience and age, will be within one of the following bands:

£1,173-£2,001 £2,184-£2,951
£1,920-£2,442 £2,904-£3,595

NJB Agreement

Please apply to: D. C. Page,
Head of Personnel Services,
Electricity Council
Research Centre,
Capenhurst, Near Chester,
quoting ref. No. N/222

(1186)

ROYAL ARMY MEDICAL COLLEGE

Ministry of Defence (Army Department)

Millbank, London, S.W.1.

PARASITOLOGIST (graded Experimental Officer/Assistant Experimental Officer) for preparation of pathology class-room for practical classes, meetings and demonstrations involving human Protozoology and Helminthology; maintenance of parasitological specimens and biological material; the setting out of macroscopic and microscopic demonstrations, and undertaking related duties including assisting in the practical tuition in parasitology of medical officers and laboratory technicians. Successful candidate may also undertake practical work in diagnosis of parasitic diseases and will have opportunities to assist in research. Duties may include work with experimental animals.

Qualifications: Degree, or equivalent in appropriate subject. Age: A.E.O. under 28; E.O. normally 26-30.

Salary: E.O. £1,590-£2,006; A.E.O. £940 (at 22)-£1,208 (at 26)-£1,454. London weighting £125.

Prospects of permanent pensionable appointment.

APPLICATION FORMS are obtainable from the Ministry of Defence (Army Department), CE2(F)(AD), Room 304 Northumberland House, Northumberland Avenue, London, WC2. **Please quote: 4/70/G.**

(1142)

ST. MARY'S HOSPITAL MEDICAL SCHOOL

(UNIVERSITY OF LONDON)

PADDINGTON, W.2

Applications are invited for a new post of

SENIOR LECTURER OR LECTURER IN BIOCHEMISTRY

established in co-operation with the Ministry of Overseas Development to assist overseas universities. The successful candidate will be a full member of the staff of the Biochemistry Department but will be expected to spend the greater part of his service on secondment overseas, mainly at the Department of Biochemistry, University of Ibadan, Nigeria, for five years.

Salary scales: Senior Lecturer, £2,770 to £3,670 plus £80 L.A.; Lecturer, £1,240 to £2,850 plus £60 L.A., together with superannuation under F.S.S.U. and overseas allowance as appropriate to the particular assignment.

Applications (two copies), together with the names of two referees, should be sent by April 30, to the Secretary, from whom further particulars should first be obtained.

(1123)

THE UNIVERSITY OF SUSSEX

SCHOOL OF BIOLOGICAL SCIENCES

LECTURESHIP IN NEUROBIOLOGY

To assist in the teaching of a new degree course in Neurobiology with special responsibilities for functional neuroanatomy for either 1970/71 or 1971/72.

Applicants with interests in other areas of neurobiology will be considered. The course is jointly organised by biologists and experimental psychologists within the School of Biological Sciences. Studies of brain function in a wide range of disciplines are actively pursued in the School and the Biology group within which the appointment would be made, intends to develop neuroanatomical approaches to studies of vertebrate motor control and motivation.

Salary will be within the range £1,240 to £2,850 per annum plus F.S.S.U. benefits.

Application forms should be obtained from the Secretary of Science, Science Office (Establishment), The University of Sussex, Falmer, Brighton, BN1 9QH. Any inquiries should be made direct to Professor R. J. Andrew.

(1190)

THE OPEN UNIVERSITY

RESEARCH STUDENTSHIP IN BIOLOGY

The Research Student will work under Professor Steven Rose on aspects of the Biochemical correlates of learning and environmental change; and will be able to register as a postgraduate student for a Higher Degree of the Open University. The value of the Studentship will be not less than £530 per annum.

JUNIOR TECHNICIAN IN BIOLOGY

To work partly in the Biological and Biochemical laboratory and partly in the Animal House. Salary at a point on the scale £377 to £637 per annum.

Detailed applications, with names and addresses of two referees, should reach the Establishments Office (3), The Open University, Walton Hall, Walton, Bletchley, Bucks, by Monday, April 6, 1970.

(1204)

QUALIFIED CHEMIST

[Female]

FOR TECHNICAL LIBRARY

We wish to appoint a chemist in our library in Birmingham to undertake the classification and indexing of information, the preparation of abstracts and literature searching.

Applicants must possess an honours degree, or be an Associate of the Royal Institute of Chemistry. Preference will be given to applicants with previous experience of information work, and a knowledge of French or German would be an advantage.

Starting salary from £1200, depending upon age and experience.

Please write for full Job Description and application form to:—

The Personnel Manager (Ref: HSP),
Thermosetting Division,
Bakelite Xylonite Limited, Redfern Road,
Tyseley, Birmingham 11.

BXL

BAKELITE XYLONITE LIMITED

(1212)

TROPICAL PRODUCTS INSTITUTE
MINISTRY OF OVERSEAS DEVELOPMENT
56-62 Grays Inn Road London WC1

Microbiologist

MICROBIOLOGIST (graded Senior Scientific Officer) to take charge of the Microbiology and Fermentation Section of the Institute. A major part of the responsibility will be to continue the Institute's pioneering researches into the microbial production of protein from nutritionally inferior carbohydrate foodstuffs, such as cassava. This work forms a part of the UK contribution to the International Biological Programme. The Section is also responsible for investigational work on the microbial deterioration of foodstuffs and for dealing with enquiries from developing countries on a wide variety of bacteriological problems in foods of tropical origin.

The successful candidate may be required to undertake occasional short term overseas visits.

Qualifications: 1st or 2nd class honours degree in microbiology and at least 3 years' post-graduate experience. Ability to conduct independent research in this subject necessary. Experience of research relevant to the production of micro-organisms in bulk and of the fermentation industries an advantage. Knowledge of microbiological control in relation to foodstuffs desirable. Age: SSO normally 26-31.

Salary: £2,146-£2,616.

Prospects of permanent pensionable appointment. Prospects of promotion.

APPLICATION FORMS from the Administrative Secretary at the above address.

(1119)

AKZO

Centrale Engineering Amsterdam, Netherlands

expert in thermodynamics or physical chemistry

The Chemical Engineering Development Department at Hengelo (Holland) has an opening for an Engineer or a physical chemist with special knowledge of thermodynamics. The development of chemical processes from the laboratory to industrial plants requires knowledge and experience in the application of thermodynamic theories and calculations, especially in the field of the phaserules. Distillation calculations for the separation of multicomponent systems call for advanced mathematical methods and the use of computers.
To a scientist (graduate of an institute of technology or a university) sufficiently interested in these fields this opening offers an interesting scope of work; having given proof of his capability he will work highly independent and be entrusted with a large measure of responsibility. Applications in writing with recent passport photograph should be addressed to: Centrale Engineering Amsterdam, Dept. Personeelszaken, Sloterweg 11, Amsterdam-W, Netherlands.

AKZO incorporates the activities of a large number of companies, both national and international, manufacturing synthetic fibres and a great variety of chemical and petro chemical products as well as foodstuffs, paints, lacquers and pharmaceuticals.

(1185)

NATURE

MACMILLAN (JOURNALS) LTD

No. 5239 SATURDAY MARCH 28 1970 Vol. 225

CONTENTS

SENATOR MANSFIELD, HIS MARK—PROSPECTS FOR SALT 1177

Old World—Libel—Computer Policy—Pollution—Third London Airport—Pollution—Medicine—Archaeology—Royal Society—Kidney Patients—Parliament in Britain—Miscellaneous Intelligence 1179

New World—MORE GRUMBLING ABOUT MANSFIELD—Communications—Bureau of Standards 1185

News and Views—THE MAN WHO LIVED WITH AUSTRALOPITHECINES—Differentiation—Nucleic Acid—Nucleosides—Cell Cultures—Chromosomes—Marine Biology—Invertebrates—Cancer—Vaccines—Crystallography—Moon—Galaxies—Tribology 1188

PROBABILITY, INFORMATION AND ENTROPY by J. R. Rowlinson. 1196

AMERICAN MUSEUM OF NATURAL HISTORY AT 100 by James A. Oliver. . . 1199

THERMODYNAMICS MAKES PROGRESS by P. T. Landsberg 1204

TRAVELLING WAVES AS FREQUENCY ANALYSERS IN THE COCHLEA by Georg Von Békésy 1207

FOUR BASE-SPECIFIC NUCLEOSIDE 5′-TRIPHOSPHATASES IN THE SUBVIRAL CORE OF REOVIRUS by A. M. Kapuler, N. Mendelsohn, H. Klett and G. Acs 1209

SPATIAL ORGANIZATION AND INTERACTION OF THE TWO PHOTOSYSTEMS IN PHOTOSYNTHESIS by W. P. Williams 1214

NEW FINDS AT THE SWARTKRANS AUSTRALOPITHECINE SITE (*contd*) by J. T. Robinson; R. J. Clarke, F. Clark Howell and C. K. Brain; M. D. Leakey . . 1217

CONCEPTUAL MODEL OF THE BAHAMIAN PLATFORM FOR THE LAST 135 MILLION YEARS by George W. Lynts 1226

Letters to the Editor (see overleaf) 1229

Book Reviews by Alex Comfort, V. B. Wigglesworth, F. B. O'Connor, W. B. Whalley, J. E. Roberts, H. Gutfreund, A. Kelly, M. J. Hall, D. Davies and James Hirschfeld 1265

Obituaries—Dr G. S. Carter 1268

Correspondence—Should Slides be seen Blind ? by P. Grasso; Another *Acanthaster* Disaster by Lowell P. Thomas ; Pulses, Units, Pitts and Hertz by Alexandru T. Balaban 1269

University News—Appointments—International Meetings—Sabbatical Itinerants 1270

RECENT SCIENTIFIC AND TECHNICAL BOOKS XXI

Nature in North America: The *Nature* office in Washington DC has opened and contributors in the United States and Canada are invited to submit manuscripts for publication either to the address given below or to the present London office. The intention is that each office will be able to answer inquiries about all manuscripts and other matters. This is one of several steps by which it is hoped to provide a better service for readers and contributors.

London Office
4 Little Essex Street,
London WC2.
01-836-6633

Washington Office
711 National Press Building,
Washington DC 20004.
(202) 737-2355

(*Continued overleaf*)

Unicam SP1800 Ultraviolet Spectrophotometer
Power
Deuterium Lamp
Scale divisions
Absorbance or Concentration
Meter Range
Zero
Concentration
Band Width
Unicam SP 8000 Ultraviolet Spectrophotometer

Unicam
UV Spectrophotometers
make light work

What does this mean to you? In research, results you can trust and repeat. In routine applications, error-free operation by the least experienced member of your staff.

When you come to Pye Unicam, even the selection of your UV spectrophotometer is simple. There is a choice of instruments broad enough to meet your every requirement and to match your budget, with a staff of experienced application specialists to back them up.

Just look at what is available . . . **SP500 Series 2** and **SP600 Series 2** quantitative single beam spectrophotometers, **SP700** for research, **SP1800** for low-cost double-beam operation, **SP3000** automatic digital instrument, **SP8000** for the ultimate in flexibility, and th **SP1300 Series 2** low-cost colorimeter.

Write or 'phone for free information today. That's simple too, isn't it.

Pye Unicam Ltd
York Street Cambridge, CB1 2PX, England
Telephone (0223) 58866 Telex 81215

1966 1969

PYE UNICAM

LETTERS TO THE EDITOR

Page

PHYSICAL SCIENCES

Short period binaries—Origin of WZ Sagittae—VILA (Pennsylvania) 1229

Pulsars—Reply to Simon and Strange—CHIU and CANUTO (NASA) 1230

Zodiacal light—New interpretation of data on particle motion—VANÝSEK (Charles University, Prague) and HARWIT (Czechoslovak Academy of Sciences) . 1231

Meteors—A new meteor stream—HINDLEY and HOULDEN (Liverpool) 1232

Electrodynamics—Reply to Katz, Ornan and Schwartz—HOYLE and NARLIKAR (Cambridge) . 1233

Moon—Desert channels resembling lunar rilles—BURKE, BRERETON and MULLER (California Institute of Technology) 1234

Stonehenge—Role of the Heel Stone—ROBINSON (Florida) 1236

Seismology—Earthquakes and explosions: comment on Molnar *et al.*—THIRLAWAY (UKAEA) . . 1237

—Reply to Thirlaway—MOLNAR, SAVINO, SYKES, LIEBERMANN, HADE (Columbia) and POMEROY (Michigan) 1238

Quarks—Measurements on cloud chamber tracks—WILSON (Leeds) 1238

Light scattering—Frequency mixing for scattering by harmonically bound electrons—ANDREOU and BALI (London) 1239

Metallurgy—Measurements of electrical potential for detecting crack extension in crack opening displacement tests—DAVIES, CANNON and ALLEN (British Railways Board Research Department) . . . 1240

Seawater—Occurrence of indium—MATTHEWS and RILEY (Liverpool) 1242

Palaeontology—Late Maastrichtian nannoplankton provinces—WORSLEY (Washington) and MARTINI (Frankfurt) 1242

BIOLOGICAL SCIENCES

mRNA sequences—Indirect assignment from data on Hb β chain variants—LANKS (Columbia Institute of Cancer Research) 1244

mRNA—Model for control of translation in eukaryotes—SUSSMAN (Brandeis) 1245

Phospholipid esters—Histological localization of hydrolysis by cultured cells—NORRIS and REPORTER (Kettering Research Laboratory) . 1246

Adenosine phosphates—Non-enzymatic hydrolysis—HULETT (Stanford) 1248

Dental decay—Basis for cariostatic effects of phosphates—PRUITT, JAMIESON and CALDWELL (Alabama) 1249

Hormones—Production of "sulphation factor" by liver after treatment with growth hormone—McCONAGHEY and SLEDGE (Harvard) . . . 1249

Oestrogen receptors—Iodination destroys binding activity—PUCA and BRESCIANI (Messina and Rome) 1251

Plant growth retardants—Differential inhibition of rat liver cholesterol biosynthesis—PALEG (Waite Agricultural Research Institute) 1252

Nitrogen fixation—Soluble nitrogenase from vegetative cells of *Anabaena cylindrica*—SMITH and EVANS (London) 1253

Placenta—Evidence against porosity to maternal cells in the mouse—SELLER (London) 1254

Antibodies—Lymphocyte cytotoxic antibodies in systemic lupus erythematosus—MITTAL, ROSSEN, SHARP, LIDSKY and BUTLER (Baylor) . . 1255

Trypanosoma cruzi—Demonstration of *in vitro* delayed type hypersensitivity in mice—SEAH (London) 1256

Lipid bilayers—Ion dependent switching in the presence of monamycin and alamethicin—GOODALL (American Medical Association) 1257

Neurophysiology—Strychnine, glycine and vertebrate postsynaptic inhibition—CURTIS and JOHNSTON (Australian National) 1258

—Strychnine antagonism and glycine: a reply—ROPER AND DIAMOND (London) . 1259

Serotonin and depression—Effect of probenecid on amine metabolism in cerebrospinal fluid—VAN PRAAG, KORF and PUITE (Groningen) . . 1259

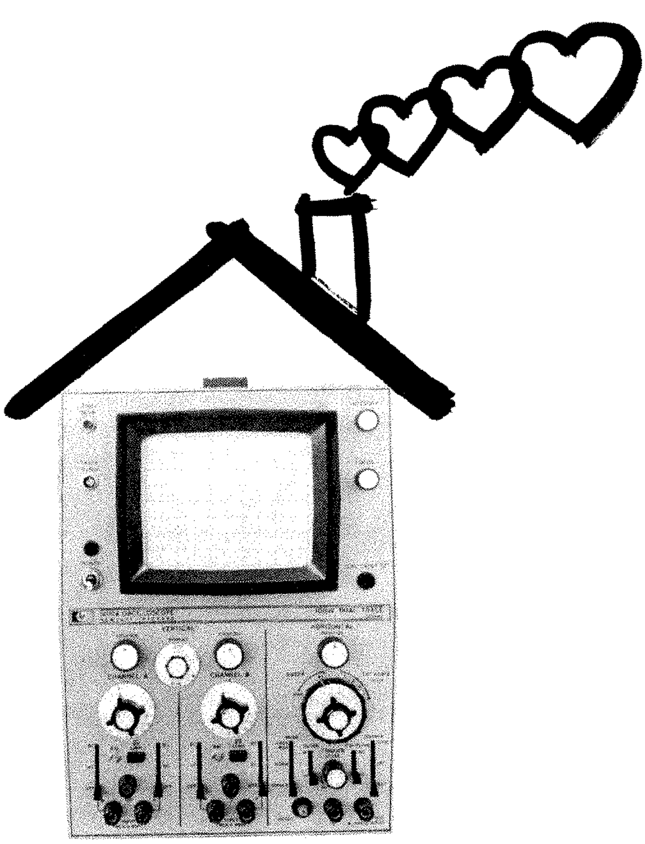
Chemical mutagenesis—Abnormal zygotes born to female mice mated with males treated with triethylenephosphoramide — EPSTEIN, JOSHI, ARNOLD, PAGE and BISHOP (Harvard) 1260

Drugs—Effects on the way mice build nests—SCHNEIDER and CHENOWETH (Dow Chemical Company) 1262

Locusts—Antennae as wind-sensitive receptor system—GEWECKE (Düsseldorf) 1263

The titles on this page are prepared by the staff of *Nature* on the basis of the titles submitted by authors which appear in full on the pages indicated

LFO



HOW TO MAKE 100 μV SENSITIVITY AND 50 μV DRIFT LIVE HAPPILY TOGETHER IN A LOW FREQUENCY SCOPE

Easy. We've done it twice at Hewlett-Packard. In the 1200A 2-channel and the 1202A 1-channel scopes. The answer? All solid state circuitry with matched FET inputs.

Typical drift at the 100 μV level is reduced to less than 50 μV per hour. Trace shifts due to shocks or vibrations are eliminated. Noise is less than 50 μV P-P at the full 500 kHz bandwidth and may be reduced to as low as 15 μV P-P by means of a bandwidth limit switch. Other features of the 1200A/1202A series include: 17 calibrated sensitivity ranges with full bandwidth at all ranges, large 8 x 10 cm CRT with internal graticule to eliminate parallax, 100 db common mode rejection, single-ended or differential input on all ranges, perfectly stable input balance, complete triggering versatility, external horizontal input, beamfinder and d-c coupled z-axis input.

Both the 2-channel 1200A (price £ 454) and the 1-channel 1202A (price £ 421) are also available in 5¼" high rack mounts.

If you are looking for accurate, stable measurement at low levels, then write to your local HP office for the full specifications, on these new, high-performance LF scopes.

Hewlett-Packard Ltd.
224 Bath Road
Slough, Bucks, Great Britain, Tel. 33341

European Headquarters:
Hewlett-Packard S.A.
Rue du Bois-du-Lan 7
1217 Meyrin 2, Geneva
Tel. (022) 41 54 00

HEWLETT hp PACKARD

NATURE Volume 225
MARCH 28, 1970

Senator Mansfield, His Mark

THE irony of the scandal which has arisen in the United States (see page 1185) about the continued financing of basic research originally supported by mission oriented agencies is that Senator Mansfield, to whom belongs section 203 of the defence appropriations bill for 1970, is essentially a liberal man. His quarrel last December was with the Pentagon, not with American science. In other circumstances, it is easy to see how he could have been the champion of academic research, for is it not one of the ways in which the United States has become not merely economically overwhelming but intellectually important? The first conclusion, then, is that it is not the senator who should be reformed, but the system.

What has now happened in the United States is that the Congress has imposed a test of relevance on the scientific research which may be supported by the mission oriented agencies, and there is a danger that the result may be such a rapid shift of emphasis that many parts of science will be seriously harmed. Although the budgets of the two agencies—the National Science Foundation and the National Institutes of Health—which are chiefly responsible for detached research have been increased, the increase is not enough to keep pace with the inflation of two years and certainly not enough to make sure that the research projects being abandoned by the mission agencies will be continued. And it is always possible that Congress will cut back the funds asked for by the National Science Foundation and the National Institutes of Health—the foundation has usually been unlucky.

The first thing to be said is that, in principle, it is entirely proper that there should be a shift from the American tradition that most basic research is a pensioner of mission oriented activity to one in which it is supported for its own sake. For several years it has been assumed that the National Science Foundation should be a haven of last resort, able to hold the ring between the richer agencies to which the support of academic research is a peripheral activity, and even now the foundation accounts for only 14 per cent of government support for academic science. The boot should properly be on the other foot, and the National Science Foundation should be able to provide for the hard core of academic science, not just the frosting on the cake. The demand by the congressional panel (see page 1186) that the right of mission agencies to support academic research should be regarded as a permanency is therefore mistaken—indeed, it is already intolerable that the National Aeronautics and Space Administration should be the chief supporter of academic research in astronomy simply because congressmen do not know the difference between that and space research. In the same way, it is shabby that in the coming year the US Atomic Energy Commission should have had to withdraw support from the accelerator operated jointly by Princeton and the University of Pennsylvania so as to accommodate the new 200 GeV accelerator within a more or less fixed budget.

This is why the most serious failure of the past few years has been the indifference of the Administration to the need for a greater concentration of support for basic research on agencies which are explicitly concerned with that. There have been suggestions that the National Science Foundation should grow, in the course of time, but neither the Administration as a whole nor the Office of Science and Technology has ever given a strong lead. What proportion should be the target? To be sure, there are difficulties. Many of the committees of Congress, for example, would resist the notion that exciting areas of research should become the property, so to speak, of others. It is also likely that the mission oriented agencies would resist a concentration of responsibility for basic research elsewhere, but the national interest that agencies such as the Department of Defense should have strong links with basic science can be safeguarded in other ways. And what, in any case, are administrations for if not to work out a rational balance between the necessarily conflicting interests of different agencies? Is Congress really able to prevent the Administration making up its own mind about the headings under which it applies for public money? One sign of the Administration's weakness on this issue is that it has so far conspicuously shied away from comment on the two reports produced by the National Science Board, the first of which, a year ago, argued the case for an explicit federal commitment to graduate education, and the second, only a few weeks ago, asked for just the concentration of resources in the National Science Foundation now shown to be urgently necessary.

But should the concentration of resources go as far as a government department, with a Secretary for Science and Technology able to rub shoulders (and break lances) with the big departments? This has often been advocated as a solution for problems like those of the present. The chances are that it would go too far. In strictly administrative terms, the immediate need is modest. Some way has to be found for providing continuity and a good sense of priorities in the spending of $1,600 million a year directly in universities and a comparable sum on the support of laboratories such as the great accelerator centres and the observatories which are widely used by academics. A suitably strengthened National Science Foundation should be able to manage well enough. For the time being it will be quite sufficient if the machinery which exists can be put to better use.

Prospects for SALT

THE way in which the Soviet Union and the United States are both making public statements about strategic weapons is at least a sign that both of them are prepared to stake a lot on the next round of SALT talks, which resume in Vienna on April 16. That, at least, is some small comfort. The danger, also by now apparent, is that both sides may go so far in their preliminary posturings that they will find themselves committed to needlessly bellicose policies if the talks should come to nothing. This is why it is important that everybody, not just the participants, should appreciate what has been happening, in the past few months, to the declaration of military policy in the United States and the Soviet Union.

In the United States, the most striking change has been the public acknowledgment by the Secretary of Defense, Mr Melvin Laird, that the old strategic supremacy of the United States has been melting away, chiefly because of the pace of military development in the Soviet Union. Thus Mr Laird told the congressional committees on February 20 that the Soviet Union now has more "operational" launchers for long-range ballistic missiles than has the United States, although it seems clear that the sites concerned will not be equipped with rockets for a long time yet. By the mid-seventies, however, according to Mr Laird, the tables could have been turned, which is why "we are proceeding with a program to place MIRVs (Multiple Independent Reentry Vehicles) on our Minuteman and Poseidon missiles". By all accounts, Mr Laird had not meant his colleague Dr Robert Seamans to say, a few days afterwards, that the installation of the Minuteman III missiles, which carry multiple warheads, had already begun, but that is no great surprise—the Department of Defense has not been testing all those warheads underground for the fun of it. The fact that Mr Laird has also raised, in his request to Congress for money, the prospect of adding another site to the two at which anti-ballistic missiles are already being deployed raises other issues. For one thing, it is significant that the United States Government has now entirely abandoned Mr McNamara's patently thin argument that anti-ballistic missiles were a necessary insurance against the possibility that an irresponsible attack by the Chinese could set the world in flames—now, the argument goes, the ABMs are necessary to help make less vulnerable to a first strike the Minuteman missiles which form the largest part of the land-based strategic striking force of the United States. The snag, which Mr Laird himself admitted, is that even a full deployment of the Safeguard ABM system, involving twelve and not three sites, would not guarantee immunity from an attack by the Soviet Union. In circumstances like these, it is only reasonable to expect that the United States Congress will give the ABM proposals an even closer scrutiny than they received last year, when the President's cause scraped through with only the narrowest of margins.

For students of strategic armaments, Mr Laird's statement on February 20 also contains some alarming news of the pace of change in fields which have recently seemed comparatively stable. One strategically puzzling development is the decision to build a supersonic strategic bomber—it will be interesting to see whether the Department of Defense has better luck than the British Government with the TSR2. More significant, there is also a plan to equip nuclear submarines with missiles of such long range that the launching vehicles can stay permanently submerged within the coastal waters of the United States, there remaining more or less free from attack by the increasingly capable hunter submarines. This, in the long run, will be the tendency in all countries with strategic pretensions.

Developments like these are, of course, enough to persuade observers outside the United States that a new period of military innovation has begun. This, certainly, is what *Pravda* has been saying, most conspicuously on March 7. And it is clear that some of these developments are unstoppable—the MIRVs and the submarines cannot be identified from the outside, for example. It is therefore only fair to say that the Soviet Union has itself done much to encourage the sense of bellicosity which now seems such a tasteless preliminary to the SALT negotiations. It is therefore as well to remember that the Soviet Union has been testing warheads underground with all the abandon of the United States, and has also been claiming that there is an anti-ballistic missile system capable of destroying hostile ballistic rockets from the United States. The truth, mercifully, seems to be simply that work has begun on the building of a rudimentary defensive system about Moscow. And on balance, in spite of Mr Laird's somewhat dramatic talk about the "technological threat" from the increasing pace of research in the Soviet Union, it is probably a simple truth, for the next several years at least, that the United States is in a strong position to anticipate and outwit any strategic innovations of which the Soviet Union may be capable.

What does this imply for the resumption of the SALT negotiations? Especially because the matter is important, there is much to be said for making a dispassionate appraisal of what may emerge. A few simple facts stand out. For one thing, it is inconceivable that any agreement at Vienna in 1970 will allow for mutual inspection of military equipment in the major powers, which in turn implies that any decision not to deploy multiple warheads will have to be taken on trust by the two sides. Most probably, neither will think this to be a justifiable risk, which in turn implies that people had better behave as if that opportunity has already been lost. The fact that the Soviet Union seems not to have got very far with the deployment of these instruments is beside the point—the testing has been done.

A second simple truth is that the quality of intelligence seems now to be good enough to allow governments to count each others' launching sites without actually paying visits to them. Satellites have no doubt played a part in this, although it may be less than some enthusiasts have liked to think. In practice, however, this means that it would be feasible to think of imposing an agreed limit on the numbers of strategic ground-based missiles on each side. The ideal would be the simplest agreement—the limitation of missiles to, say, 200 on each side—enough for a retaliatory blow of great destructiveness but not enough to make a first strike seem tempting.

On the face of things, this is the best agreement that SALT could offer. Public declarations not to work towards the development of anti-ballistic missiles will be illusory—far better simply to require that each side should decide whether to put its efforts into offensive weapons or the equally destabilizing defensive rockets. There remains, however, one obvious and hopeful avenue—the extension of the test-ban to weapons tested underground. In the past few months it has become clear that the remote detection of underground explosions is more than ever feasible. In the long run, this is also the only way in which it will be possible to impede the development of advanced weapons technology. The signature of the non-proliferation treaty three weeks ago, and the willingness of the United States Government to extend its renunciation of biological weapons to include biologically derived toxins, is a sign that things are moving in a cheerful direction. Everybody will be hoping that recent bellicosity about strategic weapons is only a preliminary to hard bargaining at Vienna.

100 Years Ago

It is stated by the *Athenæum* that a new idea has been broached in a recent lecture by Mr. Bloxam, the lecturer on chemistry to the department of artillery studies. He suggests that the committee on explosives, abandoning gun cotton, should collect the germs of small-pox and similar malignant diseases, in cotton or other dust-collecting substances, and load shells with them. We should then hear of an enemy dislodged from his position by a volley of typhus, or a few rounds of Asiatic cholera. We shall expect to receive the particulars of a new "Sale of Poisons" Act, imposing the strictest regulations on the sale by chemists of packets of "cholera germs" or "small-pox seed." Probably none will be allowed to be sold without bearing the stamp of the Royal Institution, certifying that they have been examined by the microscope and are warranted to be the genuine article.

From Nature, **1**, 562, *March* 31, 1870.

OLD WORLD

LIBEL

Science in Court

A LIBEL action which has serious implications for the scientific community will be brought before the courts sometime next year. Mr Stanley Lithgow Drummond-Jackson, a dental surgeon, is suing the British Medical Association and the authors of a paper published in the association's journal, the *British Medical Journal*, because he claims that the paper is defamatory of him in the conduct of his profession. A Court of Appeal has already decided, by two votes to one, that the paper is capable of bearing a meaning likely to be damaging to the dental surgeon, and the stage is now set for what could be a long battle in the High Court.

There is more at stake in this action than the damages being claimed by Mr Drummond-Jackson, as was recently pointed out by J. R. Lewis and his colleagues (*Nature*, **225**, 1081; 1970). The case could change not only the way that scientific papers are written, but it may also deter some journals from publishing criticisms of techniques involving commercial or professional interests. The basis of Mr Drummond-Jackson's claim seems to be that the paper was critical of a technique for dental anaesthesia which was introduced, recommended and used by him, and is, therefore, closely associated with his name. The paper, which was written by Professor J. S. Robinson, Dr C. C. Wise, Dr M. J. Heath and Dr P. J. Tomlin, of the University of Birmingham, also referred to Mr Drummond-Jackson by name when citing his published papers. Such practices are, of course, common in scientific literature, and Lord Denning, Master of the Rolls, clearly saw the effect that such an action could have on the scientific community. He was reported as saying at the Court of Appeal that "it would be a sorry day if scientists were to be deterred from publishing their findings for fear of libel actions. So long as they refrained from personal attacks, they should be free to criticize the systems and techniques of others. Were it otherwise, no scientific journal would be safe" (*The Times*, February 14, 1970).

Lord Denning also said that Mr Drummond-Jackson has chosen the course of action which suits him best. The defendants must now prove the correctness of all their tests and experiments, and justify their conclusions to the jury. "To place such a burden on the defendants", he said, "should not be permitted." But the other two Appeals Court judges thought otherwise. Sir Gordon Willmer, for example, said that he could see no danger to scientific communication in dismissing the appeal, and for him the essential feature of the case seems to be that Mr Drummond-Jackson is a practising dental surgeon whose professional reputation is at stake.

The usual way of attacking the views expressed in a scientific paper is to publish another paper stating the opposing case. Such paper discussions are, of course, common among members of the scientific community, but the weapon of a libel action does not seem to have been used before to settle a scientific dispute. There are no exactly comparable legal precedents and this action may be seen by some scientists as a test case, the outcome of which could determine whether they bring similar actions in respect of papers already published.

COMPUTER POLICY

Users caught in a Vicious Circle

Delving into the obscurities of the British Government's policy toward the computer industry, as subcommittee D of the House of Commons Select Committee on Science and Technology is trying to do, is no easy matter. Last week's session saw the chairman, Mr Airey Neave, confiding how difficult it is to discern what government policy is, never mind deciding how the government should encourage the computer industry in the future. The representatives of the British Steel Corporation and Imperial Chemical Industries, both big users of computers, who appeared before the subcommittee during last week's session, served to confuse rather than clarify the situation.

One thing that is clear, however, is that the British computer company, ICL, is up against the fact that the IBM line of machines has fast become almost a standard gauge. The chief representative of the ICL team, Mr J. K. Steward, who is director of the management services division, confessed that the 2,000 man years of programming which has been invested in ICL's portfolio of computers—largely IBM machines—is a strong disincentive to change. The same sentiment was expressed by representatives of the British Steel Corporation, where, although there is rough equality between IBM and ICL, individual divisions tend to be committed to one or the other.

BSC, which appeared first, told the subcommittee of its plans to reduce the number of computers on its books by replacing them with IBM 360/40's and 360/50's. Within minutes of the changeover of places with the ICL team, however, Mr Eric Lubbock had persuaded Mr Steward to say how surprising it would be for a company to concentrate on these machines. To be fair, the BSC representatives were talking of their IBM committed divisions, beginning with the South Wales group, but if the subcommittee was looking for hints on how to run a computer installation it was clearly in for a thin time.

The representatives from BSC, led by Mr N. C. Pollock, director of management services, also drew some critical comparisons between IBM and ICL. British companies have been less reliable than IBM in the delivery of software, and the ICL business machines used by the corporation seem to break down more often (although it was not clear whether the one per cent difference in serviceability index between IBM and ICL is significant). BSC also alleged that ICL is being unnecessarily stringent in its restrictions on the competitive tapes and disks which can be used on ICL machines.

But neither team could help a great deal when it came to defining the government's present policy, although BSC thought the policy was more protectionist than statements from Mintech indicate, or deciding what government action would help computer users to break the vicious circle of dependence on IBM.

POLLUTION

Beware of the Drinking Water

Britain's water supplies could become contaminated with unusual toxic chemicals which would not show up in the routine tests conducted by water authorities. That is the conclusion of a report published last week by the Institution of Water Engineers (evidence submitted to the Central Advisory Water Committee, 10*s*), which also proposes sweeping changes in the organization of water resources.

The institution points out that chemicals of unknown toxicity are being poured into rivers and may end up in the water supplies. But water authorities are also faced with the possibility that illicit dumping and unexpected events, such as the accidental leakage of chemicals and oil from road tankers, could result in serious contamination. During the last five years 312 cases of source waters being polluted have been reported by 140 authorities. The institution estimates that about 25 per cent of Britain's water supplies are drawn from polluted waters and another 25 per cent come from rivers which are liable to sudden, unexpected pollution. The report concludes that "safety margins are becoming too narrow in relation to the increased use of frequently or heavily polluted waters for public supply sources".

The present method for dealing with unexpected pollution is that the appropriate authority is immediately notified, and steps are taken to shut off supply from the affected source. Such procedures are inadequate, the institution says, and although there can be no foolproof way of ensuring that unexpected pollutants do not enter the supply system, there is a need for more scientists, using modern laboratory equipment, to monitor the risks.

The institution is also strongly critical of the Redcliffe-Maud proposals for water supply and sewerage. It says that these "might well act adversely on the efficiency of that service and the safety of supplies", and proposes instead that authorities should be set up charged with triple responsibility for rivers, water supply and sewage disposal. Fifteen such authorities would control regional water supplies and drainage, and a Central Water and Drainage Authority would be responsible to the Minister of Housing and Local Government. The regional boards would take over the functions of the present river authorities, water undertakings and sewage disposal boards, together with the responsibilities of local authorities for sewage. The Central Board would take over the functions of the present Water Resources Board, but it would also have responsibilities for water supply and sewage disposal.

THIRD LONDON AIRPORT

Unfairness to Foulness

by our Planning Correspondent

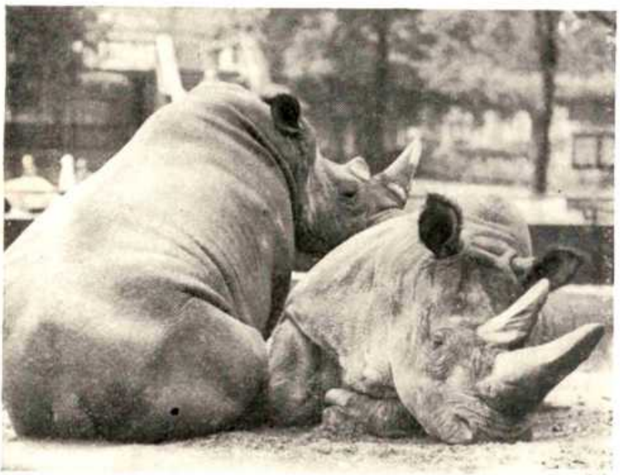
The Roskill Commission, which is looking into the siting of the third London airport, has ignored the regional advantages of siting the airport at Foulness, according to the independent research organization, Political and Economic Planning (PEP). Mr Ray Thomas and Miss Anne Whalley, who prepared the PEP evidence, say that the Roskill Commission has failed to make a full comparison of costs and benefits relevant to each of the four short-listed sites—Foulness in Essex, Nuthampstead in Hertfordshire, Cublington in Buckinghamshire and Thurleigh in Bedfordshire.

When the commuting and migration patterns of people within the four sub-regions are taken into account, the real choice before the commission, if it takes a regional point of view, is between a third London airport at Foulness, and, at the other extreme, a new national airport at Thurleigh. Cublington and Nuthampstead occupy intermediate positions. Differences in the travelling costs incurred by airport users—critical items in the Roskill Commission's study—are related to these differences in function. If Foulness were London's third airport, for example, the commission should consider the development of Elmdon or Castle Donington as an airport for the Midlands and, in such circumstances, the commission would need to reconsider airport users' travel costs.

At present, the PEP report says, the Foulness region is the most extensive dormitory area in Britain—more than 45,000 people travel daily from there to work in London—and it has an employment deficit of some 50,000 jobs. London, on the other hand, has a surplus of some 400,000 jobs. It follows that if London's third airport went to Foulness, tens of thousands of people would be given an opportunity to live nearer their place of work. For this and other reasons, most of the local authorities in Essex favour the siting of the airport at Foulness, whereas at the three inland sites there has been widespread opposition from the authorities to the siting of the airport in their area.

PEP cannot say which of the four sites is the best—it urges further research on the estimates made for critical cost–benefit items. It does, however, attempt to estimate the benefits which would accrue to the region where the airport is situated, taking into account changes in commuting patterns and the probable reductions in the levels of unemployment and under-employment. When these benefits are added up, Foulness is favoured over the other three sites by a capital sum of more than £100 million at 1968 prices.

Further sums have been calculated by the South-East Economic Planning Council which reported to the Roskill Commission last Monday. The council believes that the choice of Foulness as the site for London's third airport would cost the British taxpayer £35 million less in capital costs than would inland sites. The council's decision in favour of the coastal site, taken after a study of the regional and economic implications of a new airport there, follows the PEP and other bodies in recommending Foulness. Altogether, the evidence should provide the commission with an argument which has the air of impregnability.



Twenty white rhinoceroses are coming to Whipsnade Zoo from the Hluhluwe reserve in Natal to make up the first European breeding herd. They are of the southern square-lipped species (*Diceros s. simus*) which until recently was in danger of becoming extinct but has now joined the select number of conservation success stories. By 1966, according to the International Union for the Conservation of Nature, there were about 800 southern square-lipped rhinoceroses in the Hluhluwe and Umfolozi reserves in Natal, and about 150 more in national parks in southern Africa.

POLLUTION

Knock at Lead

Recent announcements of lead-free petrol have been prompted not because of fears that lead is a hazard to health but because it shortens the life of the catalysts used to treat automobile exhaust fumes (see *Nature*, **225**, 990; 1970). The catalytic treaters oxidize such compounds as waste hydrocarbons, carbon monoxide, and oxides of nitrogen, which are more serious pollutants, at least in the first degree, than is the tetraethyl lead that is added to petrol to inhibit knocking. Concerning the health hazard presented by lead, the Shell News Service, for example, says "at present levels there is no danger to health in the use of lead in gasoline". This view is contested in a Swedish report now published in English which concludes that "the safety margin between the present lead absorption for a person living in the city and that absorption which gives rise to chronic damage in man is apparently very small, possibly completely non-existent" (*Gasoline Containing Lead*, Lennart Danielson, Natural Science Research Council, Stockholm, S.kr.10).

Prepared for a Swedish Royal Commission on Natural Resources, the report seems to have encouraged Sweden to limit the amount of lead in petrol to 0·7 μg per litre from January 1 this year. Although this is a lenient limit that nicely corresponds to the average amount of lead already added to petrol in Sweden, there is talk of reducing the level in future.

According to the report, the amount of lead in the atmosphere—typically about 3 μg per cubic metre for a city such as Stockholm—is keeping step with the increase in the number of motor cars. As an extreme example, Danielson cites a single measurement of 71·3 μg per cubic metre on the Hollywood Freeway on a Saturday, and about 25 μg per cubic metre seems a typical value for rush hour in Los Angeles. The question is how much of this material, which is chiefly in the form of micron-sized particles that can remain suspended for about a month, is absorbed by man. Danielson claims that about half of the inhaled lead is absorbed. But the absorption of lead from food is already close to his estimate of the maximum permissible daily dose of about 5–10 μg of lead, which means that there is a good case for reducing the contribution of lead from petrol to nil. The adsorption of lead from inhaled air is estimated by Danielson as about 22 μg per day for a person breathing a concentration of 3 μg per cubic metre.

Danielson also finds fault with the conclusions of Dr R. A. Kehoe, formerly medical consultant to the Ethyl Corporation which pioneered the introduction of leaded petrol. Dr Kehoe carried out a series of experiments on the prolonged consumption of lead which seemed to indicate a safety value of 80 μg lead per 100 g of blood. (An average concentration for city dwellers is about 20 μg lead per 100 g blood.) Danielson disputes this safety value and also criticizes experiments in which Kehoe claimed to show that the lead pollution of city air is harmless.

Danielson recommends that more research should be undertaken on the accumulation of lead in the biosphere, and that the amount of lead allowed in petrol should be reduced to nothing, the phasing out taking place over five years so as to allow for the introduction of engines with low compression ratios. Essentially this is the policy which Sweden seems likely to adopt, and other countries may follow suit though for different reasons.

MEDICINE

Neutrons to treat Tumours

The first neutron generators designed to treat cancer will soon be installed at the Christie Hospital in Manchester and in the Glasgow Institute of Radiotherapy at the Belvidere Hospital. Neutron therapy may have advantages over conventional radiation therapy in treating tumours, but the difficulty in developing the technique has been to find a powerful enough source of fast neutrons. The new generating tubes may provide an effective source for routine neutron irradiation.

A prototype of the machine has been in operation in Manchester since last summer, and doctors at the Christie Hospital hope that it will be used to treat patients within the next month or so. Two commercial versions of the machine, made by Elliott Automation, will be installed in Glasgow and Manchester within the next few months. Dr J. M. A. Lenihan from the Glasgow Institute estimates that he and his staff will begin treating patients during 1971.

The neutron generating tubes, the most powerful of their kind in the world, were developed at the Services Electronics Research Laboratory, Baldock. A beam of fast, 14 MeV, neutrons is generated in a sealed tube by the deuterium–tritium reaction, and by an ingenious selection of the target material the design team has succeeded in pushing the output of the tube up to 10^{12} neutrons per second. This is high enough to be able to use the beam for therapy without unduly long treatment times, although even higher outputs would be preferable. The designers hope that they will be able to get close to the theoretical maximum of three times this figure by improving the target design.

Neutrons are already being used for cancer therapy at Hammersmith Hospital where doctors in the Medical Research Council's cyclotron unit are using neutrons generated in the cyclotron to treat patients with particularly advanced tumours. Some encouraging results have been obtained, but it is still too early to say whether the sensitivity of poorly oxygenated tumour cells to neutron radiation offers a distinct clinical advantage over X-ray or γ-ray treatment. The Hammersmith doctors are now planning a carefully controlled clinical investigation of the technique on a larger group of specially selected patients.

One difficulty in using a cyclotron to generate the neutrons is that the equipment is too expensive for most radiotherapy departments. But as Professor J. F. Fowler, at Hammersmith Hospital, pointed out, part of the cost of a cyclotron, which can be up to £0·3 million, can be borne by the other uses to which it can be put. The advantage of the new neutron therapy machine is that the generating tube is movable and also cheaper than a cyclotron—the whole installation costs about £150,000 and the replaceable tubes, which have an operating life of some 200 hours, cost only £3,000.

ARCHAEOLOGY

Real Maths

Archaeologists and mathematicians will have a fresh chance next autumn to discover how much they can help one another. The Royal Society has arranged with the Academy of the Socialist Republic of Romania to hold a conference in Bucharest on the role of mathematics in the archaeological and historical sciences, opening on September 16. Professor D. G. Kendall, director of the Statistical Laboratory at the University of Cambridge, is convinced that the conference will be "something of a novelty", not least in that it will put forward a hard sell for "real mathematics and not just arithmetic".

Fortifying archaeology with the powers of statistics in particular has a respectable history during the past decade or so, but some archaeologists seem to have retained a suspicion of mathematics. Some have a methodological bone to pick with the "statniks", to use the terminology of Jacquetta Hawkes (*Antiquity*, **42**, 255; 1968). More often, however, the problem is that some archaeologists feel out of their depth in a sea of statistics, and the view of the conference's scientific secretary, Dr F. R. Hodson, is that the Bucharest conference will help them find their feet.

The kind of "real mathematics" that can be brought to bear on archaeological problems is illustrated by Kendall's method of seriation which can be used, for example, to reconstruct the chronology of a group of tombs from stylistic variations in the pottery they contain. The Bucharest conference should pinpoint other mathematical tools for which there is an archaeological demand—cluster analysis perhaps—as well as exploring other potential applications in fields such as historical demography. Full details of the conference can be obtained from Dr F. R. Hodson at the Institute of Archaeology, 31–34 Gordon Square, London WC1.

ROYAL SOCIETY

Admissions to Carlton House Terrace

The Royal Society announced last week the batch of 32 people elected annually to join its select body. This year the 700 fellows of the society proposed some 350 candidates, the roll of which was decimated by the twelve specialist committees that mull over the candidates' claim to scientific excellence and somehow juggle their respective quotas to fill the thirty-two places. The survivors of this year's initiation rites, with one of their chief fields of interest, are as follows: C. C. Addison (University of Nottingham)—inorganic chemistry; F. F. Bonsall (University of Edinburgh)—Banach algebras; C. C. Butler (Rothamsted Experimental Station)—honey bees; R. Casey (Geological Survey, London)—Mesozoic stratigraphy and palaeontology; C. A. Clarke (University of Liverpool)—haemolytic disease in newborn infants.

J. F. Coales (University of Cambridge)—control systems; A. E. Douglas (National Research Council of Canada, Ottawa)—spectroscopy; C. Eaborn (University of Sussex)—organo-silicon compounds; I. M. Glynn (University of Cambridge)—cell membranes; J. Heslop-Harrison (University of Wisconsin)—control of sex in flowering plants; J. M. Hirst (Rothamsted Experimental Station)—control of plant diseases.

V. M. Ingram (Massachusetts Institute of Technology)—sickle-cell haemoglobin; J. C. Jaeger (Australian National University, Canberra)—rock mechanics; J. L. Jinks (University of Birmingham)—genetics of animal behaviour; B. D. Josephson (University of Cambridge)—the Josephson effect; O. A. Kerensky (Freeman, Fox and Partners, London)—design of bridges; L. F. La Cour (John Innes Institute, Norwich)—chromosome structure.

N. B. Marshall (British Museum (Natural History))—ichthyology; M. G. K. Menon (Tata Institute of Fundamental Research, Bombay)—K-mesons; R. Miledi (University College, London)—synaptic transmission; J. F. A. P. Miller (Walter and Eliza Hall Institute of Medical Research, Melbourne)—thymus and immunity; S. M. Partridge (Meat Research Institute, Bristol)—protein structure; J. S. Rowlinson (Imperial College, London)—thermodynamics of fluids.

A. E. Russell (British Aircraft Corporation, Bristol)—design of aircraft; F. G. Smith (University of Manchester)—magnetic field of the galaxy; R. W. Stewart (University of British Columbia, Vancouver)—boundary layers of ocean and atmosphere; J. B. Taylor (UKAEA's Culham Laboratory)—plasma physics; D. A. J. Tyrrell (MRC's Common Cold Unit, Salisbury)—the common cold; E. J. Underwood (University of Western Australia, Perth)—animal nutrition.

J. P. Wild (CSIRO, Sydney)—radio noise bursts from the Sun; C. G. Wynne (Imperial College, London)—optical instruments; V. C. Wynne-Edwards (University of Aberdeen)—ecology of birds.

KIDNEY PATIENTS

Demand for Machines and Organs

SOME two thousand five hundred kidney patients in England and Wales die each year because they cannot find places in renal dialysis units. In countries such as Sweden, most people who would benefit from dialysis receive it. Why not in Britain ?

Haemodialysis was first introduced as a routine treatment for kidney failure about five years ago, and there are now more than five hundred patients on dialysis in hospitals and nearly four hundred receiving dialysis at home (see page 1184). The Department of Health and Social Security (DHSS) says the chief difficulty in increasing these numbers is the shortage of trained staff needed to man the hospital units. According to Dr A. J. Wing of St Thomas's Hospital, London, the difficulty with home dialysis is the time taken by local authorities in making the necessary structural alterations to the houses, as well as the time needed to train patients in the use of their own units.

The cost of the treatment is also an important factor. The DHSS estimates that with twice-weekly dialysis, it costs about £2,000 a year to maintain a patient on dialysis in hospital, and £1,500 at his home. The capital costs of the electrical monitoring and fractionating equipment for each machine are between £1,500 and £2,000 and the widely used Kiil filters, in which the waste products are removed from the blood, cost about £400. Each filter has a life of about three years and in most hospitals is used by one patient only, so as to reduce the risks of transferring viral infections such as hepatitis. A safe but more expensive type of filter is a disposable parallel plate dialyser being marketed in Britain this month by the Swedish company Aga. Each filter costs £12 and is used for one dialysis only.

The existing dialysis centres were for the most part established by direct grants from the DHSS, but hospitals now have to find the money for maintaining and expanding their dialysis programmes from their block grants. In most hospitals, the emphasis at the moment seems to be on increasing the number of patients on home dialysis. Besides the psychological benefit, the advantages of home dialysis are its cheapness and the fact that patients can be dialysed more often. The DHSS seems to be cautiously encouraging the expansion of home dialysis programmes but unwilling to over-commit itself for fear that the increasing success of kidney transplantation as a form of treatment may in time make some of the expensive dialysis units redundant.

At present about 100 transplants are performed each year in Britain. A serious limitation is the number of donor kidneys available and this, according to Professor Shackman at Hammersmith Hospital, has arisen more from a lack of cooperation in the medical profession than reluctance on the part of the public to donate their kidneys. The other problem is to find the most suitable recipient for any kidney that does become available; a transplant is noticeably more successful the closer the tissue match between the donor and the recipient. Worldwide four-year survival rates, for example, are 94 per cent for live exchanges between matched siblings and 40 per cent between unmatched siblings; the latest two-year survival figures are 75 per cent using matched kidneys from unrelated dead donors, and 30 per cent with unmatched kidneys. Techniques for tissue typing have been steadily improving and now a total of twenty histocompatibility lymphocyte antigens have been identified and may be used for the purposes of matching. Kidneys are generally regarded as well matched if not more than two of these antigens differ between donor and recipient. Doctors at the London Hospital Transplant Immunology Centre estimate that a pool of 500 possible recipients is necessary to ensure that each donor kidney is well matched.

One way of organizing such a pool is for hospitals to cooperate in a computer network in which the tissue types and blood groups of all possible recipients are registered. Fourteen hospitals, mostly in London, are already involved in such a scheme and have a group of 120 patients waiting for transplants. In establishing such a system, another factor to be taken into account is the time needed to tissue-type and transport donor kidneys.

The question at the moment is whether there should be nation-wide cooperation along the lines of the London scheme, or whether, bearing in mind the problems of transport, a series of regional schemes would be preferable. The transplantation committee of the DHSS under the chairmanship of Sir Hedley Atkins, the former president of the Royal College of Surgeons, has been looking into this, and its recommendations are expected shortly.

Parliament in Britain

Kidney Machines

In January this year, 278 hospitals were equipped with kidney machines capable of treating a total of 509 patients and another 378 patients had installations in their homes. The exact number of people who require this type of treatment is not known. This information, which was given by Baroness Serota, Minister of State, Department of Health and Social Security, will, according to Lord Auckland, give only limited satisfaction because too few kidney machines are available in regional hospitals. Lord Auckland said that the large amount of regional development, and the growing numbers of people moving out of London, constitute a strong case for making more of these units available outside London. Baroness Serota replied that in 1965 there were only a handful of small renal dialysis research units, but in January this year there were thirty-one major units in England, six in Scotland, and one in Wales. Two more units have started work since then, five are being extended and three more are planned, she said.

Baroness Brooke of Ystradfellte also asked how many kidney machines are not being used because of a lack of trained manpower to look after them. Baroness Serota had, however, no figures either for the shortage of manpower or for the number of machines which are not being used. (Oral answers, Lords, March 17.)

Science Research Council

The alleged bias of the Science Research Council against Scotland has received the attention of Mr Ross, Secretary of State for Scotland, and Mr Short, Secretary of State for Education and Science. Mr Ross told Mrs Winifred Ewing that both ministers are concerned that Scottish interests are taken into account in questions of this kind, and that the number of Scottish academics on the Science Research Council boards has been increased from two to four. (Written answers, March 19.)

Uranium Supplies

Dr Ernest A. Davies, Joint Parliamentary Secretary, Ministry of Technology, said that the Atomic Energy Authority is confident that the intense exploration for uranium now being undertaken in many countries will result in sufficient uranium being available at reasonable prices for the UK programme. He also said that uranium reconnaissance in selected areas of the UK has been sponsored by the AEA, through the agency of the Institute of Geological Sciences. The latest programme began in March 1968 and is continuing. Mr Alexander Eadie had asked whether the Minister of Technology is satisfied that sufficient supplies of uranium will be available, in view of the shortage of indigenous resources envisaged by the European Economic Community. (Written answers, March 16.)

Nuclear Powered Ships

The government has no proposals at present for the production of a nuclear powered merchant ship, but representations from interested parties are still being studied, Dr Ernest Davies said in reply to questions from Mr Edwin Brooks. Dr Davies pointed out that although the situation has altered in a technical sense over the last few years, the chief concern is whether there would be a market for such vessels. (Written answers, March 16.)

Miscellaneous Intelligence

M. le secretaire perpetuel of the Royal Academy of Sciences, Letters and Arts of Belgium has announced that his functions as perpetual secretary came to a close at the end of last year.

The cyclamate farce has romped its way through many curious interludes, but what must surely be the last scene of all is the announcement that saccharin, too, is under suspicion of causing bladder tumours. The rats that got cyclamate banned, it will be remembered, were, of course, fed not with cyclamate but a combination of cyclamate and saccharin. With the unerring certainty of a water diviner, the United States Department of Health Education and Welfare spotted that the cyclamates were the villain of the pair and banned them forthwith. The ban was withdrawn in part but too late to save health authorities in other countries, such as Britain, from stampeding down the Gadarene slope. The belief that saccharin, too, is carcinogenic, voiced by G. Bryan of the University of Wisconsin in evidence to the United States Government, is all that was needed to complete the logical somersault into a Wonderland that even Alice never dreamed of.

Found in a junk-shop in Greenwich, London: a copy of *A Classification of Living Animals* by Lord Rothschild, inscribed by the author, "This is an exceedingly boring book". These modest words belie its opening sentence, "During my studies of spermatozoa, I have often been frustrated by having to consult a number of books. . . . " which, for a catalogue, has a rare sparkle.

An advertisement for American scientists to take up jobs in Britain would "attract about 10,000 replies", according to Mr Harry Hoff, the chairman of the selection board that tours North America on behalf of the Scientific Civil Service to recruit British scientists. If this is the epitaph of that ever-interesting topic, the brain drain, perhaps Mr Hoff's should be "Is his the face that lured ten thousand home, And spurned the topmost towers of IBM ?"

A hand-out from the United States Department of Commerce begins with the arresting words, "As civilization continues to contaminate the atmosphere. . . ." Were the ideals of the noble savage ever so succinctly, if unwittingly, expressed ? If the Department of Commerce continues to use words without thought, it cannot but hasten the return of the good old days when barbarism kept the Earth pure.

Also in the best Doomsday spirit is a press notice from the US Department of Commerce that begins with the arresting declaration: "Lightning, the biggest killer in nature's arsenal . . .".

A blatant invitation to spendthrifts is held out in a current issue of *Developmental Psychology* (**2**, 154; 1970). Dangled in a footnote to an article entitled "Birth Order and Avoidance of Dangerous Activities" is the insidious alternative, "An extended report of this study may be obtained without charge from (the author) or for a fee from the National Auxiliary Publication Service".

NEW WORLD

More Grumbling about Mansfield

SENATOR MANSFIELD has more to answer for as the days go by. The authorization hearing of the National Science Foundation in the House of Representatives finished last week (on March 19) with a recitation by four academic scientists of the ways in which useful projects would be harmed by the requirement that in future the Department of Defense must not support basic research unless this has "a direct or apparent relationship to a specific military function or operation". The fear that the National Science Foundation will not have the funds to take care of the projects dropped by the military departments—one speaker last week estimated that between $30 and $50 million extra would be needed—has been a constant theme in the hearings this year (see *Nature*, **225**, 785; 1970). This point has also been made in a special report to the subcommittee (*Mission Agency Support of Basic Research* dated February 25) by its Research Management Advisory Panel, a group of outside advisers. Everybody is clearly somewhat at a loss to know how the Administration has come to make such a muddle of the transfer of basic research from the military to the civil payroll. At least one member of Mr Daddario's subcommittee blames Congress as well. Representative Charles A. Mosher said at the hearings last week that "under the present arrangements, we cannot expect Congress to know its own mind. It's a sad indictment, I know, but it's the truth".

At several points last week, members of the subcommittee and their witnesses echoed each other's conviction that the implications of the new restriction on the research policy of the Department of Defense cannot have been fully appreciated when, last December, Senator Mansfield was able to attach Section 203 to the defence appropriation bill for the current financial year. Mr Daddario was concerned that the lack of a formal mechanism for the transfer of responsibilities for basic research from the military to civilian agencies might mean "a lost generation of scientific manpower". Dr Ivan L. Bennett, director of the New York University Medical Center and a member of the Research Management Advisory Panel, told the subcommittee last week that "for various reasons, it has not been possible within the executive branch to effect the transfer in a coordinated manner".

Dr Bennett provided a calculation of how much money would be needed for the National Science Foundation to be able to take over the projects that the Department of Defense had already dropped from its basic research programme. The reduction of the Department of Defense budget allocation for research in universities from $247 million in the current financial year to $215 million in that beginning in July this year implied, he said, that at least $32 million a year would be needed to keep the universities in a style to which the Department of Defense had accustomed them. But this calculation takes no account of the projects being dropped by other agencies—the University Sustaining Program which NASA is this year having to abandon. The total cost might be $50 million. Dr Bennett argued that the National Science Foundation is not provided in the new budget with enough money to take over these funds—Dr William McElroy has talked of an extra $10 million in the basic research programme for drop-out projects (see *Nature*, **225**, **785**; 1970). There is also the fear that the Department of Defense may be unable to defend all of its present budget for university research against Congressmen who are moved by the spirit of the Mansfield amendment in the defence authorization hearings now under way.

Some of the practical effects of the cuts which have already been made were described to the committee last week by Dr B. Lax, director of the Francis Bitter National Magnet Laboratory at MIT. The laboratory was set up in 1960, chiefly by the US Air Force, and is an outgrowth of the Lincoln Laboratories. Professor Lax described his laboratory as a unique facility which served not only as a mission-oriented research laboratory, chiefly in military electronics, but also as an academic laboratory used by more than 100 visiting scientists each year and contributing to teaching as well. Professor Lax explained that the financial pressures of the past few years had already forced the laboratory to trim its sails—the research staff has been reduced from 27 to 19. But "section 203 also declared the magnet programme irrelevant to the work of the Air Force" and, "we shall have to make very drastic cuts in the next few months".

The Magnet Laboratory has been trying to find support for some of its work from organizations like the National Institutes of Health, but "promising projects have run up against a brick wall". The result, Professor Lax said, is that the laboratory is now operating at one eighth of its full capacity. He was afraid of what will happen now that the laboratory could not count on being able to build the advanced equipment which it had designed. "Unless the National Science Foundation takes on the responsibility for the facility and supports our version of such an advanced project, we shall within two or three years relinquish the leadership we have worked so hard to attain."

The Haystack facility at MIT, which has contributed in important ways to radioastronomy in the United States, is also threatened by the impending cuts. Professor Lax said that "unless relatively modest support for this operation is found, this superb instrument will be irretrievably lost to the academic community". If the economy drive prevents the construction of new radio telescopes elsewhere, at least it should be possible to make the best use of the facilities which do exist. But MIT will also suffer from the way in which the research budget of NASA's Space Science Center at MIT, an interdisciplinary laboratory, has been "cut from $1,000,000 to zero in 1972".

Interdisciplinary laboratories are especially at risk in the present climate, according to Professor Lax, who pointed last week to the irony that these are the kinds of laboratories which need to be encouraged if science is to be applied successfully to social problems.

But academic science also stimulates industrial innovation and prosperity, and MIT has Route 128 around Boston to prove it. But "if this formula was so successful, why are we abandoning it ? . . . Science and technology are in retreat, not only in the universities but in industry and in the country as a whole".

The effects on the cuts on the balance between graduate and undergraduate education were the chief concern of Dr Charles L. Hosler, who also provided the most elaborate oratory last week. He said that the proportion of undergraduates staying on for graduate schools was already declining, even though it was more than ever clear that "much of our BS training has become superficial . . . and there is not enough time in the four-year program to give a student the breadth and depth that many of today's programs require". In Dr Hosler's experience BS graduates in the United States are now being diverted from the graduate schools by the attractive starting salaries which they are offered in industry. Like other speakers, he was anxious that there should be enough highly trained people "to solve tomorrow's unknown problems". For the benefit of those who are more directly concerned with simple profit and loss, he produced a calculation that the extra $100,000 which a PhD graduate could expect to earn in the course of his working life would yield an extra $20,000 in taxes, more than enough to pay for the cost of a graduate school stipend.

President Nixon's campaign statement that "scientific research cannot be turned off and on like a faucet" was thrown back last week by Dr Thomas Jones, president of the University of South Carolina, who feared that the effects of the Mansfield amendment would be especially severe for universities not in the first flight of academic research institutions. Surely the Federal Government, which some years ago was urging universities like his own to develop new graduate schools, now had a duty to continue to provide for them. In the event, at times like the present when institutions are competing for diminishing funds, "the ripples spread outwards as the prestigious institutions" shoulder aside their weaker fellows. One result had been that in the University of South Carolina, the amount of new research grant support for 1970 will be only a half that obtained in 1969.

Dr Davies also regretted the way in which the first report of the National Science Board, which in 1969 urged that graduate education should be accepted as a federal responsibility, "has received so little attention". He thought it essential that graduate education should be widely spread across the United States, with at least one strong centre of scientific research in every state. The alternative methods for financing graduate schools spelled out by the National Science Board deserved consideration.

The word "catastrophe" was used on several occasions during the session last week to describe the consequences of the impending shortage of money for basic research. Several witnesses urged that the rate at which grants are reduced should at least be matched to the time-scale of forward planning in the universities. The chairman of the subcommittee, Mr Daddario, said that arranging that universities could safely plan ahead is largely "an institutional problem". The season of talk about the creation of new agencies is evidently about to be reopened.

Consequences of Mansfield

THE report of the Research Management Advisory Panel (*Mission Agency Support of Basic Research*) is a brief but forceful document. "The colleges and universities of the United States are an essential national resource, central to stability, economic progress and security" and "the present science establishment of the United States is unique". But "most universities in the United States are experiencing severe financial strains which threaten their very existence as economically sound national resources in the sciences". Only the Federal Government can sustain academic science. "National policy has recognized the justification for Federal Government support of science for the past 25 years. At the heart of this crisis in our universities is academic science, both research and training. These are in jeopardy."

The report goes on to list the pressures on academic research—larger enrolments, increasingly expensive equipment and facilities, inflation, the development of interdisciplinary programmes and the involvement of scholars in several kinds of public service. Yet federal spending on academic science has not kept pace with inflation and "we enter the 1970s with a greater need for scientific knowledge but faltering support for scientific research".

The panel is alarmed because this trend threatens to undermine "our recently won leadership in science". . . . Government support for university research has become unstable and unpredictable. It is actually decreasing in several fields of science and is now inadequate in virtually all scientific disciplines. The Federal Government . . . is now treating university basic science as a luxury it can no longer afford . . . the always precarious balance of university financial needs and funding support for science is being destroyed. The universities must be, and are, willing to accept budget constraints, but "recent abrupt actions of the Federal Government have set arbitrary ceilings, cut appropriations for the National Science Foundation and redesignated the criteria under which Department of Defense appropriations may be spent. The results of these actions have been most serious. University administrators are unable to maintain coherent programs in the face of sudden changes in Government practice, for which there has been little warning".

The report goes on to say that the Mansfield amendment is "potentially disruptive" and urges that the "Federal Government carefully consider the disruption that an overly restrictive interpretation . . . would have on the close contacts of those who work in applied science and engineering". The panel also draws attention to the way in which the new developments put in hazard the "historic national policy that each mission department and agency which draws upon science in its operation should support its proper share of the country's research".

COMMUNICATIONS

Arrivederci Intelsat

WILL there be an international satellite treaty in 1970? Perhaps, perhaps not. The Intelsat conference to write a permanent agreement for the world's communications satellite network broke up on Friday in a brave and hopeful mood but without an agreement. It will have to meet again. The third of these huge unwieldy and expensive conferences had produced enough accord to provide about half a treaty.

The United States had finally agreed to let the organization which it had founded in 1964 be managed by an international directorate, rather than by the semi-public American company Comsat. And Zambia joined Intelsat. When an organization can recruit several new members—Yugoslavia was another—while it is still building itself, it cannot be too sickly.

There are some serious disputes still to be worked out before the full membership meets again. The small nations of Africa and the Middle East are passionate to have Intelsat governed by a powerful assembly in which the representatives of governments, not of telecommunications entities, would have a vote, one to a country, all countries equal. Needless to say, the Americans oppose this, but the smaller nations have the sympathy of the British and European bloc which believes that the assembly should be given teeth, even if it is prevented from biting. To sort out this and other contentious issues, Intelsat will set an intersessional committee to work. It will meet in May, possibly in Washington, possibly in Guatemala City. Yet even that decision is being subjected to a kind of small nation–big nation tug of war.

BUREAU OF STANDARDS

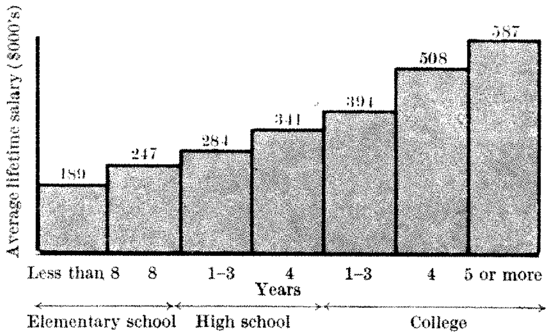
Boom and Diversity

THE National Bureau of Standards is one of the few scientific institutions in the United States which is clearly destined to grow in the next few years. It is also likely to find itself engaged in several new kinds of projects which, in time, are likely to bring about a shift of emphasis from the traditional preoccupations of standards laboratories with measurement, standardization and compilation to more general responsibilities for the technical aspects of consumer protection and the evolution of government policy towards industrial technology. The way in which the wind is blowing can be seen clearly enough in the annual report of the bureau for 1969, now published (US Government Printing Office, C13/1 : 325, $1.25). The changes which are afoot have the enthusiastic support of the director of the bureau since September 1969, Dr Lewis M. Branscomb.

Some of the bureau's new work, however welcome, has been thrust on it from outside. Thus the bureau has become the instrument in the United States for the development of technical standards of flammability of the kind necessary to provide protection for innocent consumers under the Flammable Fabrics Act. The bureau seems already to have identified the kinds of fabrics for which new standards will be needed, and is at work on the development of test methods and sampling techniques. Evidently it is going to become the fountainhead of wisdom in the regulation of flammability standards for the consumer market. In much the same spirit, the bureau has also been given responsibility for safety in the design of automobiles, and has in the past year been hard at work on the development not merely of standards of performance for tyres but on methods of conveying such information to the ultimate consumers. In the past few months, the bureau has also been given responsibility under the Fire Research and Safety Act for the development of methods of fire protection and of standards for making sure that these are properly applied, although at the spanking new headquarters at Gaithersburg, thirty miles into Maryland from Washington, there is a tinge of regret that on this occasion Congress has willed the ends but not the means.

The National Bureau of Standards is also now the organization responsible for making a technical study of the "advantages and disadvantages of increased use of the metric system in the United States". If there is ever to be a change to the SI system, the National Bureau of Standards will provide the starting point—and has already worked out rules for the use of SI units in its own scientific publications. The bureau's annual report says that the Metric Study is now confident of being able to provide "reasonable estimates" of the cost of a changeover to the metric system in several industries of the United States. The report says that the study is starting from the basis "of looking at potential costs as they would accrue if metric measurements were to be applied to new or redesigned products, stressing the concept of planning a transition . . . at an optimal rate, without going back and changing all the drawings that are in the files". This procedure is intended to avoid the "exaggerations and astronomical costs" which come from a "doctrinaire approach based on an all or nothing plunge into metric measures". The report goes on to explain that a changeover would be more practicable in industries where innovation is rapid than in those where equipment remains substantially unchanged for long periods of time, and cites the railroad and oilfield industries as ones in which "changes in existing equipment do not appear to be warranted". One of the jobs now under way is the development of a questionnaire for the collection of information about the needs of particular industries. On the face of things, at least, it looks a little as if the National Bureau of Standards, and the bodies such as the American National Standards Institute with which it is working closely, will recommend a kind of patchwork of standardization.

Money Value of Education



NEWS AND VIEWS

The Man who Lived with Australopithecines

An eminent archaeologist once divided early classical Greek sculpture into two periods, the type for the first period being a head in a museum at Berlin and the type for the second a torso in a museum in Athens. The two periods were quickly reunited when a diligent student spotted a similarity between the two type specimens, arranged for casts to be made, and found that the Berlin head fitted on to the Athenian torso. An equally serendipitous matching, though more constructive in its effects, has been made at the Transvaal Museum in Pretoria. Mr R. J. Clarke describes (this issue, page 1219) how he was sifting through the museum's rich collection of australopithecine material (*Paranthropus robustus*) from the Swartkrans site when he noticed that parts of a cranium seemed untypical. Remembering that one of the first investigators of the site, Dr J. T. Robinson, had argued for the existence of a second, more advanced, hominid at Swartkrans, Clarke immediately wondered if the cranium might have a bearing on Robinson's case. Unfortunately Robinson had managed to assemble only a handful of fragments that he considered to belong to the second hominid, but, looking them over, Clarke discovered that a piece of upper jaw fitted exactly with the suspect piece of cranium. The two fragments together tell far more than they did apart and enough of the newly reconstructed skull is present for Clarke and his colleagues to assign it to the genus *Homo*. This confirms Robinson's original supposition that Swartkrans was inhabited both by australopithecines (the descendants, on man's side of the line, of the common ancestor of men and apes) and by a form of early man. The discovery also greatly enhances the importance of the site.

Swartkrans urgently needs to be dated before it can figure prominently on palaeontologists' maps, and the article by Dr Mary Leakey (this issue, page 1222) provides the first real clue to its chronology. Despite the distance between Swartkrans and Olduvai, she finds a "suggestive similarity" between the stone industries of the two sites. If this is taken to imply that the two sites were roughly contemporary it would mean that Swartkrans was inhabited some 1·75 million years ago.

This and other straws in the wind suggest that the Swartkrans site may become of major significance for unravelling the evolution of man, particularly the transition between the two genera, *Australopithecus* and *Homo*, which are represented there. As Dr C. K. Brain described last week (*Nature*, **225**, 1112; 1970), excavations were begun at the site in 1948 but not resumed until five years ago. In the meantime a seam of limestone that had been uncovered in the cave was spotted by a miner whose activities left the site in a chaotic state. The site had also received the attention of lime miners in the 1930s who dumped some 200 tons of fossil bearing rock on the hillside below the cave. Several important fossils, including, for example, the two hominid vertebrae described by Dr Robinson (this issue, page 1217) come from the miners' rubble. Nevertheless, enough material has been left *in situ* to indicate the original form of the cave, on the basis of which Dr Brain has ingeniously inferred the existence of a second cave, now vanished because of the erosion of the hillside, from which detritus was funnelled down into the cave below.

If the Swartkrans site turns out to be as significant as the present reports suggest, the wheel will, in a sense, have come full circle. It was at the southern African site of Taung in Botswana that Professor Raymond Dart discovered the first australopithecine in the 1920s. The southern African sites were eclipsed by the discoveries in the late 1950s at Olduvai in Tanzania and more recently attention has shifted to the finds in the Omo Valley and other sites in East Africa. The southern African sites have now been pushed back into the palaeontological limelight, but whatever future surprises they may hold, none is likely to be more felicitous than Mr Clarke's matching of the two *Homo* fragments in the Transvaal museum and its vindication of the suggestion made by Dr Robinson more than 20 years ago.

DIFFERENTIATION

Sorting Cells Out

from our Cell Biology Correspondent

Recent advances in the cell biology of differentiation have been repeatedly held up by the lack of a means to fractionate a population of differentiating cells into sub-populations at different stages of differentiation. It is only necessary to recall how molecular separation techniques have transformed biochemistry to realize the potential of a reliable cell separation technique.

In the past ten years several separation techniques designed to exploit differences in cell density have been described, but none has found widespread application, no doubt because differences in cell density are small. Cells differ much more in size, however, and in the early 1960s Mel and his colleagues tried to use this difference as a basis of cell fractionation. Their so-called STAFLO system was designed to separate cells by their sedimentation velocity through gradients under the Earth's gravitational field. But the method was bedevilled by wall effects and other flow phenomena which reduced its usefulness.

In 1967 Peterson and Evans (*Nature*, **214**, 824) described an improved sedimentation velocity apparatus which they used to fractionate bone marrow cells, but little use seems to have been made of the technique until it was taken up recently by two groups in Toronto, at the University and the Ontario Cancer Institute. These groups seem to have provided a widely applicable and easily assembled apparatus for the biologically meaningful separation of various differentiating cell populations.

Last year Miller and Phillips (*J. Cell. Physiol.*, **73**, 191; 1969) described the theory behind 1 g velocity sedimentation and showed how to make their STAPUT apparatus. They were then using it to separate a wide range of differentiating cell populations, including haemopoietic stem cells and antibody forming cells. Now the first full accounts of examples of cell separation on STAPUT gradients are appearing in print. Worton, McCulloch and Till (*J. Cell. Physiol.*, **74**, 171; 1970), for example, report the separation of two types of haemopoietic cells, which are capable of forming colonies in tissue culture, from a suspension of mouse bone marrow. McCool *et al.* (*Cell Tissue Kinet.*, **3**, 55; 1970) have separated populations of erythropoietin-sensitive cells from haemoglobin synthesizing cells in rat bone marrow. And in the latest issue of *Proceedings of the US National Academy of Science* (**65**, 192; 1970) Lam, Furrer and Bruce describe the fractionation of mouse spermatogonial cells into seven distinct populations with biologically different properties. These populations represent cells at the various stages of spermatogenesis, and by labelling the mice with tritiated thymidine before making the separation, Lam *et al.* have been able to follow some of the kinetics of the process. The fact that spermatids are separated into two fractions, early and late, both distinct from the mature spermatozoa fraction, gives some idea of the impressive resolving power of velocity sedimentation. The implications of the technique for cell biology are hard to overestimate. No doubt glass blowers in countless laboratories will be summoned hastily to assemble the Miller and Phillips apparatus in the next few months if they have not already done so.

NUCLEIC ACID

DNA in New Guises

from our Molecular Biology Correspondent

THERE is more to DNA than meets the spectrophotometer. The remarkable complexity of morphological forms found in different systems has provided a rich pasture in which a small herd of physically minded molecular biologists have browsed to no little effect. An interesting new study by Lee, Davis and Davidson (*J. Mol. Biol.*, **48**, 1; 1970) concerns the DNA of phage particles induced in certain strains of *E. coli* that are host to a prophage. The phage DNA is linear before lysis, but becomes circular on maturation, and the contour length of DNA round the circles is less by 7·5 per cent than that of the linear helices. This suggested to Lee *et al.* the presence of a previously recognized phenomenon, that of sequence repetition at the ends of the chains. This leads to certain well defined consequences if the strands of the linear DNA are separated and then allowed to renature. Because the chains terminate at various points in the repeating sequence, annealing of the mixture must lead to populations of double helices with loose single stranded ends of varying lengths. If these tails at opposite ends of the duplex are long enough, they will contain mutually complementary lengths of bases; the molecules can therefore maximize the degree of base-pairing by cyclizing to allow the ends to pair as far as they are capable. One will therefore hope to see in the electron microscope circles, containing some residual unpaired ends, where the chains terminate, and these are indeed observed as small brush-like formations at varyingly separated points on the circles.

It is possible to derive the length of the repeated terminal sequences with extraordinary precision with the electron microscope in conditions leading to extension of the single strands, using samples of φX 174 DNA on the same grid as a length standard. The lengths of the ends projecting from the circles measured in this way come out gratifyingly to 7·4 per cent. This value, however, represents an appreciable spread, thought to correspond to a real heterogeneity of length in the repeating ends. A related phenomenon that Lee *et al.* have uncovered is referred to as branch migration, and consists in the displacement of a paired end of a chain by the unpaired loose end abutting on it, which has the same sequence. In some cases, therefore, both chain ends have short loose ends emanating from the point on the duplex at which they meet, and this gives the appearance of a fork. All the indications are that the beginnings of the chains are randomly distributed around the chromosome.

The effect of intercalation of a dye, ethidium bromide, on the supercoiling of circular DNA was studied by Vinograd and his associates. They demonstrated that the insertion of the dye, which causes a change in the pitch of the primary duplex and therefore generates some slack in the chain, changes the number of turns in the supercoil in a predictable manner. Bauer and Vinograd (*J. Mol. Biol.*, **47**, 419; 1970) now show how the phenomenon can be used to derive the energetics of the supercoiling process. From the binding isotherms for the dye it is found that if the DNA is intact (though not if it contains a single-strand break) the binding free energy varies steeply with the degree of saturation. Intercalation in an "overwound" helix allows the strain to be released. As the pitch increases beyond the point at which the supercoil is fully undone, the DNA has to supercoil in the opposite sense to take up the further slack that is generated by the intercalation of more dye, and binding therefore becomes progressively less favourable. From the statistical mechanics of such a model a free energy for the supercoiling process can be extracted. This turns out to be 18 calories/base pair, and this energy can be used to assist a process such as dye intercalation which unwinds the superhelix, and must in turn be supplied if the superhelix is to be wound tighter. One of the consequences of this energy of destabilization is a diminution in the melting temperature, compared with linear DNA (or circular DNA with a single-strand break). When a few base pairs have melted and more slack is again introduced the strain vanishes, and so does the difference in melting temperature. This accounts for the asymmetrical melting curves observed in supercoiled samples.

A different case of asymmetrical melting occurs in mitochondrial DNA from several strains of yeast (Bernardi *et al.*, *J. Mol. Biol.*, **48**, 23; 1970). The mitochondrial species, by dint of new preparative methods introduced by Bernardi, are now, by comparison with earlier times, made by the hogshead; that is to say, sufficient for spectroscopic and chemical studies. The DNAs contain from 83 to 87 per cent of (A+T), which is remarkable enough in itself. In addition, the shape of the melting curves shows that there is strong compositional polarization in the chains. These curious mitochondrial DNA species are evidently to be correlated with the observed cytoplasmic mutations.

NUCLEOSIDES

Syn Expected and Unexpected

from our Biomolecular Structure Correspondent

Anti and *syn* denote the two stereochemically preferred ranges in the orientation about the covalent bond linking sugar and base in nucleosides. Most nucleosides and nucleotides crystallize with the *anti* conformation, and this is also the conformation in the DNA double helix. But the observation by Travale and Sobell (*J. Mol. Biol.*, **48**, 109; 1970) that 8-bromoadenosine and 8-bromoguanosine crystallize in the *syn* conformation is by no means unexpected, for even a crude analysis with space-filling models shows considerably more steric-hindrance between the bromine atom and the sugar for the *anti* range than for the *syn*. A much more unexpected member of the select group of nucleosides and nucleotides which crystallize in *syn* (and the only pyrimidine) is 4-thiouridine (Saenger and Scheit, *Angew. Chemie*, **8**, 139; 1969).

Uridine itself and its 5-substituted derivatives all crystallize with the *anti* conformation and the peculiarity of 4-thiouridine must be attributed to intermolecular interactions, suggesting that although there may be a considerable energy barrier between the *syn* and *anti* ranges their conformational energies are not very different. Several workers have suggested that the conformation about the glycosidic link is correlated with the pucker of the sugar ring. The sugar ring is most frequently puckered with C2 and C3 about 0·5 Å out of the best plane through the other four atoms of the ring. C2 endo indicates that C2 is out of plane on the C5 side of the sugar ring and C2 exo that it is out of plane on the O1 side and similarly C3 endo and C3 exo. Lakshminarayanan and Sasisekharan (*Biopolymers*, **8**, 475; 1969) have calculated conformational energies for purine and pyrimidine nucleosides with all four idealized sugar puckers as a function of the orientation about the sugar-base bond. They conclude that for pyrimidines with a C2 endo sugar there is an energy minimum in the *syn* angular range of comparable energy to that in the *anti* range. Although the sugar conformation in 4-thiouridine approximates to C2 endo, the observed orientation about the sugar-base bond is some 30° outside the allowed range proposed by Lakshminarayanan and Sasisekharan. This is not a serious discrepancy and can be attributed to the pucker in this structure departing from the idealized C2 endo, but it emphasizes that preferred orientations predicted from such calculations should be treated with caution.

As in deoxyguanosine (which also has the *syn* configuration), 8-bromoadenosine and 8-bromoguanosine have an intramolecular hydrogen bond between the 5′OH of the sugar and the base N3. This is the only type of intramolecular bond which has been observed in a nucleoside or nucleotide crystal from a sugar hydroxyl to either a base or a phosphate atom. Intramolecular hydrogen bonds from the 2′OH to N3 purine, O2 pyrimidine and to a phosphate oxygen have been proposed to account for observations in solution but have never been observed in the crystal.

The observation of the *syn* conformation for 4-thiouridine is of considerable interest, particularly because Scheit and Gaerter (*Biochim. Biophys. Acta*, **182**, 10; 1969) have evidence that in solution both the monomer and single stranded homopolymer assume the *anti* conformation. One of the chief stimulants to studying 4-thiouridine was its occurrence in transfer RNA and no doubt the army of model builders addicted to this molecule will be taking a new look at their creations. But it is hard to escape from the fact that the significance of the *syn* conformation in 4-thiouridine is for pyrimidine structures as a whole, which have hitherto been almost universally assumed to have the *anti* conformation in polynucleotides.

CELL CULTURES

Stable Haploid Line

from our Cell Biology Correspondent

THE fusion of plant cell protoplasts, reported a fortnight ago (*Nature*, **225**, 1016; 1970), raises none of the alarums which have come to surround similar experiments with animal cells. The next step, fusing a plant cell with an animal cell, is another story, however. Imagine the evening papers emblazoned with "Human Cabbages" or "Bacon with the Beans" and science fiction writers will have a field day populating planets with green men for whom a sunbathe is an epicurean treat.

Mercifully all that is still in the future, although the day when plant and animal cells are fused may not be so very distant to judge from the rate at which somatic cell genetics progresses. Freed and Mezger-Freed, for example, have recently isolated two stable haploid cell lines which point the way to analysis of recessive mutants (*Proc. US Nat. Acad. Sci.*, **65**, 337; 1970). These two lines arose in cultures of explants from haploid androgenetic embryos of *Rana pipiens* obtained by removing the female nucleus from the fertilized frog's eggs. For more than fifty subcultures, or at least 150 mitotic generations, these haploid cells have outgrown diploid variants, which, arising by endomitosis or cell fusion, usually overgrow haploid cells. Presumably on rare occasions, as a result of segregation, the frog sperm nucleus contains in a haploid karyotype all the genes necessary for vigorous growth in culture. Of course Freed and Mezger-Freed cannot rule out the possibility that duplications of small but crucial regions of the genome have occurred to account for the stability of their two lines, but such duplications cannot involve much of the genome. The haploid lines have almost exactly half the DNA of diploid cells, half the volume of diploid cells and the haploid chromosome number in 85 per cent of the cells in one line and 95 per cent in the other. There are, however, small but significant differences in the length of the chromosomes of the haploid cell lines compared with the standard karyotype.

Once auxotrophic mutants have been isolated, and this is apparently under way, it should be possible to exploit these two lines for mapping both dominant and recessive mutations, but the really important consequence of this work lies in the stimulus it should give to attempts to isolate haploid lines of mammalian cells. Apart from the strain of *Xenopus* that lacks nucleoli, amphibian genetics is undistinguished. What is needed is a haploid cell line from a mammalian species, such as the mouse, the genetics of which has already been worked out in some detail. The problem, of course, is to obtain a large enough population of haploid mammalian cells to give some hope of finding one cell with a genetic constitution compatible with growth in culture. One approach, being tried at Oxford, is to grow a haploid mouse embryo, but it may be technically much simpler to isolate a population of spermatids by velocity sedimentation (see page 1188) or some other cell separation technique and, with luck, establish a haploid cell line from them.

CHROMOSOMES

Chromosomes Manipulated

from our Cytogenetics Correspondent

TECHNIQUES for manipulating and changing chromosomes within living cells could provide somatic cell geneticists with more useful tools, if recent achievements with microneedles and laser beams live up to their promise.

In 1967, R. B. Nicklas and his colleagues manipulated chromosomes in living spermatocytes of the grasshopper *Melanoplus* with the aim of elucidating how they become exactly divided at mesons. Nicklas (*Chromosoma*, **21**, 1, 17; 1967) introduced a fine glass needle into the cell and pushed and pulled the chromosomes into new positions. He found that the bivalents were firmly attached to the spindle apparatus by their kinetochores; but they could be detached and then reattached to the spindle in a new orientation. The bivalent then segregated normally and the cell divided, but the resulting nuclei contained different chromosomes than would have been the case without manipulation.

Nicklas could also make bivalents behave in unusual ways. Both halves of a bivalent could be attracted to the same pole of the spindle if the kinetochores were suitably orientated by manipulation. Such a bivalent would always revert to a normal orientation, however. It seemed that bivalents with both kinetochores orientated towards the same pole (unipolar orientation) underwent reorientation because the forces of tension between bivalent and spindle necessary for a stable metaphase configuration in some way were not satisfied. To test this, Nicklas and Koch (*J. Cell Biol.*, **43**, 40; 1969) induced unipolar orientation in a metaphase bivalent, and then applied a force from a microneedle in the opposite direction to the poleward pull of the spindle fibres running to both kinetochores. Such bivalents under tension never reorientated; they only did so when the source of tension was removed. Thus it seems that a state of tension between the bivalent and spindle is required for a stable bivalent orientation that leads to orderly segregation.

Henderson and Koch have extended this observation recently (*Chromosoma*, **29**, 207; 1970). They induced two unipolar bivalents and caused them to interlock through micromanipulation, so that each exerted a force on the other. As expected, such a configuration was stable; it also resulted in the non-disjunction of both pairs of half bivalents. Such a manipulation allows the cell to bring about its own feat of genetic engineering without further intervention from the experimenter.

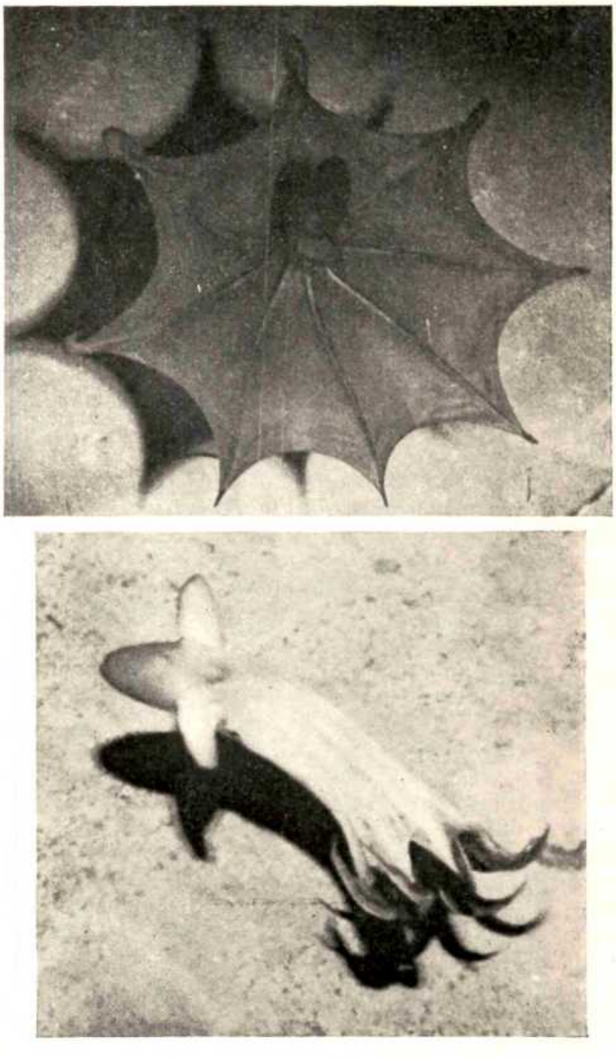
Other attempts at tinkering with chromosomes have been described by Berns and Rounds (*Sci. Amer.*, **222**, 98; 1970). Using laser beams they have been able to change, or destroy at will, segments of chromosomes in living dividing cells of salamander. One of their experiments was to destroy the secondary constriction in metaphase chromosomes—as might be expected, the daughter cells in the following interphase lacked a nucleolus. The laser beam can also remove pieces of chromosome.

This form of chromosome surgery, together with the other techniques for manipulation, would seem to offer considerable scope for influencing the structure and behaviour of genomes in somatic cells.

MARINE BIOLOGY

Strange Creatures of the Deep

THE objects in the photograph are not the open and closed version of an umbrella but deep sea octopods found at a depth of 13,000 feet off St Croix in the Virgin Islands. Scientists from the Naval Research Laboratory, Washington, photographed the creatures with a "fish", a versatile box of underwater cameras



and electronic devices, which has already helped to search for lost hydrogen bombs and the nuclear submarine Alvin. According to Dr Clyde F. E. Roper of the Division of Mollusks of the Smithsonian Institution, the creatures belong to the little known sub-order Cirrata. Further identification to family and even genus may be possible on the basis of the pictures.

INVERTEBRATES

Self Assembly of Cuticle

from our Insect Physiology Correspondent

MUCH has been learnt recently about the basic architecture of the fibrous cuticle of insects, notably by A. C. Neville and his colleagues (*Tissue and Cell*, **1**, 183, 355, 689; 1969), from the diversity in form of the pore canals. From this can be deduced the arrangement of the structural fibres of the cuticle which could not themselves be resolved.

In the layers of locust cuticle deposited during the day the microfibrils are all orientated in a preferred direction and so the pore canals run a straight vertical course through the cuticle in the form of untwisted ribbons. On the other hand, cuticle laid down during the night is lamellate: the microfibrils follow a helicoidal course changing direction 180° across the thickness of each lamella. This change in direction is imposed on the pore canals, which are then shaped like regularly twisted ribbons. They are not themselves helicoidal, as has been commonly believed. It has been known for more than a century that in Coleoptera and Hemiptera the direction of the fibre bundles in the cuticle changes 60° or 90° from one thick layer to the next. This transition has now been shown to be effected by means of a very narrow helicoidal zone of "lamellate cuticle".

Neville and Caveney (*Biol. Rev.*, **44**, 531; 1969) have applied this knowledge in a study of certain Scarabaeid beetles in which the metallic colours of the elytra are associated with the property of reflecting circularly polarized light in a manner precisely analogous to cholesteric liquid crystals. (This analogy has been pointed out particularly by Conmar Robinson, who demonstrated the same effect with systems formed from synthetic polypeptides.) Neville and Caveney have found evidence that these special interference colours in Scarabaeid beetles are determined by the helicoidal pitch of the microfibrils of the outer layers of the cuticle. The colours follow a bilaterally symmetrical pattern; but the sense of helicoidal rotation is the same all over the beetle, and is therefore bilaterally asymmetrical.

Thus the helicoid pitch seems to be under local control by the epidermal cells which secrete the cuticle; whereas the sense of rotation is presumably determined by the extracellular self-assembly or crystallization of an asymmetrical unit, comparable with the self-assembly of DNA, RNA, synthetic polypeptides and cholesterol derivatives to form helicoidal systems. Curiously enough, this system, by which asymmetrical units condense to form helicoidal microfibrils, seems to be the primitive system; it alone is found in the cuticle of the primitive Apterygota. The system with a preferred orientation, giving non-lamellate cuticle, is the more sophisticated since it requires specialized direction control by the cells. The microfibrils of the cuticle consist of protein and chitin; it is presumed to be the protein component that regulates the self-assembly into cholesteric liquid crystals, but the chemistry of the process is not known.

CANCER

Spread through the Body

from our Medical Biochemistry Correspondent

THE way that tumour cells can spread and produce tumours in other parts of the body—a process known as metastasis—is one of the most peculiar and dangerous aspects of cancer. Some recent work with heparin has shown that the progress of metastasis can be affected by changes in the surface charge of the cells. The effect of heparin on the production of metastasis was thought to be related only to its anticoagulant properties. But B. Hagmar and K. Noorby (*Intern. J. Cancer*, **5**, 72; 1970) have shown that in the concentrations normally used it interacts *in vitro* with cancer cells, and that its *in vivo* effects may sometimes result from interaction with the cell surface.

There was a small but significant reduction in the diameter of cells of the tumour MCG1-SS in suspension when they were incubated with heparin. The formation of aggregates and the viability of the cells were unaffected. There were fewer metastases in the lungs than in other parts of the body when animals received heparin before intravenous injection of tumour cells, probably due to anticoagulation effects. When cells were mixed with heparin and then injected, however, there was a significant increase in the number of metastases in the lungs, but a significant decrease in the volume of each new growth, so that the total volume of tumour in the lungs was reduced. There was also a considerable reduction in the volume of metastases in the liver.

The difference in behaviour when heparin is allowed to interact with cells before injection is probably due to effects on the electrical character of the cell surface, for chondroitin sulphate, another polyanion like heparin, had similar though smaller effects, while the polycation protamine had the opposite effect. Thus one of the factors influencing the spread of tumours and size of secondary tumours, at least for cells of this type, is the surface charge on the cell. Methods of modifying the charge might therefore reduce the spread of cancer.

In some conditions many tumours arise very quickly, and it is not always possible to tell whether the process involves several primary tumours or metastasis. Analysis of enzyme variants has shown that some multifocal tumours are derived from a single clone, while others have arisen from more than one cell and are presumably due to malignant change in several cells.

Recently Fialkow *et al.* have looked at the glucose 6-phosphate dehydrogenase isoenzymes in seven patients with Burkitt's lymphoma, who were heterozygous for this enzyme (*Lancet*, i, 384; 1970). Electrophoresis revealed two bands of activity in all normal tissues. Samples from twelve different tumours, however, all contained but one variant of glucose 6-phosphate dehydrogenase. Fialkow *et al.* point out that their results do not necessarily mean that all these tumours are caused by malignant transformations of a single cell. Nevertheless, one clone of cells does seem to become dominant in each tumour, which must be explained for an understanding of this condition.

VACCINES

Polysaccharides against Bacteria

from a Correspondent

A POLYSACCHARIDE vaccine has been used recently for successful immunization of soldiers against meningococcal disease. Polysaccharide antigens are found in many bacterial species; their ability to stimulate antibodies in man and animals is variable and has provided an abundance of problems related to animal species, dose and physical and chemical characteristics (E. A. Kabat and M. M. Mayer, *Experimental Immunochemistry*, second edition, Thomas, Springfield, Illinois, 1964). Last year the group-specific polysaccharides of *Neisseria meningitidis* were isolated by a new method (E. C. Gotschlich, T. Y. Liu and M. S. Artenstein, *J. Exp. Med.*, **129**, 1349; 1969) using the cationic detergent 'Cetavlon' to precipitate the polysaccharides from the whole bacterial culture.

The principal constituent of the group A polysaccharide is N-acetyl, O-acetyl mannosamine phosphate; the group B polysaccharide consists primarily of N-acetyl neuraminic acid; the group C polysaccharide contains N-acetyl, O-acetyl neuraminic acid. Meningococcal polysaccharides prepared in this way have a molecular weight of more than 100,000, which probably accounts for their immunogenicity in man. Antibodies produced by injection of 50 μg of polysaccharide can be measured by passive haemagglutination, immunofluorescence and bactericidal tests (E. C. Gotschlich, I. Goldschneider and M. S. Artenstein, *J. Exp. Med.*, **129**, 1367; 1969). Opsonic antibodies are also produced (R. Roberts, *J. Exp. Med.*, **131**, 1970). These polysaccharides stimulate antibodies of the three major immunoglobulin classes, although the bactericidal activity resides in the IGG moiety alone. Antibodies which develop after immunization with the A or C polysaccharides are group specific, as is the protection against meningococcal disease which is conferred by polysaccharide vaccination (M. S. Artenstein, R. Gold, J. Zimmerly, F. A. Wyle, H. Schneider and C. Harkins, *New Engl. J. Med.*, **282**, 417; 1970). Artenstein *et al.* have administered a group C polysaccharide vaccine to military recruits and obtained an 87 per cent reduction in disease caused by group C meningococci, although disease caused by group B organisms was not prevented.

The specificity of the antibody induced by polysaccharide immunization is different from the antibody response to natural infection. After systemic or local nasopharyngeal infection with meningococci, bactericidal antibodies with heterologous activity can be detected as well as antipolysaccharide antibodies (Goldschneider, Gotschlich and Artenstein, *J. Exp. Med.*, **129**, 1327; 1969). In addition to humoral antibodies and immunity, administration of group C meningococcal polysaccharide to humans induces a local nasopharyngeal immunity to the group C meningococcal carrier state (Gotschlich, Goldschneider and Artenstein, *J. Exp. Med.*, **129**, 1385; 1969). The mechanism of this local resistance has not been established so far.

CRYSTALLOGRAPHY

International Discrepancy

from a Correspondent

X-RAY intensity measurements have been carried out with single crystal diffractometers on crystals of the same compound in sixteen laboratories throughout the world. The occasion was the Single Crystal Intensity Measurement Project promoted by the International Union of Crystallography to determine the quality of X-ray structure determination. The sets of intensities were subjected to exhaustive statistical analysis followed by individual least squares structure refinements, and further comparisons of the results of the refinements. The first results have been published by S. C. Abrahams, W. C. Hamilton and A. McL. Mathieson (*Acta Cryst.*, **A26**, 1, 18; 1970).

A low-symmetry, low-absorption organic material was considered most suitable for the project, and so each participant was supplied with about twelve crystals of D(+)-tartaric acid, all from the same batch. The spacegroup is $P2_1$, which is non-centrosymmetric and monoclinic, commonly adopted by organic molecules. Each participant measured all $hk0$ reflexions including equivalent reflexions and all other reflexions with positive k and l out to $\sin\theta/\lambda = 0{\cdot}5$ Å^{-1}, using their normal procedures. Of the seventeen participants, one used neutrons and was not considered in the study, and one submitted two sets of data, believing the first to be liable to error, although the second was actually worse than the first.

At first the results appear profoundly disturbing. Although internal comparison of equivalent $hk0$ reflexions for each experiment revealed mean internal disagreements of less than 1 per cent for seven experiments and greater than 3 per cent for only two at 4 per cent and 5·5 per cent, inter-experimental comparison showed that mean deviations for the all-experiment average values of the intensities ranged between 3 per cent and 9·5 per cent for fifteen experiments, with two outliers having 14 per cent and 48 per cent disagreement. Yet both these outliers had shown quite acceptable internal disagreements of 1 per cent and 1·9 per cent respectively. These two sets of poor data were produced by machines of the equi-inclination type.

A careful analysis of variance was carried out in terms of the Miller indices h, k and l, the angular range in which the reflexion was observed, d^*, and the intensity of the reflexion. This analysis showed a larger range among the slopes of the interaction effects (indicating greater systematic trends) for the angular variables h, k, l and d^* for the equi-inclination diffractometers than for the four-circle diffractometers. The slight trends with intensity for experiments using CuKα radiation were opposite to those with MoKα radiation.

The first conclusion was that the results were more mutually concordant for four-circle diffractometers in which the crystal was rotated about three mutually perpendicular axes, while the detector was rotated co-axially with one of the crystal rotation axes—a mode of operation which can be likened to the generation of a stereogram—than for the equi-inclination devices with geometry analogous to that of traditional cylindrical crystallographic cameras. Second, the absolute accuracy of any set of data is at best 5–6 per cent, but may be as bad as 50 per cent with no internal evidence of malfunction. But, on the basis of an analysis of these results and experience with an equi-inclination machine, it seems that the two outstandingly poor sets of data are the result of an alignment problem peculiar to equi-inclination devices when mounting any triclinic crystal, or a monoclinic crystal to rotate about the *a* or *c* axes, as was the case here. This problem does not arise for monoclinic crystals mounted about the *b* axes or for any higher class of crystal.

The results of the individual structure refinements with these sets of data, however, are comforting. The poorer sets of data either failed to refine properly or else produced satisfactory coordinates. Indeed, the worst example which had shown 48 per cent average disagreement refined to a residual R of 4·7 per cent (R is the average disagreement between the observed and calculated structure factor amplitudes) which was the fourth best, with quite satisfactory coordinates. However, the anisotropic temperature factors (β_{ij}), which are related to the direction and magnitude of vibration of the atoms, were very poor indeed for the suspect data sets. For the worst set of data the value of $\beta33$, which is directly related to the amplitude squared of vibration in the *c* axes direction, was about four times the most likely value. The divergence of the thermal parameters was otherwise greatest when absorption corrections had not been applied.

The first lesson for non-crystallographers is that the coordinates of any X-ray structure determination can safely be accepted, but the quoted internally derived standard deviations should be doubled, unless precautions have been taken to improve the accuracy, in which case a factor of $\sqrt{2}$ is more appropriate. The second lesson is that the thermal vibration parameters should be regarded with suspicion, especially if absorption corrections were not applied, and the quoted standard deviations multiplied by a factor of about 5. For any structure which shows one or two of the axial vibration components (β_{ii} and U_{ii}) to be consistently large or small for all atoms, the cross terms (β_{ij} or U_{ij} where $i \neq j$), which determine the angles between the thermal vibration ellipsoid axes and the crystal axes, are probably meaningless, though the other axial components may be acceptable.

MOON

Latest Report from Houston

by our Astronomy Correspondent

PRELIMINARY examination of the Apollo 12 samples at Houston is now complete, and it is clear that there are some significant differences from the Apollo 11 material (*Science*, **167**, 1325; 1970). The reason in most cases is that the Apollo 12 site in Oceanus Procellarum is younger than Mare Tranquillitatis, and the most interesting observation singled out from the Apollo 12 report by the preliminary examination team is the K–Ar age. Once again this indicates that the maria are very old, but that with crystallization occurring about $2 \cdot 3 \times 10^9$ years ago the material of Oceanus Procellarum is roughly 10^9 years younger than Mare Tranquillitatis.

Other facts also point to the Apollo 12 site being less mature than that of Apollo 11, indicating that maria formation lasted at least 10^9 years. The surface layer of fragmented material, for example, is thinner than at the Apollo 11 site, and craters as shallow as 3 metres seem to have penetrated to the harder rock below. And the lower content of gases from the solar wind in the Apollo 12 dust indicates that the material has not been exposed quite as long as the Apollo 11 material.

The landing site in Oceanus Procellarum is on one of the broad rays centred on Copernicus, and the astronauts reported several areas of dust which is lighter grey than normal. Whether the dust which troubled the Apollo 12 astronauts more than expected during landing was due to a difference in soil mechanics between the two maria, or to a difference in Sun angle or in descent path, is not known yet. But the most important clues to the history of the dust layer are the core samples. One tube reached a depth of 70 cm—compared with 15 cm for the Apollo 11 tubes, although a different bit probably helped—and contains ten identifiable layers representing presumably different ejecta blankets. This is backed up by a sample of what looks like volcanic ash taken from a 20 cm deep trench excavated by the astronauts. Coupled with the different distribution of particle types in the dust from Oceanus Procellarum, it is clear that the dust layer does not have a uniform composition from mare to mare.

Although the rocks are similar to the finds in Mare Tranquillitatis, there are some notable differences. Fewer breccias were picked up, thought to be due once again to the thinner surface layer so that excavated material is more likely to be bedrock than to be formed from the stressing and hardening of surface material into breccias. The chemical indications are indeed that the Apollo 12 breccias were formed from fine material with a lower content of solar wind gases than the present dust layer. It is odd that the Apollo 12 rocks have a wider range of texture and composition than expected from the Apollo 11 mission, but the preliminary examination team say that the rocks are consistent with a fractional crystallization sequence, so that they seem to come from a single intrusive sequence or from several similar sequences.

GALAXIES

Rotation of Andromeda Nebula

from our Observatories Correspondent

IN many respects it is easier to study the rotation of the Andromeda Nebula (M31) than that of the galaxy. The Sun's situation in the dust clouds of the Milky Way makes it impossible to take optical measurements of the Doppler shifts of distant stars. This is why the rotation of the galaxy has been studied most often through the Doppler shift of the 21 cm line of neutral hydrogen.

This approach has the advantage that the neutral hydrogen gas clouds might be expected to be moving in circular orbits about the centre of the galaxy. Its principal disadvantage is that it is only possible to make indirect inferences about the distance of a cloud having a given Doppler shift. For these reasons the rotation curve of the galaxy—the run of rotational velocity with radius—is imperfectly known for the galaxy. This is particularly true for the regions close to the galactic centre and outside the Sun's radius (about 10 kiloparsecs). The total mass of the galaxy depends on the way in which the rotation curve behaves at large distances from the centre while the mass distribution with radius near the centre depends on the detailed behaviour of the rotation curve. Consequently both these quantities are poorly known for the galaxy.

The Andromeda Nebula is important because it resembles our galaxy, because it subtends a large angle in the sky, and because radial distances can easily be measured. M31 has been studied using optical techniques and the 21 cm line. For such a distant object the optical methods have an advantage because the angular resolving power of radio telescopes is low. Individual stars in M31 are faint and so the Doppler shifts of HII regions—bright clouds of ionized gas around young hot stars—are measured. Vera Rubin and Kent Ford of the Department of Terrestrial Magnetism of the Carnegie Institute of Washington have recently made a detailed study of the rotation curve of M31 (*Astrophys. J.*, **159**, 379; 1970). Kent Ford has been one of the pioneers of the application of image tubes to astronomical spectroscopy.

The gain in speed of the image tube over photography has enabled them to determine the Doppler shifts of 67 HII regions between 3 and 24 kpc from the centre of M31 with an accuracy of ± 10 km/s^{-1}. These emission regions were mapped several years ago by Arp and Baade. Inside 3 kpc there are no individual HII regions but instead there is a diffuse background of emitting gas. Rubin and Ford have used this to define the rotation curve inside 3 kpc.

Ford and Rubin's rotation curve for M31 is well defined and exhibits a complicated structure. Inside 3 kpc the situation is complicated by non-circular motions which they interpret as being caused by gas which has been ejected from the nucleus of M31. Subtracting out these radial motions, they conclude that the nucleus is rapidly rotating, with a maximum velocity of 225 km s^{-1} at $R=400$ pc. The rotation curve falls to a deep minimum at $R=2$ kpc and then rises to 270 km s^{-1} at $R=11$ kpc, after which the rotation velocity falls off gradually. Rubin and Ford deduce a total mass for M31 out to 24 kpc, the last measured point, of $1{\cdot}85 \pm 0{\cdot}1 \times 10^{11}$ solar masses. On this basis M31 is about 20 per cent more massive than the galaxy. The rapidly rotating nucleus has a mass of $6 \pm 1 \times 10^{9}$ Suns with a mean density of 4,000 solar masses per cubic parsec inside $R=400$ pc. (This can be compared with a mean density of 0·1 solar masses per cubic parsec in the solar vicinity.)

The final implications of Rubin and Ford's work will require further theoretical study. For example, in their model the mass density falls to zero near $R=1$ kpc, which is physically unreasonable. But theirs is the most complete study yet made of the rotation of a galaxy, and it will form a solid foundation for future investigations of the dynamics of a typical spiral galaxy.

TRIBOLOGY

On the Interface

from a Correspondent

EARLIER this month, Manchester was the scene of the latest phase in industry's war with friction. With its symposium on profit from tribology, the Ministry of Technology, in association with the National Centre of Tribology (NCT), Risley, the Institution of Mechanical Engineers and the Confederation of British Industries, sought to inculcate in local industry some idea of the potential rewards from the application of tribology—the science and technology of interacting surfaces in relative motion.

On March 9 an exhibition and film were directed at senior management from more than 200 companies. Lord Delacourt-Smith (Minister of State, Ministry of Technology) recalled that in 1966 the Jost Report estimated industry's frictional losses as £500 million per year. He pointed out that this seemed an underestimate, the true figure now being nearer £1,200 million per year, of which £500 million could easily be saved by applying existing tribological expertise. In the NCT, the industrial units for tribology and the universities, Britain had this capability. Referring specifically to the NCT, he noted the pioneering work on plastic gas-bearings. These promise to be cheap, efficient, free of contamination (being greaseless) and silent.

On March 10 a technical meeting was aimed at engineers from the same companies. Dr J. F. Archard (University of Leicester) maintained that at present "the economics of tribology is more important than the science of tribology". Valuable knowledge was available, the problem was to disseminate it.

Four reasons for this under-utilization were suggested: tribology was new, it was interdisciplinary, academics were not problem-oriented, and industrialists were scared to ask too many simple questions. The obvious savings, like energy, manpower, lubricants and increases in mechanical efficiency, he said, were typically only one-eighth of the total saving tribology could provide.

The major benefits derived from consequentials such as less maintenance and longer machine life. These factors, it was claimed, sat squarely on the interface between management and technology. This interface "must not become a barricade".

Dr D. Summers-Smith (lubrication adviser, ICI Ltd) emphasized that the process industries put a high premium on reliability, since a single pump-bearing failure could shut a complete modern integrated chemical works for days and cost many thousands of pounds in lost production, whereas to replace a part could cost only shillings.

But to duplicate each component would incur a vast capital cost. Where should the line be drawn? A tribological understanding aided the choice of the cheapest bearing design commensurate with an acceptable component lifetime. In other cases, techniques like noise spectrum analysis of vibrating bearings permitted replacement weeks before failure occurred. Britain's oil industry spends several million pounds per year on research and development, maintains advanced technical services departments, and will even give companies detailed assistance with machine design.

Mr B. E. Hurley claimed (British Petroleum Ltd) many companies still adopt a costly *ad hoc* approach to lubrication problems. Dr W. H. Roberts (NCT), however, discussing case histories of problems overcome at his centre, gave some examples of huge savings achieved through the thoughtful application of tribological knowledge.

Probability, Information and Entropy

by
J. S. ROWLINSON
Department of Chemical Engineering and Chemical Technology,
Imperial College of Science and Technology,
London SW7

The claim that information theory provides a foundation for statistical thermodynamics which is independent of the use of ensembles can be sustained only if probabilities can be determined numerically without treating them as frequencies. The use of Shannon's measure of information to assign probabilities is correct only in the same conditions as justify Gibbs's use of ensembles.

If we are to perform an experiment which can have one of n different results, and the probability of result i is p_i, where

$$p_i \geq 0 \qquad \sum_{i=1}^{n} p_i = 1 \tag{1}$$

Shannon[1] showed that a unique and self-consistent measure of the ignorance of the outcome is provided by

$$H = -\sum_{i=1}^{n} p_i \ln p_i \tag{2}$$

If one result is certain, its probability is unity and ignorance is zero. Conversely, H is greatest if the p_i are all equal; $p_i = 1/n$, $H = \ln n$. Ignorance increases with the number n of possible results, that is, results for which $p > 0$. The definition of H recalls that of entropy in statistical mechanics[2], and Shannon used this name for it. The formal link between statistical mechanics and information theory was made by Jaynes[3] and has led to many useful insights. Jaynes, Tribus[4] and Katz[5] have used it as a basis for teaching thermodynamics and statistical mechanics.

The subject of this article is not the enrichment of ideas that has followed from this use of information theory, but the claim of Jaynes and Tribus that the use of Shannon's measure provides a foundation for statistical mechanics which is independent of the use of ensembles. Such a claim requires an inverse use of equation (2). We do not assume that we know p_i and calculate H, but determine the p_i by maximizing H. To examine the validity of this procedure we must ask what is meant by the symbol p_i.

Definition of Probability

Most scientists would say that the probability of an event is (or represents) the frequency with which it occurs in a given situation. If pressed further, a scientist might admit that the frequency was often taken not from an actual series of events but from an imaginary series appropriate to the model which is his idealized representation of the situation. Thus if he has a cube of sugar[4] with the faces marked, as on a die, from 1 to 6, and is asked for the probability of scoring 6 on the next throw, he will reply, 1/6. He recognizes that the cube is not strong enough for him to measure the frequency of 6 in a long series of throws, but, almost without thought, replaces the real cube by an imaginary ensemble of identical cubes each of which he supposes to be thrown once.

Such a view of probability recalls the methods used by Gibbs to establish the rules of statistical mechanics. It is rejected by Jaynes and Tribus for whom a probability is neither a frequency nor does it represent one[6]. The definition of probability is an old problem; Edwards[7] has described briefly the current spectrum of views, of which one wing is occupied by those who divorce probability from frequency, although they recognize that an observed frequency may be evidence for the value of a probability. This group can be divided into those who take an objective view (Keynes[8], Jeffreys[9]) and those who take a subjective view (Ramsey[10], Good[11], Savage[12]). The latter often assess a personal probability as the odds at which a man will bet. But because we hope that rational men will arrive at the same probability on the same evidence we need not explore this distinction here.

Probability need not have a numerical value. To take a point currently under discussion, we do not say that there is a probability of 0·7 that vapour pressure curves are non-analytic functions of temperature at critical points, although we do say that they are probably so. It would be hard to believe that we were discussing a frequency, and equally hard to attach a meaning to the number 0·7. Carnap[13] recognized a distinction between logical probability and probability as a frequency, and attempted, without real success, to find a means of assigning numbers to the former. But we must have numbers in order to use probabilities in the physical sciences. How do those who eschew the frequency definition assign them ?

The classical answer is Bernoulli's principle of insufficient reason or, to give it the name used by Keynes, the principle of indifference. In its crudest form this asserts that if there is no known reason for preferring one alternative to another, then they are equally probable. This is too sweeping, and can be used to generate inconsistencies of which Keynes and others have given numerous examples. Cox[14] has proved formally that the proposition is false. As early as 1842 Ellis (quoted on page 85, ref. 8) went to the root of the fallacy—"Mere ignorance is no ground for any inference whatever. *Ex nihilo nihil.*" Keynes reformulated the principle in a way that removes the more obvious defects, but his qualifications need not be considered here, for they do not avoid the difficulties discussed later. The modern and apparently more powerful principle of maximizing the entropy has much in common with this principle when ignorance is almost complete. For if we know only that an experiment has n possible results and maximize H subject to equation (1), then $p_i = 1/n$, as would be found from the principle of indifference.

Games of Chance

I shall now consider a problem posed by Jaynes which is relevant to statistical mechanics. A man has been playing a game of chance in which the score for each turn can be an integer from 1 to 6. He says that his average score so far is 4·5 and asks for an estimate of the probability of his scoring 1 on the next throw. Jaynes uses all the information and maximizes H subject to equation (1) and to the constraint

$$\langle i \rangle = \sum_{i=1}^{n} i\, p_i \tag{3}$$

where $n = 6$, $\langle i \rangle = 4{\cdot}5$. By using Lagrange's undetermined multipliers we find

$$p_i = z^{-1} e^{-\beta i} \qquad z = (1 - e^{-\beta n})(e^{\beta} - 1)^{-1} \tag{4}$$

$$\langle i \rangle = e^{\beta}(e^{\beta} - 1)^{-1} - n(e^{n\beta} - 1)^{-1} \tag{5}$$

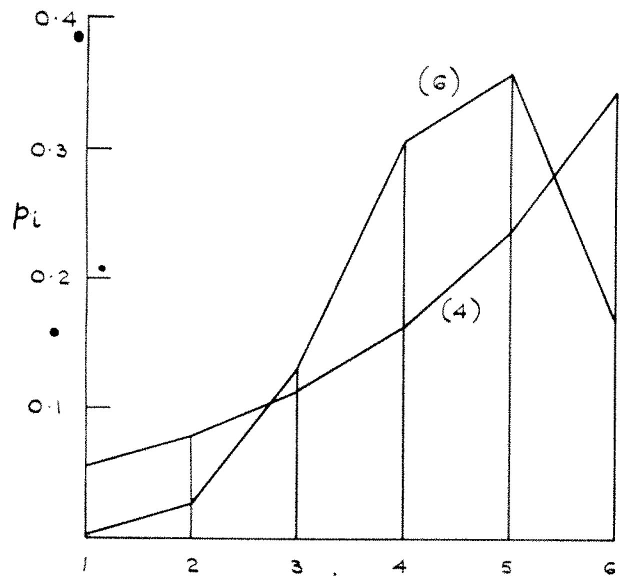


Fig. 1. Probabilities calculated from equations (4) and (6).

This distribution is shown in Fig. 1. The parameter β has the same role as reciprocal temperature $(1/kT)$ in classical statistical mechanics. At infinite temperature $(\beta = 0)$ the mean molecular energy, or score, $\langle i \rangle$, is equal to $\frac{1}{2}(n+1)$. The probability of a molecule being in level i is $1/n$. In Fig. 1, $\beta = -0{\cdot}3711$; that is, the temperature is negative because Jaynes specified a mean score which is greater than the average of the numbers 1 to 6. A physical analogue is an assembly of nuclear spins, each with six equally spaced energy levels, for which the spin–spin relaxation time is short compared with the spin-lattice time. If the mean energy per spin exceeds 3·5, then the spin temperature is negative and we have the inverted Boltzmann distribution shown in Fig. 1.

I shall consider again the game of chance. If the mean score is not known, then ignorance of the result of the next throw is ln 6 = 1·7918. When the mean score is known it follows from equation (4) that H has fallen to 1·6136, and the probability of scoring 1 is assessed at $p_1 = 0{\cdot}05435$. But what basis is there for trusting in this last number? I believe none, unless something is known of the nature of this game of chance. It might, for example, be the following.

An urn contains seven white and three black balls. It is sampled five times, and the player scores i if $(i-1)$ of the samples are white. (To sample means, here, to withdraw a ball, note its colour, replace it, and shake the urn.) This is perhaps as simple a game as could be devised which conforms with the information. A straightforward calculation of the number of ways of drawing $(i-1)$ white balls leads to the skewed-binomial distribution, also shown in Fig. 1

$$p_i = \binom{n-1}{i-1} (n-1)^{1-n} (\langle i \rangle - 1)^{i-1} (n - \langle i \rangle)^{n-i} \quad (6)$$

Thus $p_i = 0{\cdot}00243$, a result which is less than one-twentieth of that calculated from equation (4). Those who favour the automatic use of the principle of maximum entropy would observe that the entropy of equation (6), 1·4136, is smaller than that of equation (4), and so say that in proposing equation (6) as a solution "information" has been assumed for which there is no justification. This is a valid objection only if it is admitted that maximizing the entropy is a useful way of attacking the problem. I maintain, with Ellis, that on this information alone we have no means of estimating the p.

Maximum Entropy

There is a more interesting problem of which equations (4) and (6) are special solutions. There are many urns each containing many balls, black and white. A turn in the game is now played by choosing an urn at random, sampling it as before, and replacing the urn. The chance of scoring i depends on the distribution of black and white balls in the population of urns. Let there be urns with proportions of white balls of 0, $1/m$, – –, j/m, – –, 1, and let the fraction of this population that contains a proportion (j/m) be $(m+1)^{-1} f(j/m)$. Thus

$$\frac{1}{m+1} \sum_{j=0}^{m} f\left(\frac{j}{m}\right) = 1 \quad (7)$$

$$p_i = \frac{1}{m+1} \binom{n-1}{i-1} \sum_{j=0}^{m} f\left(\frac{j}{m}\right) \left(\frac{j}{m}\right)^{i-1} \left(1 - \frac{j}{m}\right)^{n-i} \quad (8)$$

$$\langle i \rangle = \sum_{i=1}^{n} i\, p_i = 1 + \frac{n-1}{m+1} \sum_{j=0}^{m} \left(\frac{j}{m}\right) f\left(\frac{j}{m}\right) \quad (9)$$

We can now take the limit as m, the number of urns, becomes infinite; when $f(y)\mathrm{d}y$ becomes the chance of choosing an urn with a proportion of white balls between y and $y + \mathrm{d}y$, and equations (7) and (9) become

$$\int_0^1 f(y)\mathrm{d}y = 1 \quad \langle i \rangle = 1 + (n-1) \int_0^1 y f(y)\mathrm{d}y \quad (9a)$$

We cannot calculate p_i until we have chosen the prior probability distribution function $f(y)$. One choice is a delta-function at $y = 0{\cdot}7$, the value that satisfies (9a). All urns then have the same proportion of white balls and p_i are given by equation (6). We can, however, spread $f(y)$ as widely as possible between 0 and 1, consistent with equation (9a). To do this we maximize the entropy of the discrete distribution, subject to (7) and (9), and then proceed to the limit of infinite m. The result is

$$f(y) = \beta e^{-\beta y} (1 - e^{-\beta})^{-1} \quad (10)$$

$$\langle i \rangle = 1 + (n-1)[\beta^{-1} - (e^{\beta} - 1)^{-1}] \quad (11)$$

$$p_i = \binom{n-1}{i-1} \int_0^1 f(y)\, y^{i-1} (1-y)^{n-i}\, \mathrm{d}y \quad (12)$$

These equations require $\beta = -2{\cdot}6721$ and give p_i close to those of equation (4). We find $p_i = 0{\cdot}05179$ and $H = 1{\cdot}6134$, a difference of entropy of 0·0002. Equations (4) and (12) become identical if $(n-1)$, the number of times an urn is sampled, becomes infinite. Spreading the prior probabilities according to equation (10) may seem to be less arbitrary than choosing a delta-function, but there are no grounds for thinking it to be more correct if nothing is known of the principle on which the urns were filled.

In this example, maximizing the entropy has the same result as following a postulate of Bayes and Laplace[11] (not Bayes's theorem), namely, that if the prior probabilities are not known they should be distributed uniformly. If, as in this case, the information that $\langle i \rangle \neq \frac{1}{2}(n+1)$ rules out a strictly uniform distribution, $f(y) = 1$, then maximizing H gives a rule for obtaining as wide a distribution as possible. The rule is therefore an adaptation of two old principles—that of indifference and that of Bayes and Laplace—to situations where there is not total ignorance of the distribution of probabilities.

It must now be admitted that the first problem is not exactly as stated by Jaynes[15], who said that the "game of

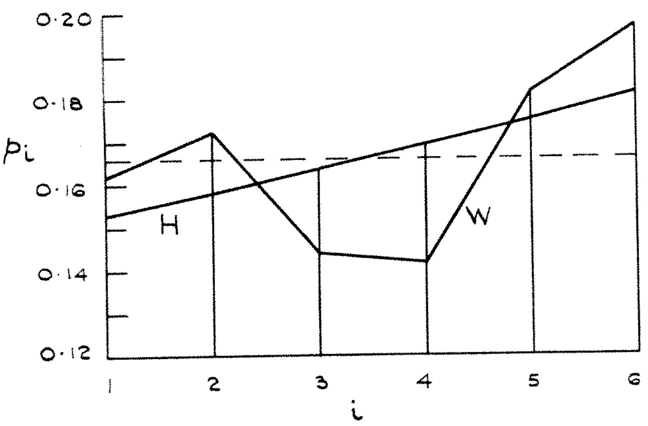


Fig. 2. The frequencies observed by Wolf (W) for a die for which $\langle i\rangle = 3{\cdot}5983$; the probabilities ($H$) calculated by maximizing the entropy for the same value of $\langle i\rangle$. The dashed line is the random result $p_i = 1/6$.

chance" was the throwing of a loaded die. How does this restriction affect the argument ? Clearly we shall now be less likely to entertain the binomial distribution (6), but is there anything in the mechanics of throwing dice which suggests that if a die is not true the probabilities of scores of 1, 2, – – 6, should form the geometrical progression (4) ? I think not. In 1883 Wolf[18] made 20,000 throws of a pair of dice; one of them, the white die, was untrue and gave $\langle i\rangle = 3{\cdot}5983$. The frequencies of the six scores are shown in Fig. 2, which shows that their departures from the random value of 1/6 bear no resemblance to those calculated from the rule of maximum entropy.

What is clearly wrong with the indiscriminate use of this rule, and of the older rules from which it stems, is that they ignore the physics of the problems. Until we know something of the mechanics of dice, or of how the balls got into the urns, we can say almost nothing about p_i. In statistical mechanics we understand sufficient of the physics; in particular we know that if we could follow the evolution of a system for a short time we should find that the final microstate was extremely sensitive to changes in the specification of the initial state. It is such knowledge, not ignorance, which justifies us in supposing that a Gibbs ensemble might be an appropriate model from which to calculate p_i. (Because the rules of statistical mechanics cannot be deduced from those of mechanics, the final justification of this supposition is, as always, the agreement of its consequences with experiment.)

Similarly, if we estimate $p_i = 1/6$ for an untested but apparently quite normal die, then we do so because we know that the result of throwing a die is also extremely sensitive to the initial state, that is, to its momenta and orientation as it hits the table. Thus, we calculate p_i by an analysis similar to that which is used to calculate the probability that a nuclear spin is in level i in a system at infinite temperature, $\beta = 0$. If we have less confidence in predicting the result of throwing a loaded die by the analysis we use for a spin system at $\beta \neq 0$, then Wolf's results show that our lack of confidence is justified. If we assert that a man who lays his bets according to the principle of maximum entropy will, in the long run, beat a man who does not, then we are expressing an opinion on the physics of the situation on which they are betting. If we specify p_i by a precise number we are stating a frequency in an ensemble of our choosing. The principle of maximum entropy is a useful rule, but its validity is no wider than can be justified by the interpretation of probability as a frequency in a Gibbs ensemble, or in any analogous ensemble if the problem is not one of physics.

Failure to make a satisfactory division between those parts of a problem that are physical and those that are mathematical, that is, between the synthetic and the analytic, may well be at the root of other difficulties in the theory of probability. If probability is defined as a frequency in an ensemble[17-19], then the analysis is straightforward in principle, even if difficult in practice. The ability of the ensemble to represent the problem is a matter of physics, not of logic, and insuperable difficulties arise if we try to bring the justification of the ensemble into the analysis of probabilities.

The paradox of the relevance of evidence discussed, in different forms, by Ayer[20], Bartlett[21] and Popper[19] may serve as a final illustration. We have an untested but apparently normal die; we maximize H and obtain $p_i = 1/6$, $H = \ln 6$. We throw the die 999 times and find that all p_i are close to 1/6; it is shown to be a true die. We are now asked to estimate p_i for the thousandth throw, and to recalculate H. We obtain the same answers, and have therefore apparently learnt nothing from our experiment. Lewis[22] admits this, and says we have gained no information from the 999 throws. He is correct if the word information is being used in the technical sense of equation (2). The experiment has, however, confirmed the initial hypothesis about the physics of the problem, namely, that in calculating p_i for a throw of this die it was appropriate to use an idealized ensemble of true dice. There are several schemes for attaching a number to this degree of confirmation[19], or support[23] or increase in likelihood[7] of the initial hypothesis, but such numbers are not probabilities (that is, they do not conform to the usual rules of the calculus of probabilities), and they play little or no part in the physical sciences.

I thank Dr K. G. Denbigh for helpful comments.

[1] Shannon, C. E., and Weaver, W., *The Mathematical Theory of Communication* (University of Illinois Press, Urbana, 1949).

[2] Tolman, R. C., *Principles of Statistical Mechanics*, 539 (Oxford University Press, 1938).

[3] Jaynes, E. T., *Phys. Rev.*, **106**, 620 (1957); *ibid.*, **108**, 171 (1957).

[4] Tribus, M., *Thermostatics and Thermodynamics* (Van Nostrand, Princeton, 1961).

[5] Katz, A., *Principles of Statistical Mechanics—The Information Theory Approach* (Freeman, San Francisco, 1967), reviewed by Jaynes, E. T., *IEEE Transactions on Information Theory*, IT-14, 61 (1968).

[6] Tribus, M., in *Recent Developments in Information and Decision Processes* (edit. by Machol, R. E., and Gray, P.), 102 (Macmillan, New York, 1962).

[7] Edwards, A. W. F., *Nature*, **222**, 1233 (1969).

[8] Keynes, J. M., *A Treatise on Probability* (Macmillan, London, 1921).

[9] Jeffreys, H., *Theory of Probability*, third ed. (Oxford University Press, 1961); *Scientific Inference*, second ed. (Cambridge University Press, 1957).

[10] Ramsey, F. P., *Foundations of Mathematics* (Kegan Paul, Trench and Trubner, London, 1931); Keynes, J. M., *Ramsey, F. P., in Essays in Biography*, second ed., 239 (Hart-Davis, London, 1951).

[11] Good, I. J., *Estimation of Probabilities: an Essay on Modern Bayesian Methods* (MIT Press, Cambridge, Massachusetts, 1965).

[12] Savage, L. J., *Foundations of Statistics* (Wiley, New York, 1954).

[13] Carnap, R., *Logical Foundations of Probability*, second ed. (University of Chicago Press, 1962).

[14] Cox, R. T., *The Algebra of Probable Inference*, 31 (Johns Hopkins Press, Baltimore, 1961).

[15] Jaynes, E. T., in *Statistical Physics* (edit. by Ford, K. W.), 181 (W. A. Benjamin, New York, 1963).

[16] Czuber, E., *Wahrscheinlichkeitsrechnung*, Part 1, 118 (Teubner, Leipzig, 1903).

[17] von Mises, R., *Probability, Statistics and Truth*, trans. Geiringer, H. (Allen and Unwin, London, 1957).

[18] Braithwaite, R. B., *Scientific Explanation* (Cambridge University Press, 1953).

[19] Popper, K. R., *The Logic of Scientific Discovery*, English ed. (Hutchinson, London, 1968); Popper, K. R., and Mackay, D. M., in *Observation and Interpretation* (edit. by Körner, S.), 65, 79, 88 (Butterworth, London, 1957).

[20] Ayer, A. J., in *Observation and Interpretation* (edit. by Körner, S.), 12 (Butterworth, London, 1957); *The Concept of a Person and other Essays*, chap. 7 (Macmillan, London, 1963).

[21] Bartlett, M. S., *J. Roy. Stat. Soc.*, A, **130**, 457 (1967).

[22] Lewis, P. M., *Information and Control*, **2**, 214 (1959).

[23] Hacking, I., *Logic of Statistical Inference* (Cambridge University Press, 1965).

▶Amino Acids—^{14}C Labeled
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700
(SEE ADVERTISEMENT THIS PAGE)

▶Amino Acids—^{3}H Labeled
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700
(SEE ADVERTISEMENT THIS PAGE)

▶Amino Acids—^{15}N and ^{35}S Labeled
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700
(WRITE FOR INFORMATION)

▶Amino Acids—Reconstituted Protein Hydrolysates (^{14}C, ^{3}H)
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700
(WRITE FOR INFORMATION)

▶Amino Acids—Special Contract Orders
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700
(WRITE FOR INFORMATION)

▶Amino Acids—t-Boc
Schwarz/Mann
We supply virtually every starting material needed for peptide synthesis: 33 Boc-amino acids, 22 ^{14}C and ^{3}H labeled Boc-amino acids, 12 Boc-amino acid resin esters, etc. Write or call for brochure.
Orangeburg, New York 10962 (914) 359-2700

▶Amino Acids—Unlabeled
Schwarz/Mann
Orangeburg, New York 10962
(914) 359-2700
(WRITE FOR INFORMATION)

▶Amino Acids-
Schwa

^{14}C Amino Acids from Schwarz/Mann

DIVISION OF BECTON, DICKINSON AND COMPANY BD

Examples of the unusually high specific activities available from Schwarz/Mann:

L-[^{14}C] Alanine	159mc/mM	L-[^{14}C] Lysine	320mc/mM
L-[^{14}C] Arginine	320mc/mM	L-[^{14}C] Methionine	255mc/mM
L-[^{14}C] Aspartic Acid	212mc/mM	L-[^{14}C] Phenylalanine	455mc/mM
[^{14}C] Glycine	106mc/mM	L-[^{14}C] Threonine	212mc/mM
L-[^{14}C] Leucine	320mc/mM	L-[^{14}C] Tyrosine	455mc/mM

Further re our high specific activities: ALL of our ^{14}C amino acids are higher than 50mc/mA. About purity. Every lot of phenylalanine and tyrosine is repurified every month. Every lot of everything else: reassayed every ten weeks and repurified if necessary. And *all* assay and reassay data goes onto the Product Analysis Report that you receive. About availability: these compounds are available *from stock*. For the complete story on our complete labeled amino acid line call (914) 359-2700 or write: Schwarz/Mann, Orangeburg, New York 10962. Division of Becton, Dickinson and Company.

(914) 359-2700

(SEE OUR OTHER ADVERTISEMENT AND LISTINGS ON THIS PAGE.)

^{3}H AMINO ACIDS FROM SCHWARZ/MANN

Examples of the unusually high specific activities available from Schwarz/Mann:

Catalytic

L-[^{3}H] Alanine	6c/mM
L-[^{3}H] Arginine	10c/mM
L-[^{3}H] Histidine	8c/mM
L-[^{3}H] Lysine	6c/mM
L-[^{3}H] Phenylalanine	7c/mM
L-[^{3}H] Valine	8c/mM

Specifically Labeled

L-[4^{3}H] Arginine	20c/mM
L-[2,3^{3}H] Aspartic Acid	15c/mM
L-[3,4^{3}H] Glutamic Acid	15c/mM
L-[4,5^{3}H] Leucine	50c/mM
L-[4,5^{3}H] Lysine	20c/mM
L-[5^{3}H] Proline	15c/mM
L-[2,3^{3}H] Valine	15c/mM

These high specific activity ^{3}H-labeled amino acids are reassayed every ten weeks—as is *every* radiochemical we make—and repurified when necessary. All relevant analytical data gets forwarded to you. For detailed information call (914) 359-2700 or write: Schwarz/Mann, Orangeburg, New York 10962. Division of Becton, Dickinson and Company.

(SEE OUR OTHER ADVERTISEMENT AND LISTINGS ON THIS PAGE.)

For the rest of the
Schwarz/Mann amino acid story
write or call Schwarz/Mann
Orangeburg, N.Y. 10962
(914) 359-2700

KABI

announce the availability from stock of

TRH-KABI 250µg

Synthetic hypothalamic thyroid release hormone

for experimental investigations in animals only.

Ampoules of 0.5 ml containing 250 microgram
of pyro-Glu-His-Pro-NH_2 in sterile
sodium chloride solution

Price per ampoule $10.

Reference: New Scientist, Jan. 29, 1970, p. 200.

KABI

AB KABI, Fack, S-104 25 STOCKHOLM 30, Sweden.

American Museum of Natural History at 100

by

JAMES A. OLIVER

American Museum of Natural History,
New York 10024

The centenary of New York's Museum of Natural History, which continues until May this year, has been marked by the beginning of some important new programmes, particularly concerned with the environment. In this article, the former director, now coordinator of the museum's scientific and environmental programme, traces its history and describes some new departures for the next hundred years.

THE American Museum of Natural History was conceived and founded when the intellectual community was experiencing either exhilaration or mental indigestion depending on the individual's viewpoint. The cause of these disparate sensations was Charles Darwin's *The Origin of Species*. In addition to producing a widespread philosophical debate, both in and out of scientific circles, the book resulted in a great increase of interest and curiosity about the natural sciences. Thus in the 1860s the climate was favourable for Albert S. Bickmore, a young student of the renowned Swiss naturalist, Louis Agassiz, to enlist the sponsorship and financial support of a group of New York City's socially prominent men for the founding of a new museum of natural science. The American Museum of Natural History was officially chartered on April 9, 1869, when the Governor of the State of New York signed the enabling legislation for "the purpose of establishing and maintaining . . . a Museum and Library of Natural Science; of advancing the general knowledge of kindred subjects, and to that end of furnishing popular instruction".

When the first small exhibition of mammals, birds, and reptiles opened in temporary quarters in the spring of 1871, visitors were largely the élite of New York's adult society coming to view the curios of nature. Today, the museum occupies its own home in nineteen connected buildings covering twenty-three acres of floor space, and its nearly four million annual visitors come from all walks of life and nearly all parts of the world. Approximately 60 per cent of them are below college age. They come to the museum for a wide variety of reasons—with interests that usually go far beyond curiosity about the strange or the odd, the dwarf or the mammoth (although some 40,000 visitors arrived in four hours recently to view a moon rock). Today most visitors are seeking answers to urgent questions that bear on the survival of man and of the hundreds of thousands of species of animal and plant life with which he shares his planet; questions about the other planets in the solar system; questions about the characteristics of the men and women of other cultures and other societies. They view the museum as a place to find new insights into the meaning of their lives and new understanding of themselves and their fellow men.

When the first permanent building of the museum was opened by the President of the United States, Rutherford B. Hayes, on December 22, 1877, Professor Othniel C. Marsh, director of Yale University's Peabody Museum of Natural History, remarked: "These vast collections will spread the elements of Natural Science among the people of New York and the surrounding region, but the quiet workers in the attic who pursue Science for its own sake, will bring the Museum renown throughout the world". Few will debate those prophetic words. In spite of the museum's many other claims to international recognition, it has been scientific research that has established the world-wide reputation of the institution.

Growth of the Collections

At first the activities of the small scientific staff were simply to collect, classify, catalogue, and sometimes exhibit the forms that occurred in remote areas. The first official expedition was launched in 1887 when Joel A. Allen set out to collect mammals in the western United States in the area later designated as the state of Montana. The 1890s saw a series of expeditions by the Department of Vertebrate Paleontology to western North America for Mesozoic and Tertiary vertebrates, and the Jesup North Pacific expeditions of the Department of Anthropology, conducted from 1897 to 1903. The first thirty years of the twentieth century were the "golden age of exploration" and many large scale, long term and highly successful expeditions were sent to various parts of the world resulting in rich collections of scientific specimens and priceless exhibition material: the Lang–Chapin expedition to the Congo for reptiles, birds, and mammals; the 1921–30 Central Asiatic expeditions led by Roy Chapman Andrews, and in 1920–29 the Whitney South Sea expeditions primarily for birds but including other recent groups.

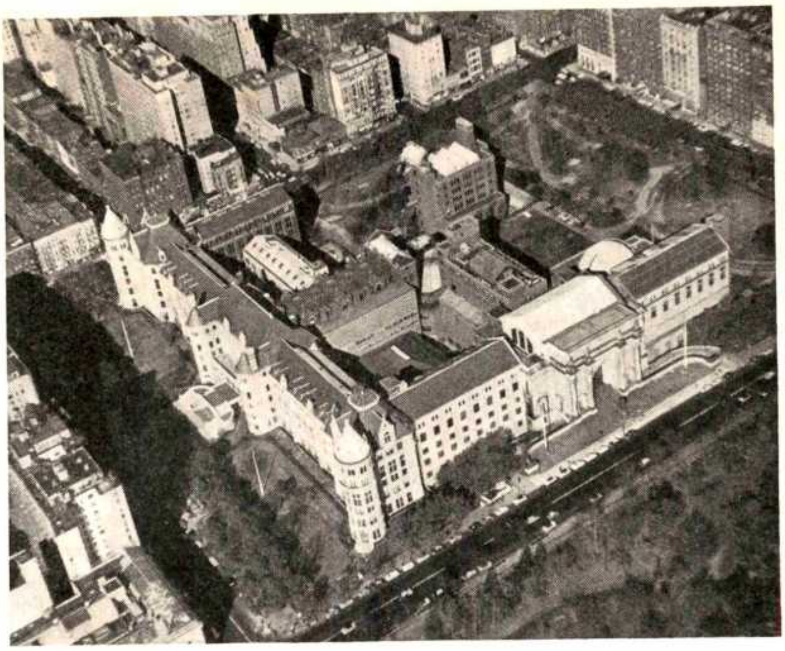
The Second World War cut down expeditions and later, when air travel became more common, the purposes and nature of expeditions, their size and span, changed. Today, two or three individuals may go for short periods of time to gather or verify observational or experimental data or to gather new information and record it on electric and electronic equipment that greatly facilitates the collection and verification of new data.

Changing Research Trends

The research activities of the scientists at the museum evolved from classical, descriptive, taxonomic and morphological studies of the collections into broader, more analytical, and more inclusive investigations. As the theories of Darwin became more widely accepted, and were verified and modified, biology turned to inquiries into the mechanisms by which evolution works, the relationships of the known forms of life, the causes of extinction, the modes of adaptation and the behaviour of organisms.

Much of the scientific information that increased knowledge of the workings and results of evolution came from vertebrate palaeontology and systematic biology. The American Museum of Natural History was fortunate in attracting a brilliant palaeontologist, Henry Fairfield Osborn, who served its cause vigorously, imaginatively, and productively as scientist, trustee, and president. Osborn was one of the most effective individuals to have been associated with the museum in its first hundred years. The programme in vertebrate palaeontology that he established in 1891 has grown into the largest centre for this kind of research in the world, and science and the museum continue to benefit directly and indirectly from his influence.

The scientists, field collectors, preparators and students attracted by Osborn established a long and flourishing



Aerial view of the New York Museum of Natural History.

line of prominent vertebrate palaeontologists. Beginning with William Diller Matthew and William King Gregory, the group also includes Walter Granger, Barnum Brown, and Roy Chapman Andrews, and George Gaylord Simpson, Edwin H. Colbert and Bobb Schaeffer. Only recently, the museum received a substantial bequest that represents yet a further indirect blessing from Henry Fairfield Osborn. In 1967 the heirs of the late Childs Frick donated to the museum Frick's incomparable collection of fossil vertebrates, including the largest and most extensive series of North American Tertiary mammals in any collection, and provided an endowment of more than $7·5 million for construction of a new building to house the collections and to provide funds for continuing studies on these fossils. The acquisition of this collection—approximately equal in bulk to the entire fossil vertebrate collections of the museum—and the personnel to look after and study it gives the museum an unrivalled position as the leading centre for the study of vertebrate palaeontology. Frick was a close personal friend and protégé of Osborn. The new building now under construction will be known as the Childs Frick Wing of Vertebrate Paleontology.

Anthropology has also been emphasized from the early days of the museum. Starting about the same time as the programme in vertebrate palaeontology, the activities that centred around the study of man first concentrated on North American archaeology and ethnology. The pioneering studies of Franz Boas, Clark Wissler and Nels Nelson contributed greatly to knowledge of pre-European and native peoples of North America. The tradition of excellent archaeological work has continued to the present, and has been complemented by notable contributions in physical anthropology, ethnology, and social anthropology through the studies of such staff members as Harry Shapiro, Junius Bird, Gordon Ekholm and Margaret Mead.

At first taxonomic studies on recent forms of animal life were mostly confined to mammals and birds, with Joel A. Allen and Frank M. Chapman in charge of the respective groups. New programmes and departments were developed to cover each class of vertebrates, insects, spiders, crustaceans, molluscs, parasitic and marine worms, and foraminifera. During its history the museum has experimented with different administrative alignments of its departments and with various specialty departments. Today, there are twelve scientific departments with a hundred active scientists conducting 300 research projects. All but two of the departments, astronomy and mineralogy, are concerned with some form of animal life.

Taxonomic studies evolved into the broader fields of systematic and evolutionary biology as knowledge accumulated. In fact, many museum staff members have made substantial contributions to the development of the "new systematics". Especially notable is the work of Ernst Mayr and George Gaylord Simpson, both of whom produced numerous major publications while they were on the staff of the museum. The emphasis on systematic and evolutionary biology has expanded the interests of the staff, enlarged the value of the collections and increased the demands for new equipment and new techniques. Studies of live animals often augment and complement investigations on the preserved collections.

Animal Behaviour

The study of animal behaviour has been described as one of the oldest preoccupations of man but one of the youngest sciences. Although many other departments study some aspects of animal behaviour in the process of their systematic research, this department is concerned solely with behavioural investigations. In 1928 Dr G. Kingsley Noble established a division of experimental biology. It became a full department in 1933 and in 1942 was renamed the Department of Animal Behavior. The staff of this department, who study both invertebrates and vertebrates including man, have been pioneers in this complex and difficult field. Much of the work in the department has been organized toward comparative studies on animals of various phylogenetic levels and is aimed at gaining an understanding of how the combined forces of external and internal stimuli interrelate to

The façade of the Theodore Roosevelt Memorial Building of the New York Museum of Natural History.

determine the behaviour of the animal. Work in progress includes studies of the different bases for and forces involved in socialization in insects, fish and mammals; the psychobiological development of emotional reactions and sexual behaviour in mammals; the role of sound production, underwater hearing and communication in fishes.

The effectiveness of researches such as those being carried out by the staff of the museum is related to the supportive facilities for research. The library of approximately a quarter of a million volumes of natural history references is one of the best in the world. Because of the excellence of the library the National Science Foundation is supporting pilot projects in bibliographic processing techniques in ichthyology and herpetology.

The museum operates five field research stations primarily for the use of its own staff, but also open to other qualified scientists. These are the Archbold Biological Station in south-central Florida; the Lerner Marine Laboratory on the island of Bimini, Bahamas; the South Western Research Station in Arizona; the Kalbfleisch Field Research Station on Long Island, New York; and Great Gull Island off the north-east coast of Long Island, New York. All but the last named have resident staffs and permanent laboratory and living facilities.

Programmes of Education and Exhibition

Albert S. Bickmore was an enthusiastic educator, and it was not surprising that he soon augmented his exhibition of real objects with lectures. His first programmes were for teachers, but he went on to include "artisans, mechanics and other citizens". Demonstrations for school children followed, attendance increased, and the success of the programme led to the establishment, in 1884, of the Department of Public Instruction. The name of the department has been changed several times in the eighty-five years of its existence, but the continuity of its function has been unbroken. Known today as the Department of Education, it has the largest teaching staff of any museum. Its activities for young people range from formal programmes for the school children of New York, which are correlated with the school syllabuses in science and social studies, to classes for both the mentally retarded and the intellectually gifted, and include visits to children confined in hospitals.

Programmes for adults range from the highly popular bird walks and field trips for laymen, conducted in the parks and sanctuaries of the metropolitan New York area, to specialized, intensive courses for biology teachers. Bickmore's early tradition of innovation and pioneering has been continued, and programmes such as a course for nurses in the anthropological and natural sciences, initiated in 1931, provide an understanding of ethnic and cultural differences. Graduate training programmes are conducted by members of the scientific staff in cooperation with universities in the New York area. Candidates for higher degrees receive both formal instruction and project guidance in this programme, which was initiated more than eighty years ago.

An important arm of the museum's education programme is the American Museum–Hayden Planetarium, directed by the Department of Astronomy. A relatively new addition to the museum complex, this has become a vigorous element in the teaching of astronomy and related Earth and space sciences. Youngsters may begin their education in these fields at the age of eight, and continue throughout their adult lives, if their interests and capabilities orient them to subjects such as celestial navigation and astronomy for engineers.

The museum has long had an active publication programme in both scientific and popular work. The *Bulletin*, the *Anthropological Papers*, and the *American Museum Novitates* are the primary scientific journals, and from time to time large monographs and miscellaneous papers are also published. For more than a quarter of a century the comprehensive *Catalogue of Foraminifera*, now in its seventieth volume, has been published at the museum. Publication of the twenty-nine volumes of the *Catalogue of Ostracoda* was undertaken in 1952 and the quarterly journal *Micropaleontology* was initiated in 1954.

Besides the scientific publications the museum has for the past thirteen years published a quarterly magazine for the profession entitled *Curator*, which covers such

Alaskan moose in the hall of North American mammals.

varied aspects of museum studies as a description of the preparation of archaeological objects for display, and the use of data storage and retrieval systems to enhance the usefulness of museum collections. Many popular books and three popular magazines have also been part of the publications programme. The first children's magazine, *Junior Natural History*, was superseded in 1963 by *Nature and Science*. *Natural History*, now in its 70th year of continuous publication, is directed toward the informed lay public. Its editorial scope and content have been broadened as the museum has expanded its fields of study and interest.

But the most potent educational force of the museum is its exhibition programme. From 1871, the date of its first public exhibit, the museum has always attempted to innovate and to set new standards of excellence in its displays. Its first modest effort at the habitat style of presentation, in 1886, was a pair of robins shown with their nest on the bough of an apple tree, and for many years the major effort was directed toward the creation of large and small, but always true to life, habitat exhibits, always constructed on the firm foundation of competent scientific authority. As knowledge has increased, the museum has endeavoured to keep pace with current scientific information, but occasionally the high cost of creating these exhibits resulted in a rather prolonged span between the desire for change and the time of the actual change. One "temporary exhibit" lasted forty years.

Habitat groups, many built under the creative and artistic direction of Carl Akeley and later James L. Clark, were the most spectacular types of exhibits shown by natural history museums for many years, and some of the largest and most ambitious of those halls were built at a cost of more than a half million dollars each. Today, although the great habitat halls still attract vast throngs of visitors, the museum is using many other methods of display to increase the educational impact of its fifty-eight exhibition halls. Exhibit labels are augmented or replaced by audio messages. Animated exhibits, films, slide displays, graphics, recorded sounds and music all complement the real, three-dimensional object or model. Many of the new techniques are seen in the halls developed in the last ten years.

Centenary Activities

In 1959 the Board of Trustees and the City of New York jointly adopted a major expansion of the exhibition programme in preparation for the centenary year. As a result, fifteen permanent exhibition halls have been completed and twenty special or temporary exhibits were opened including the halls of Indians of the eastern woodlands and plains, biology of the invertebrates, the John Lindsley hall of earth history (which has one of the few working seismic recorders on public display), ocean life and biology of fishes, man in Africa, and the hall of Mexico and Central America, which is scheduled to open in the spring of this year.

The exhibition of ocean life and biology of fishes fills the largest hall in the museum, a duplex area 80 feet wide by 135 feet long by 50 feet high. On the balcony are fifteen dioramas of life in and adjacent to the seas, and an exhibit of the phylogeny of fishes in which some 400 models are displayed. Suspended from the ceiling is a life-size model of a breeching blue whale, 94 feet long, which has rapidly become one of the most popular attractions in the museum and in the City of New York.

Another of the highly effective centennial exhibitions is the hall of man in Africa which draws many visitors from the black community. This hall, by telling the story of man in his varying African environments and showing the richness of his cultural heritage, corrects misunderstandings about the nature of traditional life in Africa.

John Canaday, art critic of *The New York Times*, described the hall as "a fine demonstration of museum craftsmanship using a superb collection of African art as its major source of raw material. . . . It illustrates the dignity and richness of African tribal life and culture before their corruption by European influences, and does so without sentimentalizing or propagandizing. . . . It is the breadth of this perspective that makes this view of man and Africa so revealing and so enlarges our perception of his art as an integral force in his life".

The most recent and striking departure from the characteristic three-dimensional museum display is the special centennial exhibit, "Can Man Survive ?". This exhibit, using very little three-dimensional material, is designed to provide an environment isolated from the rest of the museum, in which the visitor is exposed to various media, from electronic music and flashing neon lights to psychedelic murals. The effect, generally very successful, is to produce in the visitor an awareness of what man has done, and is doing, to the environment. As the visitor walks through the truss in which the

Savannah culture, religion and economy in the peoples of Africa hall.

exhibit is suspended, the noise level rises higher and higher, the passageway narrows, the lights flash with increasing intensity. All around him are examples of man's insults to the environment and the message is brought home: "It's up to you !" Man can survive only if he cares about the quality of life, protects the resources of our planet, and acts wisely—now.

Environmental Studies

The centennial exhibit is a striking re-emphasis in a century of concern by the museum about the biophysical environment and the wise use of the Earth's resources. Dr Frank M. Chapman, the first curator of birds and chairman of that department, was a leader in conservation programmes in the United States and in Latin America, and the staff of the Department of Ornithology who followed him have all been active in wildlife protection. This concern has been shared by many other staff members through the years, and the museum has at various times held formal programmes in conservation and the study of ecology.

As the museum enters its second century, it has declared its commitment to define and help solve the urgent problems of the environment. Museum scientists, who have studied the ample records of fossil life, know the tenuous nature of the existence of a species. They are aware that man, the often brilliant innovator and technologist, is primarily a biological being—dependent on requirements similar to those of other living organisms. Thus the museum has established a museum-wide, interdisciplinary programme of environmental studies to assist in mustering its scientific and educational resources to this cause. The American Museum of Natural History feels that it can help lead the way to a better understanding among men through studies of human behaviour and of individual and group differences and through explorations of the causes of aggression and conflict. It can contribute to the solution of urban problems through multidisciplinary studies by anthropologists, ecologists and behaviourists; it can help bring about awareness of the price of biological mismanagement and the dangers of overpopulation, famine and pollution that confront us, and, finally, it can help in the development of wise public policies based on public understanding of science and the natural world.

Much of the knowledge needed to achieve these goals is still to be gathered, but a great deal of the information that can illuminate these environmental problems is to be found within the collections, and in the reference materials of the scientific departments, as well as in the minds of the scientists. These resources must be mobilized and directed toward the solutions of these critical problems. Research programmes now under way can be expanded and new projects initiated, both by the scientific departments and in cooperation with other agencies, to include necessary investigations into wise methods of protecting threatened ecosystems, of increasing or expanding sources of protein foods, and of better understanding the stresses of urban life. New educational programmes and exhibits will be developed to assist in providing a wider public understanding of the urgency and scope of the problems, as well as to further the development of wise solutions to the ecological problems.

Centennial Capital Campaign

On January 9, 1970, the museum announced the inauguration of a drive to raise $25,000,000 in capital funds, in order to enhance and enlarge its facilities as it moves into its second century. It is the first such campaign in the history of the institution and the first broad public appeal the museum has made for funds.

The money will be used for the perpetuation of science teaching programmes, the conduct of scientific research, the development of new exhibition halls, and the modernization of the old, and in places creaking, physical plant. Additional funds, in the range of $35,000,000, will be sought from governmental sources for a construction programme to bring a new building for the library, a new research laboratory building, a new education building and air conditioning.

Mr Gardner D. Stout, president of the museum, announcing the drive for funds, said: "The economies of the past were necessary, and forced the deferral of needed and vital improvements. We can no longer defer these needs, and in my presidency we shall see that they are carried out." He pointed out that the museum's responsibilities were increasing at an explosive rate as scientific and environmental problems engulf the world. Dr Thomas D. Nicholson, director of the museum, said: "The museum is respected and recognized as a great teacher of science, because it has been in the forefront of the acquisition of knowledge. We teach from the authority of sound research." He added that the museum would build large exhibits in the style of the world's fairs and extend its use of multi-media presentations. Support for the interrelated science and education programmes will account for a major portion of the funds to be raised. Thus the programmes begun in the museum's first century will be developed and expanded in the challenging years ahead.

Thermodynamics makes Progress

by
P. T. LANDSBERG
Department of Applied Mathematics
and Mathematical Physics,
University College, Cardiff

On April 1–4 the Institute of Physics and the Physical Society is to hold an international conference on thermodynamics at Cardiff. This article, by one of the organizers, highlights some of the topics which are likely to provide particular interest for the participants.

WHILE it is natural to ask "What is new in pulsars ?" or "What is new in solid state devices ?", few people would ask "What is new in thermodynamics ?". Yet at the boundaries of thermodynamics, as well as at the foundations, there is considerable scientific activity. Indeed, the intrinsic interest of the subject becomes more manifest when we ask: how is it that thermodynamics, with an ever increasing entropy, permits an apparently unstructured fireball (which was the original state in a big bang cosmology) to give rise to the highly structured (low entropy ?) universe which we see around us ? This is not an easy question even for a thermodynamicist, and it illustrates well the universality as well as the difficulty of the subject.

There are, however, several more standard centres of scientific activity. In the first place, of course, the formalism of the subject expands with each really original application. There are, for example, the generalizations required to discuss the properties of ensembles which contain a small number (N) of "pieces" or systems. For an ensemble open to c chemical components[1]

$$E = TS - pV + \sum_{i=1}^{c} \mu_i N_i + \xi N$$

where E, S, V and the N_i refer to the total ensemble. The new variable

$$\xi \equiv (\partial E/\partial N)_{S,V,N_1,\ldots N_c}$$

is a kind of positive or negative "subdivision energy". It is determined by adding systems to the ensemble and evaluating the change in energy at constant entropy, volume and particle numbers N_i for each of the components i. The average small system variables such as energy, entropy, volume, particle number for component i are

$$\bar{E} = E/N, \bar{S} = S/N, \bar{V} = V/N, \bar{N}_i = N_i/N$$

It follows that the average small system energy E is not a linear homogeneous function of the small system variables, $\bar{S}$, $\bar{V}$, $\bar{N}_i$, owing to the existence of the energy ξ. This tends to zero as all $N_i \rightarrow \infty$. The terms in ξ are in the nature of correction terms which are important, for example, in colloidal systems.

Physically related, but mathematically distinct, problems arise if a system has a large surface area so that the terms arising from the nature of the surface make a notable contribution to the density-of-states function. For an ideal gas of particles of mass m in a box of surface area A and volume V, for example,

$$N(E) = a_3 \, l^3 \, V + a_2 \, l^2 A + a_1 \, l \, L + R(E)$$

Here L is a mean total curvature, $R(E)$ is a residual term, $l \equiv (2mE/\hbar^2)^{1/2}$, and the as are numerical factors of order 1/40. These correction terms lead to a purely quantum mechanical effect which requires surface and curvature tensions to be introduced into the thermodynamic quantities even of the ideal gas[2].

Rapid progress is being made at present in several closely related topics which are intermediate between thermodynamics and statistical mechanics. First, there is the behaviour of thermodynamic functions near a critical point. What happens to these functions ? Are they analytic in their arguments ? These questions are usually approached by representing a quantity which diverges at the critical point by a power of T–T_c where T_c is the transition temperature. Thus an exponent is associated with this quantity. The next question is how these exponents are related (this may be called exponentology). For example, for a van der Waals gas

$$p = AT\,(v - b)^{-1} - a\,v^{-2}$$

so that the isothermal compressibility is

$$K_T \equiv -\frac{1}{v}\left(\frac{\partial v}{\partial p}\right)_T = \left[\frac{AvT}{(v-b)^2} - \frac{2a}{v^2}\right]^{-1}$$

At the critical point $v = v_c = 3b$ and $T = T_c = 8a/27bA$, so that for $v = v_c$ indeed

$$K_T = A\,(T - T_c)^{\gamma}\ (A = 4b/3A, \gamma = -1)$$

The coefficient of volume expansion has the same form with

$$A = 2/3 \text{ and } \gamma = -1$$

Careful recent studies on argon and other fluids furnish the experimental data for this work. In ferromagnets, when the critical point is replaced by the Curie point, and in binary mixtures, similar critical phenomena occur. The values of the exponents are being estimated. Symmetry and other requirements yield relations between the exponents, and the resulting laws are called scaling laws[3]. The divergence troubles at the critical point are, roughly speaking, due to the possibility of large fluctuations in macroscopic variables and their long lifetimes.

Divergences in transport coefficients are also being studied and the laws here are sometimes called "dynamic" scaling laws[4]. There is a great deal of experimental and theoretical work to be done on this topic, and even the "static" scaling laws and the associated numerical values must be established with greater accuracy.

Limiting Behaviour

Also between statistical mechanics and thermodynamics is the question of the limiting behaviour of statistical mechanical systems when the number of particles N and the volume V both tend to infinity, subject to N/V remaining at some fixed value. This limit was investigated before the Second World War in the case of simple systems[5], and a systematic treatment is possible for all ideal gases (ref. 6, page 427). In the 1950s the limit was called "the limit L"[7]. Considerably more work has been done and the name "thermodynamic limit" has established itself, although not everyone thinks this name is a happy choice[8]. In the case of interacting particles the existence in the limit of a free energy per particle was first discussed in 1949 by van Hove for classical systems. In 1962 the results were extended by Ruelle to a more general class of systems. Existence theorems have, of course, a rather abstract flavour[9]; nonetheless, it is important that these functions are available in the limit, for this limiting procedure can be used to simplify discussions of phase transitions and theories of interacting systems in statistical mechanics.

There is a related topic intermediate between thermodynamics and statistical mechanics. When energy, volume and the number of particles are fixed for a system, one type of ensemble is needed (the microcanonical one) to describe the system in statistical mechanics. When the energy is replaced by the temperature in this specification, the so-called canonical ensemble is needed, and when, in addition, the number of particles is replaced by the chemical potential in the above specification, a grand canonical ensemble is needed. It is important to know whether these various ensembles yield similar thermodynamic functions if one and the same system is treated theoretically by any of them. This is in general not so, however, except in the thermodynamic limit. This leads to another problem which is under discussion at present, namely the asymptotic equivalence[10] of the various statistical mechanical formalisms. Except in certain special cases of phase transitions and fluctuations, these formalisms are subject to a kind of boundary condition: given a system which is described by various ensembles, its thermodynamic properties must nevertheless be ensemble-independent. The establishment of such arguments and their perfection has made considerable progress recently.

Irreversibility

The concept of irreversibility in thermodynamics and statistical mechanics has exercised scientists since Boltzmann and the Ehrenfests struggled with it. One problem is to understand thermodynamic irreversibility for a closed system in terms of mechanics and its reversible equations of motion. Where do we look for the origin of this irreversibility ? Is it in the nature of the Hamiltonian and the interaction terms which occur in it[11] ? Is it in the coarse graining of phase space, which is required to take account of the fact that all measurements are macroscopic ? (Here it is necessary to note that the fine grained entropy of a closed system remains constant in time[12].) Is it due to the impossibility of completely isolating a system from the rest of the universe[13] ? Or is the essential step the passage to the limit of an infinite number of degrees of freedom[14] ? Although a great deal of work has been done, there is still no agreement on these matters[15]. Information theory can be used[16] to elucidate some of these questions and this theory has been fully used in some books on statistical mechanics[6]. The explanation of its use in this context is being further refined[17]. At the same time the exact formal solutions of the reversible equations of mechanics have been made a starting point for the discussion of irreversibility. An important part has been played by the Liouville equation and its mathematical discussion is leading to new mathematical approaches developed particularly by Zwanzig[18] and the Brussels school[19].

Returning to purely macroscopic theories, there are noteworthy developments in formulating a broadly based theory of real solids which display thermomechanical and thermoelastic effects, and of real fluids which display viscosity and heat conduction. These theories can perhaps be called the thermodynamics of "real" materials. They operate with momentum and energy balance, with balance of moment of momentum, with the second law (also called the Gibbs–Duhem inequality by these authors[20]), and with new assumptions not present in classical thermodynamics[20]. Among these principles is that of material frame indifference, also called the principle of material objectivity. This has a long history and states that constitutive equations must be form-invariant with respect to rigid motions of the spatial frame of reference. There is also the principle of equipresence which states that a quantity which is present as an independent variable in one constituent equation must be assumed to be present in all such equations for a material (unless the contrary can be established). These theories are at present formulated in an abstract manner. While it is not clear to what extent they will yield new understanding of experimental findings, it is highly desirable that their power of synthesis be more widely understood.

One of the more obscure problems in thermodynamics proper is the discussion of self-gravitating systems[21]. In this case long range correlations cause difficulties. Relatively little is known about this topic so far. Is it possible to have a proper thermodynamics which takes account of gravitating bodies ? Or does the absence of equilibrium prevent one from establishing any form of thermodynamics for gravitating systems ?

Astrophysics and Thermodynamics

Astrophysics offers thermodynamic problems in extreme conditions of pressure and temperature. These include the possibility of surprising hypothetical systems of superdense matter in which the velocity of sound (propagated by "particles" of zero rest mass) exceeds the velocity of light in vacuum[22]. Several theoretical schemes which lead to this result have been studied. In this area lies also the question of whether cosmology can illuminate the irreversibility observed locally. Some very interesting, if speculative, discussions on this subject have recently taken place on this topic among some leading scientists. They have been edited by Gold (*The Nature of Time*, Cornell, 1967) and contain, among other things, discussions of the view that the irreversible character of physical processes can be traced back to the boundary conditions which give rise to the present expansion of the universe. It has been remarked that if we live in a universe in which contraction follows expansion periodically, then the universe itself is clearly nature's largest clock.

Arrow of Time

There are, of course, several arrows of time. The thermodynamic arrow is the first. The biological arrow is the second, and points towards age and death, which are at least in principle physico-chemical phenomena. It also points (broadly speaking) in the direction of the increases in knowledge of intelligent beings—a less obviously physico-chemical notion; but one which is a reminder of the operation of Maxwell's demon. By virtue of his knowledge he was able to reduce the entropy of a physical system, and this is a notion with which we have become familiar in the meantime. The demon has not been killed; he has been absorbed, has been given a label, and is now classified as an information-gathering mechanism. Biologists do, of course, study energy balances and energy transformations. Occasionally they even venture into estimates of entropy changes in living things. How soon can definitive relations be established between such changes and the processes of learning, ageing, and so on ?

The cosmological arrow of time is the third arrow, and points in the direction of an expanding universe. How does it come about that these three arrows all point in the same direction ? This empirical fact will surely remain a subject of speculation for a long time to come. Indeed, it may never be elucidated.

Much has also been written recently about the thermodynamic equations which should be used by an observer who is describing a system moving relative to him. This "special relativistic thermodynamics" is somewhat remote from experiment at present and there is no consensus of opinion as to how it should be formulated[24]. It leads to the equally uncharted area of relativistic statistical mechanics. Are the probabilities of statistical mechanics, are entropy and temperature, Lorentz-invariant ? The answers are not known with certainty.

There is also no very clear understanding yet of the best way of using thermodynamics to elucidate the key properties of living matter. It seems that thermodynamics should have a greater contribution to make than has so far been possible, but there are clearly several major conceptual difficulties to be overcome first (ref. 25 and references cited there). This is another speculative but potentially important area in which further work should be encouraged.

Lastly, it must not be forgotten that thermodynamics has also been turning in upon itself, and its foundations are in the process of being axiomatized[26]. It was possible to answer with a clear "No" the question "Is thermodynamics an axiomatic discipline ? " in the early 1960s[27]. It looks as if the answer may be different in the 1970s. Whereas Carathéodory was able to reach an empirical temperature and an empirical entropy without the notion of heat, it is now possible to reach the empirical entropy without even first introducing temperature (Falk and Jung), and possibly even without requiring the notions of adiabatic processes and of equilibrium states (Giles).

The notion of a negative temperature is no longer new, but it warrants a few remarks. It can be understood in terms of systems with a finite number of energy levels (more precisely with energy levels which have a finite upper bound). If the highest level is occupied, while the remaining levels are empty, the temperature may be regarded as -0 and the entropy as zero. As particles drop back, the system loses energy but gains entropy until, for a uniform distribution of particles over quantum states, the temperature is $\pm\infty$, and the entropy has its maximum value. It is most convenient to plot the entropy of a system in a fixed configuration (volume fixed, magnetic field fixed, and so on) against the variable $\tau = -1/T$ (Fig. 1). Then it is seen that as energy is pumped into the systems the temperature T suffers rather strange changes, whereas τ behaves quite smoothly:

$$T: 0, \ldots, +\infty, -\infty, \ldots, -0$$

$$\tau: -\infty, \ldots, 0, \ldots, +\infty$$

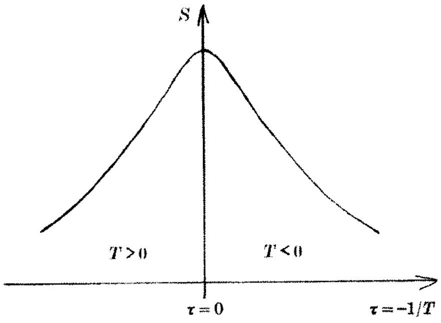


Fig. 1. The entropy of a system plotted in a fixed configuration as explained in the text.

Communicating Thermodynamics

A great many people are interested in thermodynamics. It enters into physics, chemistry, biology, engineering and casts its shadow over linguistics, economics, philosophy and theology. Why should this be ? The reason is twofold: First, the subject is general, abstract and independent of models. For example, any statistical mechanical model must agree with the thermodynamic relations. Yet, second, the subject has a very specific property, and that is the one way character of the entropy. These two observations make sense only if this one way character expresses a rather profound truth. What is this truth ? Just as the hundreds of books of thermodynamics look so different, so the answer to this question can differ widely. Perhaps this truth merely expresses the property of increasing randomness when a statistical system is left to itself in a situation in which the effect of long range forces can be neglected.

The wide interest in thermodynamics and also its abstract nature lead to problems of communication. Should thermodynamics for engineers be different from thermodynamics for physicists ? Can they understand each other's language ? What is the "core thermodynamics" to be taught to everyone ? Problems of this type will occupy us for some time.

[1] Hill, T. L., *The Thermodynamics of Small Systems*, Part I, 23 (Benjamin, 1963).

[2] Ebert, R., and Hilf, E., *J. Phys. Soc. Japan*, **26**, Suppl., 307 (1968).

[3] Tisza, L., and Quay, P. M., *Ann. Phys.*, **25**, 48 (1963); Coopersmith, M. H., *Phys. Rev.*, **172**, 230 (1968); Smith, B. L., *Contemp. Phys.*, **10**, 305 (1969); Münster, A., *Statistical Thermodynamics* (Academic Press, New York, 1969).

[4] Halperin, B. I., and Hohenberg, P. C., *Phys. Rev. Lett.*, **19**, 700 (1967).

[5] Fowler, R. H., and Jones, H., *Proc. Camb. Phil. Soc.*, **34**, 573 (1938).

[6] Landsberg, P. T., *Thermodynamics with Quantum Statistical Illustrations* (Interscience, 1961).

[7] Landsberg, P. T., *Proc. Camb. Phil. Soc.*, **50**, 65 (1954).

[8] Uhlenbeck, G. E., in *Fundamental Problems in Statistical Mechanics* (edit. by Cohen, E. G. D.), **2**, 27 (North Holland, 1968).

[9] van Hove, L., *Physica*, **15**, 951 (1949); Ruelle, D., *Statistical Mechanics: Rigorous Results* (Benjamin, 1969).

[10] Linden, J. van der, *Physica*, **32**, 642 (1966); Galgani, L., Scotti, A., and Valz Gris, F., *Physica*, **44**, 623 (1969).

[11] van Hove, L., *Physica*, **21**, 517 (1955); **25**, 268 (1969); Farquhar, I. E., *Ergodic Theory in Statistical Mechanics* (Interscience, 1964).

[12] Landsberg, P. T., *Proc. Roy. Soc*, A, **262**, 100 (1961); Kampen, N. G. van, in *Fundamental Problems in Statistical Mechanics* (edit. by Cohen, E. G. D.) (North Holland, 1962).

[13] Blatt, J. M., *Prog. Theoret. Phys.*, **22**, 745 (1959).

[14] Balescu, R., *Physica*, **36**, 433 (1967); *Phys. Lett.*, **27**A, 249 (1967).

[15] Hobson, A., *Amer. J. Phys.*, **34**, 41 (1966); *Phys. Lett.*, **26**A, 649 (1968).

[16] Jaynes, E. T., *Phys. Rev.*, **106**, 620 (1957); **108**, 171 (1957).

[17] Katz, A., *Nuovo Cimento*, **33**, 1544 and 1552 (1964); Hobson, A., *J. Chem. Phys.*, **45**, 1352 (1966).

[18] Zwanzig, R., in *Lectures in TheoreticalPhysics* (Boulder, Colorado), **3**, 106 (1960).

[19] Prigogine, I., Henin, F., and George, Cl., *Proc. US Nat. Acad. Sci.*, **59**, 7 (1968); Lefever, R., Nicolis, G., and Prigogine, I., *J. Chem. Phys.*, **47**, 1045 (1967).

[20] Coleman, B. D., and Mitzel, V. J., *J. Chem. Phys.*, **40**, 1116 (1964); Day, W. A., *Archive for Rat. Mech. and Analysis*, **31**, 1 (1968); Meixner, J., **33**, 33 (1969).

[21] Saslaw, W. C., *Mon. Not. Roy. Astron Soc.*, **141**, 1 (1968).

[22] Bludman, S., and Ruderman, M., *Phys. Rev.*, **170**, 1176 (1968); Ruderman, M., *ibid.*, **172**, 1286 (1968).

[23] Gold, T. (ed.), *The Nature of Time* (Cornell, 1967).

[24] Landsberg, P. T., and Johns, K. A., *Nuovo Cimento*, **52**B, 28 (1967).

[25] Viswanadham, C. R., *Nature*, **219**, 653 (1968).

[26] Falk, G., and Jung, H., in *Handbuch der Physik* III/2 (Springer, 1959); Giles, R., *Mathematical Foundations of Thermodynamics* (Pergamon, 1964).

[27] Landsberg, P. T., *Bull. Inst. Phys. and Phys. Soc.*, 150 (1964).

Travelling Waves as Frequency Analysers in the Cochlea

by
GEORG VON BÉKÉSY
Laboratory of Sensory Sciences,
University of Hawaii,
1993 East-West Road,
Honolulu, Hawaii 96822

The human ear can detect a pitch change of 10 per cent of a pure tone in a time interval of two cycles. The inner ear, representing the combination of a mechanical and a nervous system, therefore seems to be superior to filters based on pure resonance and energy accumulation.

THE performances of our sense organs are impressive even by today's standards of technology. The methods used in nature are in general different from those an engineer would use and are often unexpected. In hearing, it is the combination of a fast acting but rough frequency analysis by travelling waves with the inhibitory processes of the nervous system which produces a unique system superior in performance to the filters used in communication engineering.

Definition of Resonance and Travelling Waves

Fig. 1*A* shows a pendulum which is attached to an oscillating support. This is the representation of a simple resonance system. If the resonant frequency of the pendulum coincides with the driving frequency of the oscillations of the support, the amplitude of the pendulum will constantly increase. It accumulates energy. When the oscillations of the support stop, the energy of the oscillation of the pendulum can only decrease if friction is present. This system is called a true resonance system. The resonance curve is symmetrical when we plot the amplitudes of the pendulum versus the frequency deviations (plus and minus) from the resonant frequency.

If we have a set of such pendulums with different lengths and different resonant frequencies, moved by the same oscillating support, according to drawing *B*, we obtain a travelling wave when two neighbouring pendulums are coupled by a small weight between the pendulums. In this case the movements of the pendulums no longer represent true resonance. The resonance curve is no longer symmetrical around the resonant frequency of one specific pendulum. If the oscillations of the support stop, a pendulum loses its energy in two different ways. First, by the friction similar to energy lost in the case of drawing *A* and, second, by a shift of the energy from one pendulum to its neighbouring pendulum. It is convenient to call this lateral energy flow to the sides a travelling wave. If the row of pendulums is long enough, the amplitude of one specific pendulum will decrease even if there are no frictional forces present because the energy flows away.

If the mass of the coupling ball between two pendulums is small, or the coupling balls are placed very close to a supporting point, the lateral energy flow is relatively small and we have a combination of true resonance and a travelling wave. Whether or not along the cochlea we have true resonance points or a travelling wave is therefore determined by how much coupling exists between the adjacent sections along the basilar membrane.

If we could separate the basilar membrane into small strips perpendicular to the longitudinal axis, we might approach the situation of true resonance on the condition that we neglect the coupling produced by the friction of the perilymph. The question as to whether a true resonance or a travelling wave dominates the vibrations in the basilar membrane depends on the numerical value of the coupling. The statement of the numerical value of the coupling is therefore the basis for discussion of travelling waves.

If we observe a set of pendulums with a small amount of coupling between them, it is surprising how long it takes for the pendulum to establish their final oscillatory amplitudes after the support starts to oscillate. When the oscillations of the support are suddenly stopped, the decay time is also very long. But if the coupling is increased the lateral flow of energy will start and stop a specific pendulum quite quickly.

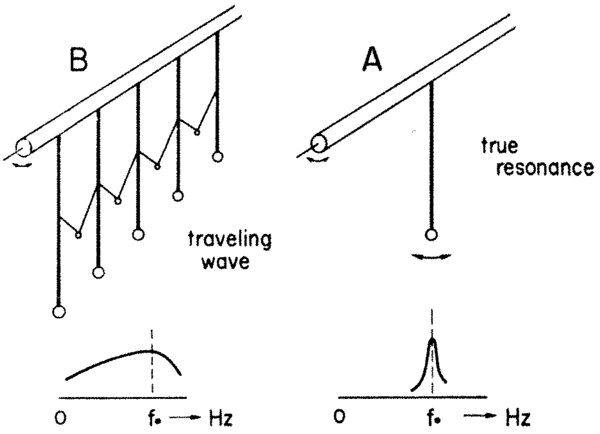


Fig. 1. *A*, A pendulum as a representation of a true resonance system which accumulates energy delivered through oscillations of the suspending point. Below the drawing is a schematic amplitude response for different frequencies of the oscillations of the suspension. *B*, Several pendulums of different resonant frequencies driven simultaneously. The coupling between the pendulums is represented by small balls between neighbouring pendulums. The energy flows constantly from the shorter pendulums to the longer ones.

To find out which type of energy dissipation is dominant in the cochlea of the human temporal bone preparation or an anaesthetized animal, I used stroboscopic illumination projected from a slit lamp made from a microscope. The microscope ocular was replaced by a mercury arc and the slit light beam thus produced was similar to that used in ophthalmology, but with a diameter of 0·01 mm. Using such a slit illumination, some of the corners of the cell membranes appear as bright points. Observing these points with another microscope with a magnification of × 150, it is easy to judge the vibration amplitude of the basilar membrane, Reissner's membrane and the organ of Corti.

With such an apparatus, if the sound to the ear is switched off, in the case of true resonance, the vibration amplitudes should collapse locally, but if a travelling wave is present, then in the stroboscopic illumination the vibrations should be observed to move away laterally. We can learn to discriminate between the two phenomena by observing the vibrations of the membrane of a telephone which is switched off suddenly and comparing it with the vibrations of the basilar membrane. It was found for different animals that the vibrations were damped by a lateral flow of the energy as the main feature.

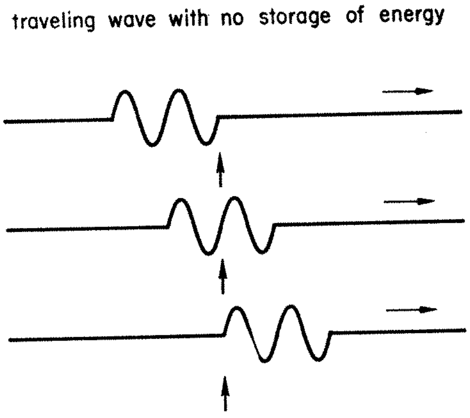


Fig. 2. Two sinusoidal oscillations moving along a rope at different time intervals. There is practically no onset and offset phenomenon at a certain point of the rope, because the rope does not accumulate energy: it just transmits it.

Why transients and storage of energy are avoided in a travelling wave can be seen from Fig. 2. A travelling wave of two cycles initiated at the left will move to the right with practically no accumulation of energy in a system like a rope, a water surface, a longitudinal membrane and so on. If we look at a certain fixed point, the moment a travelling wave reaches that point the oscillation will be instantaneously at full amplitude, and after two cycles have passed that point the oscillations will stop. If the travelling wave is absorbed at the right of the system there will be no reflexions. In the cochlea this absorption is accomplished by the helicotrema.

If the transmitting line is not uniform, during the movement of the waves the amplitude of the oscillations will for every frequency reach a maximum at a different place. This scheme fits perfectly with the anatomical structure of the inner ear, especially the basilar membrane.

Possible Mathematical Vibration Patterns of the Basilar Membrane

The many theories of hearing can be classified in four main groups: (1) the resonance; (2) the travelling wave; (3) the standing wave; and (4) the telephone theories of hearing. All four have proposed different types of vibration patterns of the basilar membrane as the main feature. For almost a century they seem to have been regarded as completely independent theories. But it was shown earlier that in a small experimental model of the size of the normal human basilar membrane, there is no difficulty in going from one type of vibration pattern continuously to another type, changing only two parameters; the volume elasticity of the basilar membrane and the coupling between neighbouring sections. Mathematically speaking, they all belong to the same equation, the difference between them arising only from the assumed numerical values of the basilar membrane[1].

The mathematical treatment of the cochlea is unfortunately to a large degree a hydrodynamic problem. To avoid some of the difficulties I always assumed a laminar flow of the fluid. This assumption later turned out to be a possibility, but it is probably not a stable mode of flow. The introduction of viscosity can change the equations significantly, and I was often reminded of the experiments of Reynold where the laminar flow in a tube with increasing speed suddenly became turbulent for reasons which were mathematically hard to define. Leaving out viscosity and the boundary conditions, it was in most cases not possible to exclude a hydrodynamic paradox[2]. Modern computer techniques should overcome this difficulty.

Longitudinal Stress in the Organ of Corti as a Mechanical Sharpening Device

Because, during evolution, the curvature of the cochlea has become continually greater, one wonders whether the curvature produces a mechanical sharpening effect. There are two principal possibilities: (1) that the curved basilar membrane itself produces a sharpening effect and (2) that the organ of Corti sharpens the discrimination, because the organ of Corti can be looked upon as an elastic transmission system between the basilar membrane and the tip of the hair cells under the tectorial membrane. Such an elastic system could act as a sort of filter.

To test the first possibility, the organ of Corti was brushed off from the basilar membrane with the use of a specially made thin brush. The basilar membrane is stiff enough for there to be no difficulty in peeling off the whole organ of Corti from the membrane. Observation, under stroboscopic illumination, of the vibrations of a cochlear partition and the basilar membrane, without the organ of Corti, showed no specific difference as compared with the organ of Corti intact.

The vibration pattern of the organ of Corti is shown in Fig. 3 (ref. 3). A new phenomenon was that the direction of the vibrations of the hair cells changes along the basilar membrane, mainly as a consequence of the curvature, and this might produce a sharpening effect in the localization. If the hair cells are able to detect such a change in the vibration direction, because the inner and outer hair cells are stimulated in opposite phase, there is a possibility of neural inhibition which might be sensitive to a change in direction. In my opinion the movement of the organ of Corti sandwiched between the basilar membrane and the tectorial membrane is an interesting problem if we take curvature into consideration. It is the same type of vibration calculated by Huxley[4] for the basilar membrane. Direct observations have, however, failed to confirm the local energy accumulation in the organ of Corti or the basilar membrane which should result from this form of vibration.

These observations were carried out with a stroboscopic illumination of a special type, an alternating red and green flash, which enabled measurement not only of the vibration amplitudes but also the phase of the vibrations. Using a slit microscope with underwater surgery in a slowly moving fluid, it was easy to do precise work.

These measurements have been confirmed recently with different methods by Johnstone and Boyle[5] and Tonndorf and Khauna[6]. The electrophysiological recordings of Tasaki[7], Katsuki[8] and Simmons and Linehan[9], and

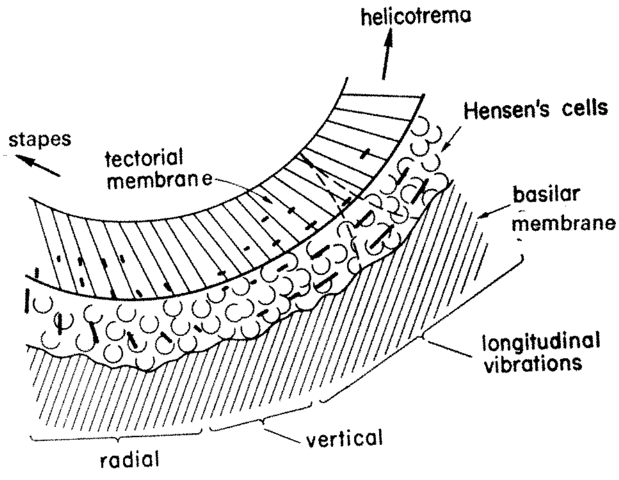


Fig. 3. The curvature in the cochlea might produce a sharpening effect of a mechanical type, because there is a change of the direction of the vibrations in the organ of Corti (taken from G. von B., *J. Acoust. Soc. Amer.*, **25**, 770; 1953).

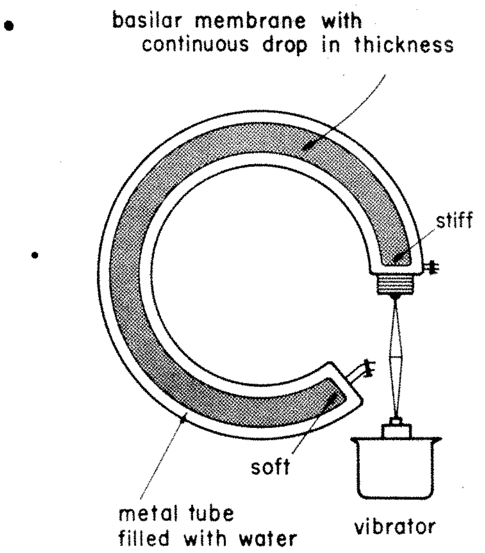

basilar membrane with continuous drop in thickness
stiff
soft
metal tube filled with water
vibrator

Fig. 4. An enlarged model of the upper turn of the cochlea circling around the helicotrema, with the human skin as nerve supply.

measurements by Galambos and Davis[10] also confirmed response curves in agreement with travelling waves. They show an asymmetric response as shown in the lower left corner of Fig. 1.

Model Experiments

In physiology it is assumed that a model is an enlarged or reduced replica of a certain system and, because not every item can be completely duplicated, something is automatically wrong. I have used modelling techniques rather differently. In a system like the cochlea there are so many variables that it would be impossible to measure them all. The question is therefore which is the variable of importance for a certain phenomenon? Modelling techniques provide us with the possibility of leaving out certain structures so that we can study the variables which determine the particular phenomenon in which we are interested[11]. For the cochlea it was determined in this manner that volume elasticity of the basilar membrane is an essential variable and therefore it was the one that was measured first in the human cochlea. The height of the cochlear channel, the width of the basilar membrane, and the length of the fluid column between the basilar membrane and the stapes foot plate would make only small changes. The same holds also for the curvature of the basilar membrane, but not for the curvature of the organ of Corti. The enlarged model of a cochlea provided a chance to replace the hearing nerves with the nerves of the skin.

Several types of models were made: straight ones for the underarm and curved ones representing the upper coil of the human cochlea with the basilar membrane circling around the helicotrema (Fig. 4). The travelling waves were about the same in the curved and in the straight model. The curved model had an outside diameter of about 8 inches. It was placed vertically on a table and adjusted so that the whole model touched the abdominal wall of a standing person. A change in the frequency moved the maximum of the vibrations. The sharpening effect[12] of the localization sensation is a surprising phenomenon because the vibrations can only be felt in a small section of the membrane. Because the sharpening effect of the organ of Corti is omitted, this model can be further improved.

Received August 19, 1969.

[1] Békésy, G. von, *Science*, **123**, 779 (1956).
[2] Birkhoff, G., *Hydrodynamics, a Study in Logic, Facts and Similitude* (Princeton University Press, 1950).
[3] Békésy, G. von, *Experiments in Hearing* (McGraw-Hill, New York, 1960).
[4] Huxley, A. F., *Nature*, **221**, 935 (1969).
[5] Johnstone, B. M., and Boyle, A. J. F., *Science*, **158**, 389 (1967).
[6] Tonndorf, T., and Khauna, S. M., *Science*, **160**, 1139 (1968).
[7] Tasaki, I., *J. Neurophysiol.*, **17**, 37 (1954).
[8] Katsuki, Y., *J. Neurophysiol.*, **21**, 569 (1958).
[9] Simmons, F. B., and Linehan, J. A., *J. Neurophysiol.*, **31**, 799 (1968).
[10] Galambos, R., and Davis, H., *J. Neurophysiol.*, **6**, 39 (1943).
[11] Békésy, G. von, *Deutsche Akademie der Naturforscher Leopoldina*, Halle, West Germany (in the press).
[12] Békésy, G. von, *Sensory Inhibition* (Princeton University Press, 1967).

Four Base-specific Nucleoside 5'-Triphosphatases in the Subviral Core of Reovirus

by

A. M. KAPULER
N. MENDELSOHN
H. KLETT
G. ACS

Institute of Muscle Disease, Inc.,
515 East 71st Street,
New York, New York 10021

By virtue of their capacity to hydrolyse the substrates of RNA polymerase, the nucleotide phosphohydrolases present in the subviral core may serve to regulate transcription. They may also have a phosphodiesterase function concerned with the processing of polynucleotide chains.

Studying the life cycle of a virus frequently provides a way of discovering and understanding biochemically important cell functions. This has been particularly so for the DNA and RNA bacteriophages of *Escherichia coli*. The discoveries that polio virus RNA codes for polypeptide precursors of functional proteins[1], and that *Vaccinia* virus[2] and reovirus[3,4] assemble their mRNA synthesizing enzymes within the mature virion, illustrate that this is also true for animal virus cell systems.

We have studied the reovirus transcriptase in an attempt to develop an *in vitro* transcription–translation system amenable to experiments relating to interferon. While characterizing this RNA polymerase, which can be isolated from infected L cells in subviral cores, we observed that a triphosphate-generating system is necessary for extensive RNA synthesis. This article describes four enzyme activities localized within the virion which are responsible for hydrolysing polymerase substrates to di-

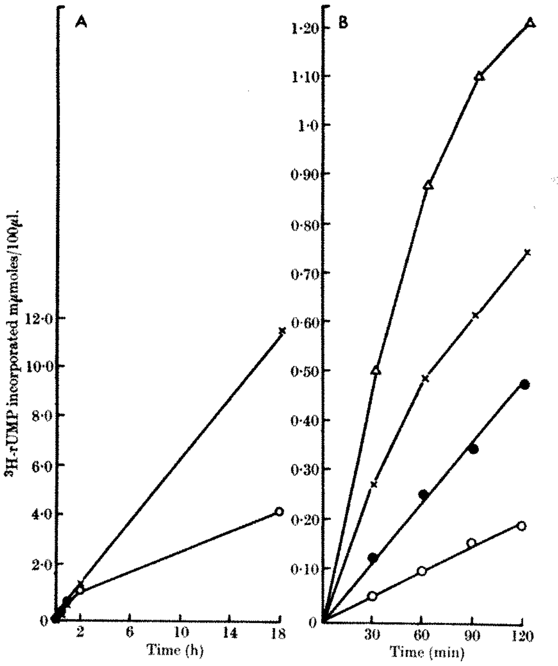


Fig. 1. Kinetics of RNA synthesis: *A*, Requirement for an rNTP generating system for linearity. Two reactions, each twice normal scale, were prepared in standard conditions and incubated at 37° C for 18 h. Aliquots of 40 μl. were withdrawn and analysed. Both reactions included 0·60 units of SVP fraction 4, and one reaction (×) also contained 5 μg of pyruvate kinase (Boehringer) and 1 μmole of sodium phosphoenolpyruvate. *B*, Effect of different amounts of enzyme on linearity. Four reactions, each twice normal scale, were prepared in standard conditions and synthesis was followed as described. SVP fraction 4 was added in the following amounts: ○, 0·13 units; ●, 0·26 units; ×, 0·39 units; △, 0·52 units.

phosphates. The activities are functionally separate from that of the polymerase and are probably associated with a single protein. They may be involved in processing mRNA, regulating the transcriptase or reorganizing the ten chromosomes[5-9] into the reovirion.

Table 1. COMPARISONS OF THE REOVIRUS SUBVIRAL PARTICLE RNA POLYMERASE AND NTPASES

	RNA polymerase	NTPases
Substrate requirement	rCTP, rGTP, rUTP, rATP necessary together; no mononucleotide polymerization	Each rNTP hydrolysed singly, independently (Figs. 2 and 4)
Substrate specificity	Only rNTP is polymerized; dATP and 8-bromo-GTP* are not substrates (Table 3)	rNTP, dNTP and rNTP analogues including 8-bromo-GTP, are hydrolysed; neither rNDP nor rNMP are substrates (Table 3)
Monovalent cation dependence	Strong dependence on M^+; 12-fold stimulation by 0·25 M K^+; $K^+ \simeq NH_4^+ > Na^+$	Inhibited 30–50 per cent by 0·25 M K^+ or Na^+ †
Thermostability	$t_{1/2}$ (Fig. 5): 60° C, 9 min; 70° C, <1 min	rATPase (Fig. 5): $t_{1/2}$: 60° C, >15 min; 70° C, 8 min

* 8-bromo-GTP is an rGTP analogue with normal hydrogen bonding sites but an abnormal conformation (*syn* rather than *anti*: A. M. K. and E. Reich, in preparation; refs. 22, 23, 24).

† rGTPase and rCTPase assays were performed in standard conditions with SVP fractions 4. Inhibition of both activities by potassium or sodium acetate followed identical dose-response curves. For example, rGTPase was reduced by 0·10, 0·25 and 0·50 M potassium acetate to 93, 67 and 27 per cent of control, respectively.

Enzymatic Activities associated with Reovirus Subviral Particles

Twelve to fourteen hours after infection, a template-bound RNA polymerase can be purified approximately 200-fold from the cellular cytoplasmic matrix (ref. 10 and G. A., D. Levin, N. M., M. Schonberg, H. K., S. Silverstein and A. M. K., unpublished results). As can be seen under the electron microscope, the purified enzyme is a component of the subviral core, and the isolation procedure involves stripping off the outer capsomeres from cell-associated virus with sodium dodecyl sulphate and chymotrypsin. The subviral particles (SVP) have a density in CsCl of 1·43–1·45 g/ml., whereas that of the mature virus is 1·36–1·38 g/ml. (refs. 2 and 3). The RNA product is single-stranded, non-self-complementary, sedimentable in sucrose gradients with coefficients of 15S, 19S and 25S and specific for the ten chromosomes of reovirus. As judged by its inability to degrade poly rC

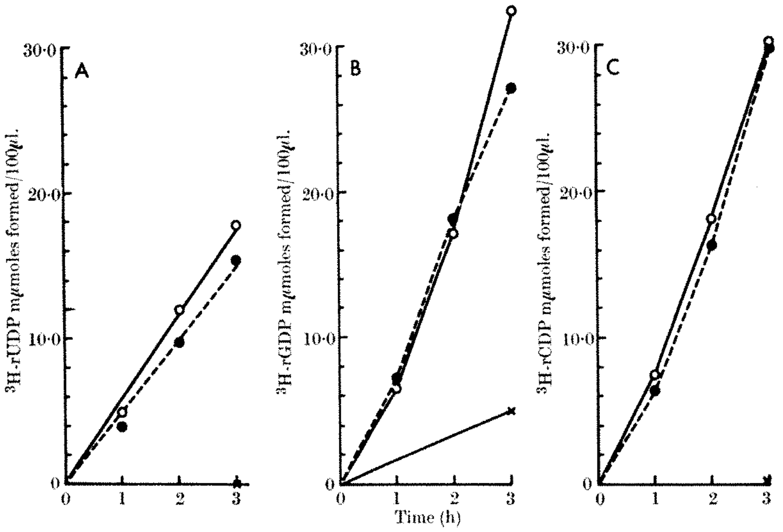


Fig. 2. Kinetics of hydrolysis of rUTP, rGTP and rCTP by subviral particles. Reaction mixtures, prepared in standard conditions in 100 μl. volumes, contained (unless otherwise indicated) 1·2 μg (0·3 polymerase units) of SVP fraction 3 and the following substrates: *A*, ○—○, ^{3}H-rUTP, 100 nmoles (specific activity 7,500 c.p.m./nmole). ● - - - ●, ^{3}H-rUTP, 100 nmoles; rGTP, rCTP, rATP, 200 nmoles each. ×—×, ^{3}H-rUTP, 100 nmoles, no enzyme. *B*, ○—○, ^{3}H-rGTP, 100 nmoles (specific activity 35,000 c.p.m./nmole). ● - - - ●, ^{3}H-rGTP, 100 nmoles; rCTP, rATP, rUTP, 200 nmoles each. ×—×, ^{3}H-rGTP, 100 nmoles, no enzyme. *C*, ○—○, ^{3}H-rCTP, 100 nmoles (specific activity 13,000 c.p.m./nmole). ● - - - ●, ^{3}H-rCTP, 100 nmoles; rATP, rUTP, rGTP, 200 nmoles each. ×—×, ^{3}H-rCTP, 100 nmoles, no enzyme.

Table 2. 8-BROMO-GTP AND dATP AS POTENTIAL SUBSTRATES FOR THE REOVIRUS POLYMERASE

Substrates (nmoles/reaction):	nmoles ^{3}H-rUMP incorporated 1	2
rATP (200), rCTP (200), rUTP (200) and:		
rGTP (20)	0·30	
rGTP (200)	0·38	
bromo GTP (140)	< 0·003	
rGTP (20), bromo-GTP (140)	0·26	
rCTP (200), rUTP (200), rGTP (200) and:		
rATP (50)		0·18
rATP (200)		0·34
dATP (200)		< 0·001
rATP (50), dATP (100)		0·15

Reactions were performed in standard conditions. Series 1: 0·36 units of SVP fraction 3 and ^{3}H-rUTP (specific activity 26, 300 c.p.m./nmole) per reaction. Series 2: 0·20 units of SVP fraction 4 and ^{3}H-rUTP (specific activity 15,000 c.p.m./nmole) per reaction; incubated for 60 min. The ability of bromo-GTP to replace rGTP was further assessed by measuring the dilution of α-^{32}P-rGTP relative to ^{3}H-rCTP in a series of reactions containing increasing amounts of bromo-GTP and fixed amounts of rGTP. In these conditions, bromo-GTP was incorporated into RNA at less than one five-hundredth the rate of rGTP.

and 28S L cell rRNA, there is no detectable ribonuclease activity associated with the polymerase. The polymerase assay and the definitions of enzyme activity are described in the legend to Fig. 1.

The reovirus polymerase requires a ribonucleoside triphosphate (rNTP) generating system such as phosphoenolpyruvate and pyruvate kinase for continued linear synthesis of RNA (Fig. 1*A*). In the absence of the kinase system, the rate of RNA synthesis decreases after 2 h whereas replenishment of substrates *in situ* permits linear synthesis for 18 h. When increasing amounts of enzyme are supplied to a series of parallel reactions, the initial velocities of RNA synthesis increase proportionately, and concomitantly the duration of linear kinetics decreases (Fig. 1*B*). These observations imply that an activity, present in the polymerase preparations, is degrading one or all of the substrates to their corresponding diphosphates.

The hydrolysis of NTP to diphosphates by SVP or chymotrypsin-activated reovirus was measured using three different techniques: electrophoresis (Fig. 2), thin-layer chromatography (Fig. 3) and colorimetry (Table 2). Reactions were performed in standard polymerase conditions in volumes of 50 or 100 µl. NTP substrates were supplied as indicated in the legends to the tables and figures, and specific radioactivities are given where appropriate. The incubation was for 60 min at 37° C.

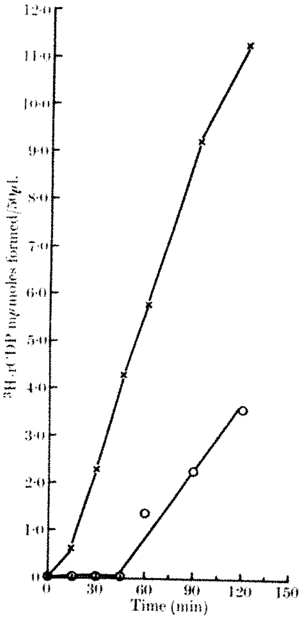


Fig. 3. Latency of rCTPase activity in the reovirion; activation by chymotrypsin. Two reactions, half normal scale, were prepared in standard conditions. Both included 2·4 μg of reovirus (0·12 polymerase units) and 100 nmoles of ^{3}H-CTP (20,000 c.p.m./nmole). ×—×, Plus 5 μg of chymotrypsin; ○—○, no chymotrypsin.

rATP, rUTP, rCTP and rGTP were all substrates for the SVP fractions 3 and 4; the results for the last three are found in Fig. 2. At equal concentrations, rATP was hydrolysed at approximately twice the rate of rCTP and rGTP, which in turn were hydrolysed 1·8–2·4 times more rapidly than rUTP. The hydrolysis of rCTP, taken as representative of the four polymerase substrates, was a linear function of enzyme concentration. In standard conditions, 2·6, 5·8, 9·0, 11·0 and 15·5 nmoles of inorganic phosphate were released in the presence of 2·5, 6·0, 10·0, 12·0 and 18·0 µg of SVP fraction 4, respectively.

The stoichiometry of the reaction was measured with rATP as substrate. Mono, di and triphosphates were measured radioisotopically after separation by thin-layer chromatography; inorganic phosphate was determined colorimetrically. In a standard reaction, 16·8 nmoles of rADP were formed and 13·6 nmoles of orthophosphate released. No monophosphate was produced in the reaction.

Demonstration of NTPase Activity in the Mature Virion

Reovirus was isolated from L cells by standard procedures[11,12] and characterized initially with regard to its RNA polymerase activity. The RNA polymerase of the virus was manifest only after chymotrypsin treatment, in confirmation of the observation of Shatkin and Sipe[3]. Thus, in standard conditions, 1·3 nmoles of rUMP were polymerized in 2 h by 4·6 µg of chymotrypsin-activated virus, but less than 0·020 nmoles were polymerized in a comparable reaction containing untreated virus. Our virus preparation also had the same requirement as the SVP enzyme (Fig. 1*A*) for an energy-generating system in order to synthesize RNA with linear kinetics.

The triphosphate hydrolase activities of the virion have latency characteristics similar to those of the polymerase. There is no rCTPase activity in the virion during 45 min at 37° C and then a spontaneous—probably heat-induced—activity is observed (heat activation of virion polymerase has been reported)[4]. Addition of chymotrypsin to the reaction gives an immediate rCTPase activity (Fig. 3). The virion NTPases, after pretreatment of the virus with chymotrypsin, can hydrolyse rCTP, rATP, rUTP and rGTP to their corresponding diphosphates. As observed for the SVP hydrolases, the chymotrypsin-treated virion degrades rATP twice as rapidly as rGTP and rCTP (Fig. 4). Hydrolysis of rUTP is at 0·4–0·6 the rate of rGTP and rCTP, in comparable conditions. Thus the mature virion, as well as subviral particles from infected cells, possesses four NTPase activities.

Table 3. SUBSTRATE SPECIFICITY OF THE SVP NTPases

Substrates	nmoles Pi formed /h/reaction 1	2
rAMP	< 1·0	
rGMP	< 1·0	
rUMP	< 1·0	
rCMP	< 1·0	
rADP	1·3	
rGDP	1·1	
rUDP	< 1·0	
rCDP	< 1·0	
dATP		19·0
dGTP		13·8
dUTP		12·1
dCTP		10·0
rATP	29·2	36·6
rGTP	13·1	15·8
rUTP	5·7	6·9
rCTP	11·7	16·2
8-Bromo-GTP		6·9
8-Oxo-GTP		4·4
Formycin triphosphate		33·0
5-Bromo-CTP		11·1
5-Fluoro-UTP		5·8
Tubercidin triphosphate		12·5

In these experiments, only 5′-phosphoryl compounds were studied. Reactions were performed in standard conditions; those in series 1 and 2 received 0·05 and 0·12 μg, respectively, of two different preparations of SVP fraction 4. 100 nmoles of each compound were provided per reaction.

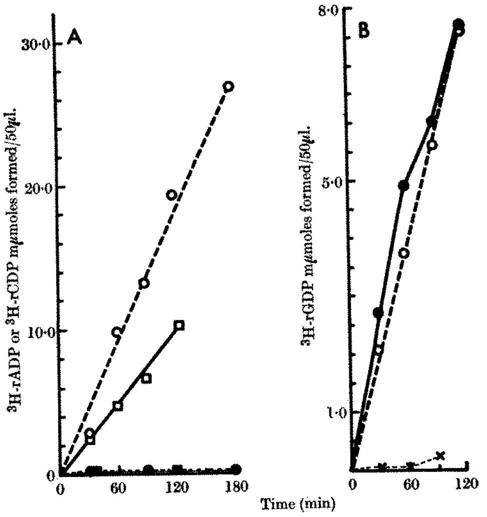


Fig. 4. Kinetics of hydrolysis of rATP, rCTP and GTP by chymotrypsin-activated reovirus. Reactions were prepared, half normal scale, in standard conditions, and (unless otherwise indicated) included 2·4 μg reovirus (0·12 polymerase units) which was activated by preincubation of 24 μg virus with 50 μg chymotrypsin for 10 min at 37° C. *A*, ○ --- ○, ^{3}H-rATP, 100 nmoles (specific activity 12,500 c.p.m./nmole); ● --- ●, ^{3}H-rATP, 100 nmoles, no enzyme. □—□, ^{3}H-rCTP, 100 nmoles (specific activity 15,000 c.p.m./nmole); ■—■, ^{3}H-rCTP, 100 nmoles, no enzyme. *B*, ○ --- ○, ^{3}H-rGTP, 100 nmoles (specific activity 11,200 c.p.m./nmole). ●—●, ^{3}H-rGTP, 100 nmoles; rATP and rUTP, 100 nmoles each. × --- ×, ^{3}H-rGTP, 100 nmoles, no enzyme. Assays were performed as described in the legend to Fig. 3.

The relative rates of hydrolysis of rCTP, rGTP and rATP by the virion NTPases (in nmoles of inorganic phosphate/h/unit of RNA polymerase) were 22·5, 15·0 and 75·0, respectively. In one preparation of SVP fraction 3 (specific activity 140 polymerase units/mg) they were 23·0, 16·0 and 87·0. In general, however, the ratios of NTPases to polymerase were 5–10-fold greater in the SVP preparations.

Analogous to the auxiliary activities of the *E. coli* DNA polymerase[13], the four rNTPase activities may correspond to four base-specific activities of the SVP polymerase not directly related to RNA synthesis. The polymerase and triphosphate hydrolases can, however, be distinguished by several criteria summarized in Table 1.

Characteristics of NTPases

rCTPase is strongly inhibited by inorganic pyrophosphate with a stoichiometry suggesting that the inhibition is achieved indirectly by removal of divalent cations. Surprisingly, inorganic phosphate inhibits neither the polymerase nor the rCTPase (Table 4). Deletion of Mg^{2+} from the rCTPase reaction inhibits hydrolysis by 55 per cent; replacement of Mg^{2+} by 1·5 mM $MnCl_2$ stimulates hydrolysis by 43 per cent beyond the unsupplemented level. In comparison, the polymerase has a more stringent divalent ion requirement, deletion of Mg^{2+} reducing the rate of polymerization by 95 per cent (G. A., D. Levin, N. M., M. Schonberg, H. K., S. Silverstein and A. M. K., unpublished results). Addition of 370 and 1,250 nmoles of EDTA to standard rATPase assays reduces hydrolysis by 80·5 and 90·0 per cent, respectively.

Table 4. EFFECT OF PHOSPHATE (Pi) AND PYROPHOSPHATE (PPi) ON THE VIRION POLYMERASE AND rCTPase ACTIVITIES

System	nmoles ^{3}H-rCDP formed (per cent of control)	nmoles ^{3}H-rUMP incorporated (per cent of control)
Control	100	100
Minus enzyme	2	5
+ Pi 2×10^{-3} M	94	92
+ Pi 1×10^{-2} M	83	88
+ PPi 2×10^{-3} M	55	31
+ PPi 1×10^{-2} M	6	5

All reactions included 2·4 μg reovirus (0·12 polymerase units). The enzyme was activated as described in the legend to Fig. 4. Polymerase reactions were performed in standard conditions for 30 min. In the control 0·13 nmoles of ^{3}H-rUMP was polymerized. rCTPase reactions, half normal scale, were incubated for 60 min and contained 100 nmoles of ^{3}H-rCTP (specific activity 15,000 c.p.m./nmole). In the control, 4·7 nmoles of rCDP were formed.

Using electrophoretic or chromatographic assays of NTPase activity, we were able to measure the conversion of a ^{3}H-rNTP to the corresponding diphosphate in the presence of other non-radioactive rNTP. Hydrolysis of rUTP, rGTP and rCTP by the SVP preparation is unaffected by the other three polymerase substrates, even though each is present in twice the amount of the substrate being analysed (Fig. 2). The virion rGTPase had the same insensitivity to other 5′-triphosphates; equal concentrations of rUTP and rATP did not affect the rate of hydrolysis of rGTP (Fig. 4). Pending a more thorough kinetic analysis, the inability of the polymerase substrates to compete with one another for their respective hydrolase activities implies that there are four separate base-specific activities. Whether they are associated with four separate polypeptide chains, or even whether they are all carried in the same subviral particle, is not yet known. It is noteworthy that both rCTPase and rATPase are heat inactivated at the same rate (Fig. 5), for this is evidence for their association with a single protein.

Interrelationship between Hydrolases and Polymerases

Induction of nucleoside triphosphate hydrolases after viral infection usually serves a specific and important function in the life cycle of the virus. The T-even bacteriophages of *E. coli*[14–17] induce virus-specific dCTPases. Because they contain the hydroxymethyl analogue of dCMP in their DNA genomes, the hydrolases function to deplete the pool of unwanted polymerase substrate and enable specific incorporation of the desired residue by the polymerase. These enzymes are remarkably specific, hydrolysing only dCTP to dCMP and inorganic pyrophosphate, and being unable to cleave rCTP[15].

The function of the reovirus-specific NTPases associated with the polymerase in the subviral core is more obscure. Potentially, the rapid hydrolysis of dNTP by the phosphohydrolases provides an explanation for the inhibition of cellular DNA synthesis observed late in reovirus infection[18]. *Vaccinia* virus, the other animal virus that assembles a transcriptase into the mature virion, also has an active rATP hydrolase in the subviral core[2,19].

The functional separation of polymerase and NTPase activities, particularly with regard to substrate specificity and thermostability, would support the notion that the rNTPase activities are not auxiliary activities of the

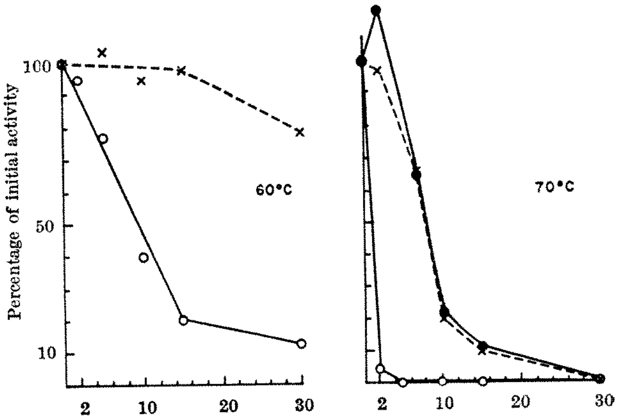


Fig. 5. Heat inactivation of the polymerase and NTPases in the reovirus subviral core. 60° C: 0·43 units of SVP fraction 4 were incubated at 60° C and two aliquots corresponding to 0·030 units each were removed at 2, 5, 10, 15 and 20 min. Polymerase and rNTPase (assayed as described in the legend to Table 3) were measured on these samples in standard conditions and compared with enzyme that was not heated. In the controls, 36·4 nmoles of Pi were released from rATP and 0·026 nmoles of ^{3}H-rUMP polymerized: ○—○, polymerase; × --- ×, rATPase. 70° C: reactions were performed as described except that 0·52 units of SVP fraction 4 were used. In the controls 0·031 nmoles of ^{3}H-rUMP were incorporated into RNA and 29·0 nmoles of rATP and 10·4 nmoles of rCTP were hydrolysed: ○—○, polymerase; × --- ×, rATPase; ●—● rCTPase. With an SVP fraction 3 having a 10-fold lower ratio of rATPase to polymerase, the half times for inactivation at 65° C were 6 and 1·7 min, respectively.

polymerase. Rather, they are activities associated with different proteins bound together in the subviral core. The NTPase activities may well be secondary functions of an enzyme, however, the primary role of which is related to the enzymatic processing of the reovirus mRNAs (such as hydrolysis of the γ-phosphate from RNA chains beginning with pppNp. . .)[20,21]. A more attractive possibility for the primary activity is based on the idea that the biologically relevant leaving group is R–O–PO$_3$ rather than PO$_4$. R could then be a long polyribonucleotide chain, in which case the NTPase would be acting as a phosphodiesterase. In the biology of reovirus replication, such a mechanism might be conceivable if the separate chromosomes were held together by pyrophosphate or tripolyphosphate linkages. Alternatively, the biological role of the NTPases might be to transfer the rNDP moiety to a polynucleotide acceptor, thus providing a mechanism for creating a pyrophosphate linkage between a polynucleotide and a mononucleotide or between two separate polynucleotides, one terminated by pppNp . . . The lack of inhibition of the NTPases by inorganic phosphate is consistent with an alternative function for the enzymes.

In any case, the presence of four hydrolases that destroy polymerase substrates provides a mechanism for regulating polymerase activity. By encapsidating the NTPases in the subviral core, the polymerase activities in the virions maturing late in infection can be reduced, and mRNA synthesis can be severely diminished. Whether the rNTPases are selectively activated at a precise time after infection by an endopeptidase process, analogous to the one found in poliovirus growth[1], remains to be determined.

Polymerase Assay and Purification

Standard assay conditions are 0·1 M Tris-HCl (*p*H 7·9)—0·25 M potassium acetate—0·075 M magnesium acetate with 2×10^{-3} M rATP, rCTP and rGTP, 1×10^{-3} M ^{3}H-rUTP (final specific activity 30–40,000 c.p.m./nmole), and 10^{-4} M dithiothreitol. Reactions are performed for 30 min at 37° C in final volumes of 100 µl. and contain less than 1·5 units of polymerase. They are terminated by addition of 5 per cent trichloroacetic acid, and the polymeric products are trapped on Balston 'GF/A' glass fibre filters. The filters are washed with 7×5 ml. portions of 5 per cent trichloroacetic acid, dried with a heat lamp and counted by standard scintillation methods. One unit of polymerase corresponds to the conversion of 1 nmole of radioactive precursor into acid precipitable material in 30 min at 37° C. Protein was measured by the procedure of Lowry *et al.*[26]. Ribo and deoxyribonucleoside 5′-triphosphates and ribonucleoside 5′-diphosphates were obtained from P–L Laboratories and 5′-monophosphates from Sigma. Tritiated 5′-triphosphates were purchased from Schwartz. Synthesis and characterization of analogue triphosphates or references thereto can be found in the papers of A. M. K. *et al.*[27] and Ward *et al.*[28].

Reovirus transcriptase was purified from infected L cells as follows: Mouse L-929 cells are infected with reovirus, type 3, at a multiplicity of 10–20 p.f.u./cell and incubated in Eagle's[5] spinner medium containing 10 per cent (v/v) foetal calf serum for 12–14 h. The cells are then pelleted by centrifugation and homogenized; the resulting suspension is centrifuged to remove nuclei and unbroken cells. This crude cytoplasmic extract is diluted to 0·5 per cent in sodium dodecyl sulphate and centrifuged at 30,000*g*; a small pellet containing virtually all RNA polymerase activity is recovered. This pellet is treated with chymotrypsin, and the polymerase is pelleted by centrifugation several times to remove polypeptides and chymotrypsin. This resuspended pellet of aggregated subviral particles is SVP fraction 3. This fraction is then made 4 M in urea, layered on a solution of RSB buffer (0·01 M Tris-HCl *p*H 7·4, 0·015 M NaCl, and 0·0015 M MgCl$_2$), 20 per cent in glycerol and 0·1 per cent in 'Brij-35', and centrifuged at 105,000*g* for 2 h in a Spinco 40 rotor. The pellet, resuspended in RSB, is SVP fraction 4. Fractions 3 and 4 have specific activities of 180–250 and 225–325 units/mg, respectively.

The complete virion was isolated from infected L cells as described by Silverstein and Dales[12] and Gomatos[11]. Digestion of the high-speed virus pellet was performed with pancreatic deoxyribonuclease alone, with the omission of both pancreatic ribonuclease A and chymotrypsin. The virus used in our experiments was isolated from a CsCl gradient at a density of 1·36 g/ml. After activation with chymotrypsin, the specific RNA polymerase activity was 50 units/mg.

Assay of NTPases

Separation of nucleotides was carried out by high-voltage paper electrophoresis on Whatman 3 *MM* paper with 0·05 M sodium citrate buffer (*p*H 3·5), for 1–2 h, at 35 V/cm with a water-cooled plate and 5 kV power supply manufactured by Savant Instruments. Aliquots of 5 µl. were removed from enzyme reactions, spotted and dried on top of a mixture of rNMP, rNDP and rNTP (0·1 µmole each) which corresponded to the radioactive rNTP in the reaction. After electrophoresis, the mono, di and triphosphate portions were revealed under ultraviolet light, marked, cut out and counted in a liquid scintillation system. The radioactive rNTPs contained 2–5 per cent of the corresponding rNDP, thus constituting a background which was subtracted from the experimental values.

Thin-layer chromatography was performed with polyethyleneimine-cellulose layers coated on plastic and supplied by Brinkman ('MN polygram cel 300 PEI'). Aliquots of 1 µl. were removed from enzymatic reactions, superimposed and dried on a mixture of mono, di and triphosphates (0·03 µmole each) which corresponded to radioactive rNTP supplied to the reaction. The chromatograms were developed to a height of 12 cm with 1·1, 1·4, 1·4 and 1·7 M LiCl solutions for uridine, adenosine, cytidine and guanosine nucleotides, respectively. In these solvents, the R_f values are, respectively, rUMP, rUDP, rUTP : 0·64, 0·37, 0·17; rAMP, rADP, rATP : 0·54, 0·40, 0·21; rCMP, rCDP, rCTP : 0·61, 0·43, 0·15; rGMP, rGDP, rGTP : 0·46, 0·35, 0·20. After chromatography, the positions of the nucleotides were marked after examination with ultraviolet light, and the di and triphosphates were cut out and counted by liquid scintillation methods. Conversion of rNTP to rNDP was measured by determining the change in the percentage of counts in rNDP relative to rNDP plus rNTP.

For the colorimetric assay of NTPases, the release of inorganic phosphate (P_i) from NTP substrates was monitored[25]. Duplicate reactions were prepared and incubated in parallel: one included enzyme at zero time; the other received enzyme immediately before addition of the acidic molybdate reagent to stop the reaction. The difference between the two reactions measures the enzymatic release of P_i from NTP substrates. All reactions were in final volumes of 50 µl.

We thank Miss Virginia Hamilton for her contributions to the preparation of this manuscript.

Received September 11; revised December 1, 1969.

[1] Summers, D. F., and Maizel, jun., J. V., *Proc. US Nat. Acad. Sci.*, **59**, 966 (1968).

[2] Kates, J. R., and McAuslan, B. R., *Proc. US Nat. Acad. Sci.*, **58**, 134 (1967).

[3] Shatkin, A. J., and Sipe, J. D., *Proc. US Nat. Acad. Sci.*, **61**, 1462 (1968).

[4] Borsa, Y., and Graham, A. F., *Biochem. Biophys. Res. Commun.*, **33**, 895 (1968).

[5] Shatkin, A. J., Sipe, J. D., and Loh, P., *J. Virol.*, **2**, 986 (1968).

[6] Bellamy, A. R., Shapiro, L., August, J. T., and Joklik, W., *J. Mol. Biol.*, **29**, 1 (1967).

[7] Bellamy, A. R., and Joklik, W. R., *J. Mol. Biol.*, **29**, 19 (1967).

[8] Vasquez, C., and Kleinschmidt, A. K., *J. Mol. Biol.*, **34**, 137 (1968).

[9] Watanabe, Y., Millward, S., and Graham, A. F., *J. Mol. Biol.*, **36**, 107 (1968).

[10] Gomatos, P. J., *J. Mol. Biol.*, **37**, 423 (1968).

[11] Gomatos, P. J., and Tamm, I., *Proc. US Nat. Acad. Sci.*, **49**, 707 (1963).

[12] Silverstein, S., and Dales, S., *J. Cell. Biol.*, **36**, 197 (1968).

[13] Kornberg, A., *Science*, **163**, 1410 (1969).

[14] Zimmerman, S. B., and Kornberg, A., *J. Biol. Chem.*, **236**, 1480 (1961).

[15] Cohen, S. S., *Ann. Rev. Biochem.*, **32**, 83 (1963).

[16] Koerner, J. F., Smith, M. S., and Buchanan, J., *J. Biol. Chem.*, **235**, 2691 (1960).

[17] Kutter, E. M., and Wiberg, J. S., *J. Virol.*, **4**, 439 (1969).

[18] Gomatos, P. J., and Tamm, I., *Biochim. Biophys. Acta*, **72**, 651 (1963).

[19] Gold, P., and Dales, S., *Proc. US Nat. Acad. Sci.*, **55**, 219 (1968).

[20] Maitra, U., Cohen, S. N., and Hurwitz, J., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 113 (1966).

[21] Maitra, U., and Dubnoff, J., *Abstr. Seventh Intern. Cong. Biochem.*, B-111 (1967).

[22] Kapuler, A. M., thesis, Rockefeller University, New York.

[23] Haeschemeyer, A. E. V., and Rich, A., *J. Mol. Biol.*, **27**, 369 (1967).

[24] Bugg, C. E., and Thewalt, U., *Biochem. Biophys. Res. Commun.*, **37**, 623 (1969).

[25] Ames, B. N., and Dubin, D. T., *J. Biol. Chem.*, **235**, 769 (1960).

[26] Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., *J. Biol. Chem.*, **193**, 265 (1951).

[27] Kapuler, A. M., Ward, D. C., Mendelsohn, N., Klett, H., and Acs, G., *Virology*, **37**, 701 (1969).

[28] Ward, D. C., Cerami, A., Reich, E., Acs, G., and Altwerger, L., *J. Biol. Chem.*, **244**, 3243 (1969).

Spatial Organization and Interaction of the Two Photosystems in Photosynthesis

by
W. P. WILLIAMS
Biophysics Group,
Physics Department,
Chelsea College,
London

The following is an account of a more generalized model of the interactions in the photosynthetic system. Its advantages and limitations are discussed and the possibility of its extension by the introduction of the concept of an "oxygen-unit" is explored.

THE interaction of the two light reactions, or photosystems, in photosynthesis is most easily explained in terms of the series model of photosynthesis (Fig. 1*a*). According to this, photosystem I (PS I, linked with a CO_2 assimilation system) interacts with photosystem II (PS II, linked with an oxygen evolution system) through an electron transport chain. The model can, however, be misleading. One manifestation of its inadequacy[1] is that it predicts that the addition of an inhibitor such as 3-(4-chlorophenyl)1,1′-dimethylurea (CMU), which has a specific effect on PS II, would necessarily isolate PS I from the oxygen evolution apparatus. The inhibition of oxygen evolution by partial poisoning with CMU should then be independent of wavelength, which is not the case[2]: inhibition of PS II can take place without the concomitant isolation of PS I.

The wavelength dependence of inhibition (compare ref. 1) can readily be explained in terms of additional cross-linkages between the two photosystems allowing the inhibition of PS II without necessitating the isolation of PS I from the oxygen evolution system. Clearly, it would be desirable to design a more formal model detailing these interactions.

If we assume that the basic photosynthetic complex comprises a PS II subunit, a PS I subunit, the electron transport chain joining them and their associated enzyme systems, the necessary extra cross-linkages could be either "intra-unit" or "inter-unit". Additional intra-unit linkages could be visualized, for example, as a number of separately inhibitable electron transport sub-chains diverging from PS II and converging to form the main chain before linking with PS I. If there were a waste of the PS II energy flowing through a sub-chain (which, normally open, was blocked by poisoning) while the PS I energy was redistributed through the remaining unpoisoned subchains, the necessary inhibition without isolation would be achieved. Inter-unit linkages, arranged so as to ensure the localization of the excitation energy of poisoned PS II subunits while allowing the redistribution of the energy absorbed by the corresponding PS I subunits among

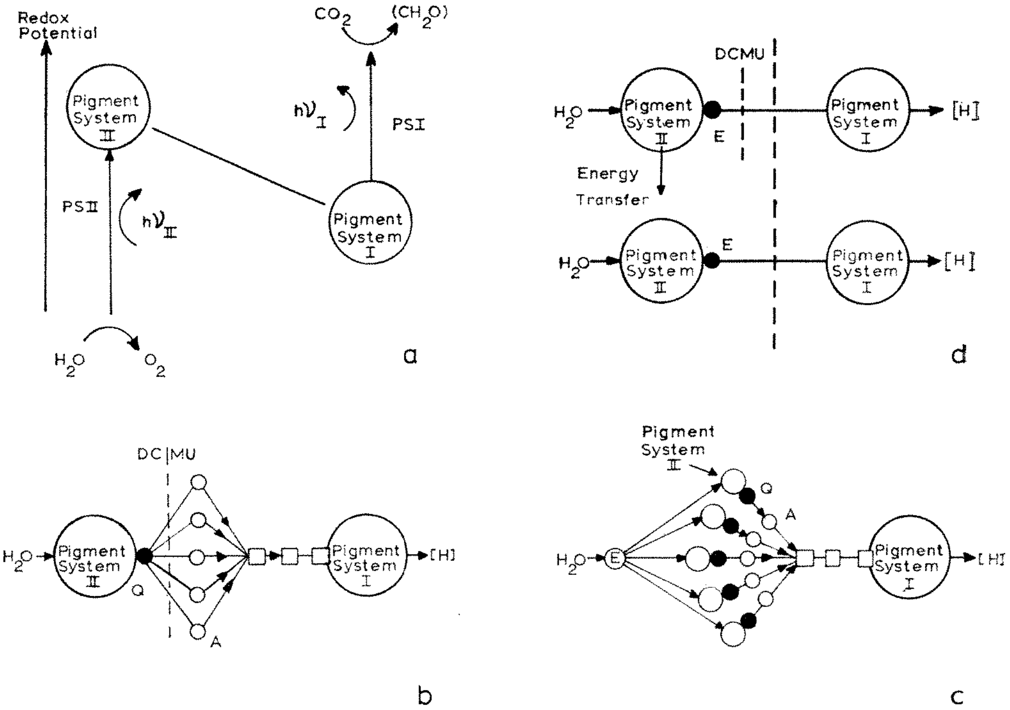


Fig. 1. Current models of the interaction of the two photosystems PS I and PS II: *a*, the simple series model; *b* and *c*, models proposed by Kok *et al.* on the basis of their investigation of the size of the primary oxidant pool available to PS II *d*, model incorporating energy transfer between PS II subunits (postulated by Joliot *et al.*[4]) and an unspecified linkage between neighbouring electron transport chains.

surrounding unpoisoned units, might also be invoked. Kok *et al.*[3], as a result of their studies on the size of the primary oxidant pool available to PS II, have proposed two possible models (Figs. 1*b* and *c*) incorporating multiple electron transport sub-chains. Joliot *et al.*[4], on the basis of two lines of evidence—the non-linearity of the relationship between the amounts of oxygen evolved by poisoned chloroplasts in response to a single short saturating flash and continuous illumination, and the linearity of the fluorescence rise curve in the presence of inhibitor—postulated the transfer of excitation energy between neighbouring PS II subunits. Such a transfer process, if correct, must necessarily infer an even more efficient system for the redistribution of the excitation energy of the corresponding PS I subunits, for otherwise poisoning would seem to involve a preferential inhibition of PS I rather than PS II. Fork and Amesz[5] have put forward evidence for energy transfer between PS I subunits. It suggests, however, a transfer efficiency rather similar to that quoted by Joliot for PS II and, as such, does not give an adequate explanation of the inhibition data. A diagrammatic representation of a model incorporating Joliot's postulated energy transfer and an unspecified linkage between neighbouring electron transport chains is given in Fig. 1*d*.

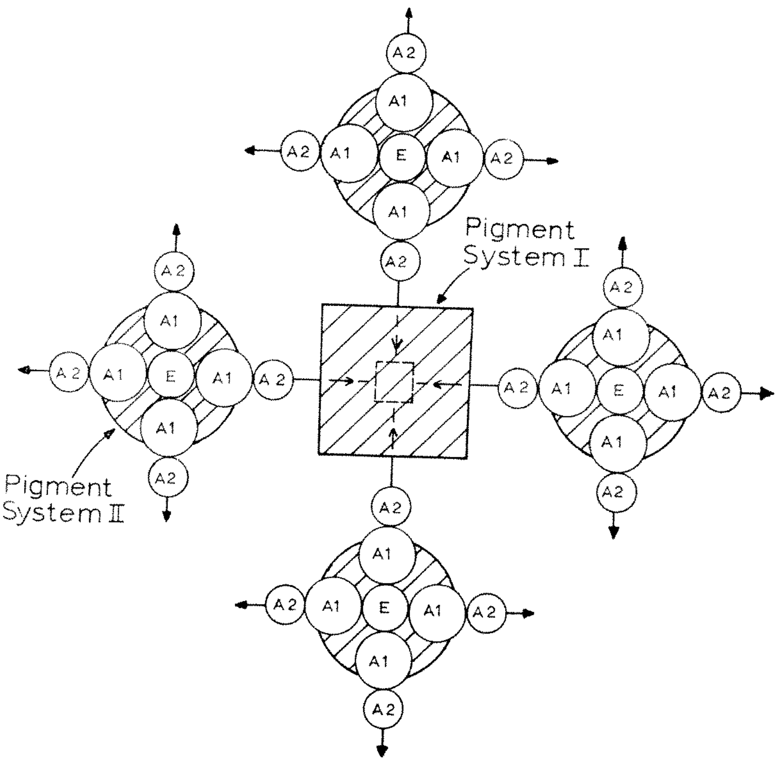


Fig. 2. Repeating unit of the matrix model of photosynthesis. The reaction centre, E, of each PS II subunit is linked to four electron transport sub-chains each of which consists of two primary oxidant molecues, A_1 and A_2. The four sub-chains linking a set of PS II subunits to a given PS I subunit are considered to converge to form the main chain at some early electron transport intermediate such as a cytochrome.

All three models, by incorporating the necessary multiple cross-linkages between the two photosystems, are capable of providing an explanation for the wavelength dependence of inhibition by inhibitors of the CMU type. A further factor which must be taken into account, however, is the extreme sensitivity of photosynthesis to these types of inhibitors. Izawa and Good[6], after investigating the effects of CMU, 3-(3,4-dichlorophenyl)-1,1′-dimethylurea (DCMU) and 'Atrazine' on the rate of the Hill reaction, reported the number of inhibition sites in isolated chloroplasts to be 1 per 2,500 chlorophyll molecules. They consequently postulated the existence of a functional "oxygen-unit" of this size possessing a single inhibition site. Assuming equal amounts of chlorophyll in PS I and PS II, and a PS II subunit size of about 300 chlorophyll molecules, this corresponds to one site per four PS II subunits. Their value is not universally accepted, but an upper limit of one site per PS II subunit seems reasonable. The models discussed so far predict values of from four to five sites per PS II subunit (if the concept of multiple individually inhibitable cross-linkages is to be maintained), down to one site per PS II subunit for the energy transfer model.

Any realistic model of the interactions between the components of the photosynthetic system should contain multiple linkages between the two photosystems (which in the case of "intra-unit" linkages, at least, can be individually inhibited), and at the same time preserve a low ratio of inhibition sites to PS II subunits. Both models proposed by Kok's group fail in that they cannot fulfil both criteria simultaneously. The energy transfer model succeeds, but only at the cost of introducing the added assumption of a system of undefined linkages between electron transport chains. I wish to outline here a new type of model which fulfils both these criteria and which may offer a deeper insight into the photosynthetic process.

The Matrix Model of Photosynthesis

The proposed new model takes into account both the approximate pool size of primary oxidants, as calculated by Kok *et al.*, and the necessity of a low ratio of inhibition sites to PS II subunits. The basic unit (Fig. 2) consists of four PS II subunits, each of which has a reaction centre, E, associated with two primary oxidant pools consisting of four molecules each of A_1 and A_2. These oxidant molecules are arranged in the form of four electron transport sub-chains. The latter, joining a set of PS II subunits to a given PS I subunit, are considered to converge into a single chain at one of the early electron transport intermediates—probably a cytochrome.

The repeating units are considered to be interconnected within a matrix embedded in, or on, the faces of the grana membranes of the chloroplast. When viewed as a matrix (Fig. 3) the ratio of PS I to PS II subunits is 1 : 1. The overall stoichiometries of all the components of the photosystems and their electron transport chains are also maintained.

The electron transport sub-chains, which serve as intra-unit linkages in the models proposed by Kok's group, also serve as inter-unit links in the matrix model. The other models could be adapted to explain the wavelength dependence of inhibition only by assuming that the sub-chains could be individually inhibited. According to the matrix model, a single molecule of inhibitor acting at, or close to, the reaction centre can be considered to inhibit a group of four sub-chains, giving a ratio of 1 : 1 for inhibition sites to PS II subunits. Thus the twin criteria previously outlined can be simultaneously fulfilled. An inhibition site/PS II subunit ratio of one is still at variance with the findings of Izawa and Good, but even this difference can be reconciled by the introduction of the oxygen-unit concept into the matrix model.

Comparison of Matrix Model with Chloroplast Ultrastructure

Electron microscope studies have revealed the existence of two sets of particles arranged in paracrystalline arrays either on, or embedded inside, the grana membranes of

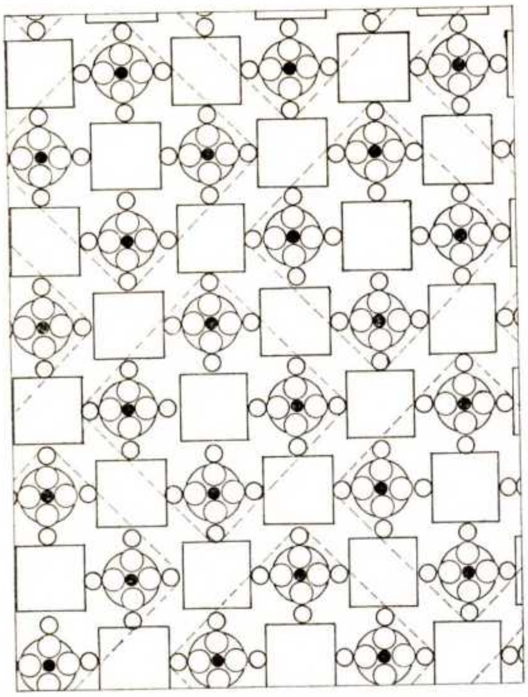


Fig. 3. A set of interconnected repeating units of the type shown in Fig. 2. Individual units are indicated by enclosure by a dashed line. It is not clear, at this stage, whether or not such a division has any structural or functional significance.

the chloroplast. Park and his co-workers[7,8] have favoured the idea that they are located inside the membrane, while Mühlethaler and his co-workers[9] are of the opinion that the two sets are embedded in the outer surfaces of the membrane. Park's group have tentatively identified one set of the particles as units they term quantasomes, which they identify as the physiological equivalent of photosynthetic units. Moudriankis *et al.*[10] confirm the existence of the particles, but believe that they are in fact phosphorylation co-factors. Despite this conflict of opinions on the exact location and function of these arrays, their existence is certain and it is reasonable to assume that they reflect the basic symmetry of the photosynthetic apparatus. A comparison of a schematic diagram of the matrix model with an electron micrograph (after Park and Pfeifhofer[8]) of one of the arrays (Fig. 4) shows that their symmetries are identical.

The Oxygen-unit Concept

The matrix model provides an explanation of the wavelength dependence of inhibition, a role for the primary oxidant pools and a direct link between the ultrastructure of the chloroplast and function of the photosynthetic apparatus, without calling for any changes in the general biochemical properties or overall stoichiometry of this apparatus. It does not explain Izawa and Good's data or throw any light on the possibility of energy transfer between PS II subunits. If, however, the model is extended by the introduction of the concept of an oxygen unit, an explanation of the former—and an alternative explanation of the latter, if one be needed—can be provided.

The oxygen unit as conceived by Izawa and Good was an undefined entity, functionally equivalent to four PS II subunits, with a single inhibition site. I shall define it as the smallest set of mutually adjacent PS II subunits capable of producing oxygen, or a freely diffusible oxygen precursor, in response to a short saturating light flash capable of turning over each subunit once only. The oxygen unit could have two forms. It could correspond to a definite structural unit—the simple form—or to a functional unit consisting of any suitable set of subunits—the generalized form. The behaviour of the two forms is similar and there is no need to distinguish between them at this stage.

To evolve a molecule of oxygen, four electrons must be transferred from water to PS II. Emerson and Arnold[11] showed that a short saturating flash of light can only produce one molecule of oxygen per 2,500 molecules of chlorophyll, that is, one oxygen molecule per four PS II subunits.

Three possible modes of operation of the oxygen evolution system suggest themselves: the product of each PS II subunit could be freely diffusible; the products of two subunits could react with a single molecule of water at a commonly adjacent site, the product so formed being freely diffusible; or the products of four subunits could combine at a single mutually adjacent site to produce a single molecule of oxygen. These three modes of operation are conveniently referred to as A(1), A(2) and A(4). They can be further subdivided depending on the minimum number of functioning subunits needed to drive a set of subunits. The principal possibilities are a single functioning subunit B(1), two subunits B(2) or four subunits B(4). Because the number of functioning subunits cannot exceed the number of subunits in a set, this leads to a total of six operational modes.

The mode normally ascribed to photosynthesis is A(1) B(1), that is, each PS II subunit, acting independently, produces an oxygen precursor with no fixed steric relationship between the sites of energy absorption and oxygen evolution. If, however, we accept the possibility that the operational mode of photosynthesis can be other than the A(1) B(1) mode, two important consequences appear. First, the actual number of inhibition sites need not necessarily coincide with the number calculated from inhibition data. Izawa and Good, assuming a B(1) mode, estimated a ratio of inhibition sites to chlorophyll molecules (before correction for free inhibitor within the chloroplast) corresponding to one site per two subunits. If, however, a B(2) mode is assumed, their data can be

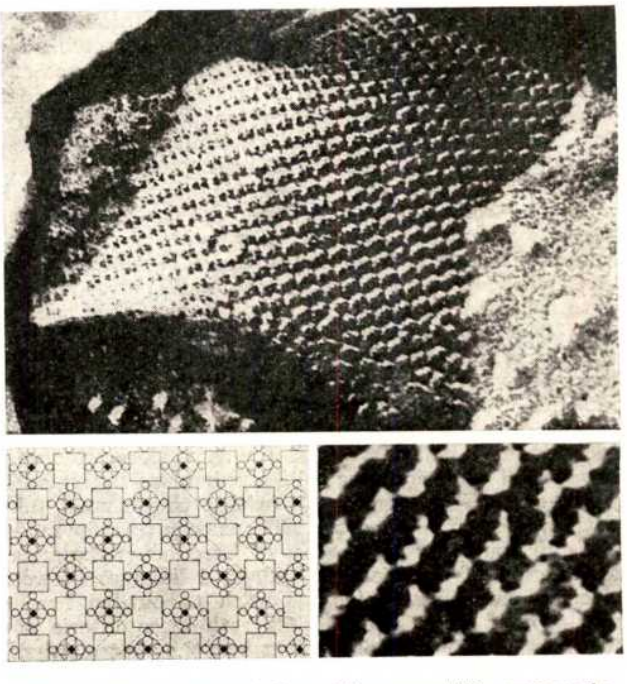


Fig. 4. Electron micrograph of one of the paracrystalline arrays on the surface of a grana membrane exposed by deep etching (after Park and Pfeifhofer[8]). An enlarged section of the array is compared with a section of the matrix model corresponding to a similar area. Their symmetries are identical, suggesting a direct relationship between the ultrastructure of the chloroplast and the matrix model. This, of course, does not necessarily imply an identity between the particles of the array and any specific feature shown in the diagrammatic representation of the model.

re-interpreted as evidence for one site per subunit. Their corrections for the free inhibitor within the chloroplast were also made on the basis of an A(1) B(1) mode and again are open to re-interpretation.

The second consequence is that the oxygen evolution of poisoned chloroplasts in response to a flash need not bear a linear relationship to that evolved by continuous illumination. Such a relationship would be expected only if the number of functioning subunits needed to drive a set of subunits is equal to the number of subunits in the set, that is, for A(4) B(4), A(2) B(2) or A(1) B(1). In these modes the oxygen unit cannot function if any one of its subunits is poisoned. In the other modes, however, a partially poisoned oxygen unit, although unable to respond to a single flash, may respond to continuous light by summing the products of successive turnovers of the remaining functioning units.

A more detailed analysis of these possibilities with the consequent formulae relating the various parameters will be presented elsewhere. Nevertheless, this preliminary analysis should suffice to illustrate how the introduction of the oxygen unit concept into the matrix model—which in itself promises to be a powerful tool—might serve to throw new light on a variety of controversial points in the field of photosynthesis research.

I thank Dr R. B. Park for kindly making available a copy of the electron micrograph of the grana membrane and Dr D. Rosen for his help in the preparation of the manuscript.

Received October 28, 1969; revised March 3, 1970.

[1] Williams, W. P., *Biochim. Biophys. Acta*, **153**, 484 (1968).

[2] Gingras, G., Lemasson, C., and Fork, D. C., *Biochim. Biophys. Acta*, **69**, 438 (1963).

[3] Kok, B., Malkin, S., Owens, O., and Forbush, B., *Brookhaven Symp. Biol.*, **19**, 446 (1966).

[4] Joliot, P., Delosme, R., and Joliot, A., in *Currents in Photosynthesis* (edit. by Thomas, J. B., and Goedheer, J. H. C.) (Ad. Donker, Rotterdam. 1966).

[5] Fork, D. C., and Amesz, J., *Biochim. Biophys. Acta*, **143**, 266 (1967).

[6] Izawa, S., and Good, N. E., *Biochim. Biophys. Acta*, **102**, 20 (1965).

[7] Park, R. B., and Shumway, L. K., in *Comparative Biochemistry and Biophysics of Photosynthesis* (edit. by Shibata, K., Takamiya, A., Jagendorf, A. T., and Fuller, R. C.) (Univ. Tokyo, Tokyo; Univ. Park, Pennsylvania, 1967).

[8] Park, R. B., and Pfeifhofer, A. O., *J. Cell. Sci.*, **5**, 299 (1969).

[9] Mühlethaler, K., Moor, H., and Sarkowski, J. W., *Planta*, **67**, 305 (1965).

[10] Moudriankis, E. N., Howell, S. H., and Karu, A. E., in *Comparative Biochemistry and Biophysics of Photosynthesis* (edit. by Shibata, K., Takamiya, A., Jagendorf, A. T., and Fuller, R. C.) (Univ. Tokyo, Tokyo; Univ. Park, Pennsylvania, 1967).

[11] Emerson, R., and Arnold, W., *J. Gen. Physiol.*, **16**, 191 (1932).

New Finds at the Swartkrans Australopithecine Site (*contd*)

Last week Dr C. K. Brain introduced the Swartkrans cave site and described some recent discoveries there. Dr J. T. Robinson now describes two hominid vertebrae which he suggests belonged to a *Paranthropus* individual ; Mr R. J. Clarke, Dr F. Clark Howell and Dr C. K. Brain present evidence for the view that two different hominids once lived at Swartkrans ; and Dr Mary Leakey considers the stone artefacts.

Two New Early Hominid Vertebrae from Swartkrans

Two hominid vertebrae have been recovered by Dr C. K. Brain from the Swartkrans cave site[1]. The specimens were obtained from blocks of breccia which were found lying just outside the site and which had been excavated by lime miners in the early 1930s. It is very likely that all of this material originated from the outer part of the site, and that it was from this breccia that almost all of the early hominid material known from Swartkrans was recovered. Among the specimens found by Dr Brain are a number which seem to belong to *Paranthropus*. The two vertebrae were found in the same block of breccia. Both belonged to adult individuals, possibly the same one, and both are clearly primate. *SK* 3981*a* is a last thoracic vertebra, while *SK* 3981*b* is a last lumbar.

The conclusion that *SK* 3981*a* is the last in the thoracic series is based on two facts: (1) the superior articular facets are typical of thoracic vertebrae but the inferior facets are typical of lumbar vertebrae; (2) complete costal facets are present for the capitula of the ribs and these are situated wholly on the vertebra, partly on the neural arch and partly on the body.

This vertebra is clearly primate and, at this site, primate material is likely to be either cercopithecoid or hominid because no other primate groups are represented in South African Pleistocene sites. The vertebra is, however, not cercopithecoid because it lacks certain cercopithecoid features—in cercopithecoids, for example, the last thoracic vertebra, as judged by the articular facets, is not the last of the rib-bearing vertebrae and therefore has hemifacets as well as a clearly defined costal articular facet on the transverse process. The probability of finding pongid vertebrae in South African sites is very small, but even if it were probable, pongid affinity of this Swartkrans vertebra can be ruled out because it lacks, for example, the relatively small neural canal, low, wide and back-swept neural spines, and the complicated transverse process which are characteristic of pongids.

This vertebra is therefore very probably hominid. Both *Paranthropus* and *Homo erectus* occur in the front cave breccia at Swartkrans, hence the vertebra could belong to either of these. So far as I am aware the last thoracic vertebra is not known in *H. erectus*. Because the postcranial skeleton of the latter seems not to have differed in principle from that of *H. sapiens* so far as is known, it seems not unreasonable to assume that the vertebrae of the two would be rather similar.

The Swartkrans vertebra can be distinguished from the *H. sapiens* homologue by the following features:

(1) It has a relatively long neural spine projecting approximately at right angles to the superior–inferior axis of the vertebra. In modern man the spine is shorter and its total length, measured from the neural canal to the apex, is usually no longer than the anterior–posterior thickness of the centrum. The spine of the fossil specimen is considerably longer than this. (2) The neural canal is relatively large. Although the fossil vertebra is appreciably smaller in overall size than the *H. sapiens* homologue the neural canal is of the same absolute size in the two. Correlated with this is the fact that in lateral view the inferior notch of the fossil specimen is relatively wide and well developed, being of about the absolute size found in modern man although it belongs in a relatively small vertebra. (3) The mammillary process–transverse process

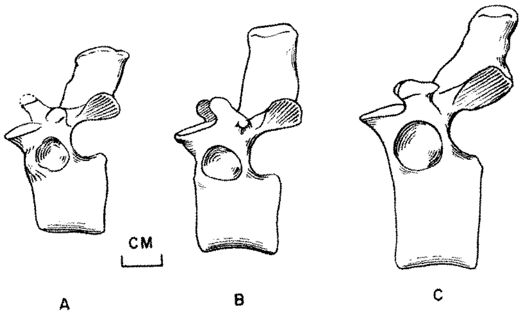


Fig. 1. Last thoracic vertebrae of (*A*), *Australopithecus* (*Sts* 14); (*B*), *Paranthropus* (*SK* 3981*a*); (*C*) *Homo sapiens* in lateral view.

complex is different in the Swartkrans specimen. The mammillary process is relatively well developed but slender, resembling a small transverse process. It is well separated from the transverse process homologue, which is no more than a small tubercle well below the mammillary process.

It is therefore unlikely that the fossil vertebra belongs to *H. erectus*.

One last thoracic vertebra of *Australopithecus africanus* is known from Sterkfontein (*Sts* 14), a description of which, along with that of others from the same vertebral column, is being published elsewhere. This specimen probably came from an adult female. There is considerable resemblance between *Sts* 14 and the new specimen from Swartkrans, more so than between the latter and the *H. sapiens* equivalent, and in the light of this I consider that the Swartkrans specimen belongs to *Paranthropus*. There are, however, some noticeable differences between the two. Because a single specimen only of this vertebra is known from each of the sites, Sterkfontein and Swartkrans, it is unfortunately impossible to know what the significance is of the differences observed because the nature of the variation in the two parent populations is unknown.

SK 3981*a* is slightly larger than *Sts* 14. The maximum width of the superior face of the centrum in the former is 123 per cent of that of the latter, while the distance from the apex of the neural spine to the anterior surface of the centrum is 126 per cent of that in the Sterkfontein specimen. The maximum superior–inferior length of the centrum (nearest the neural canal) in *SK* 3981*a*, however, is only 105 per cent of that of *Sts* 14. The neural spine is proportionately longer in *SK* 3981*a*, being 107 per cent of the maximum antero-posterior diameter of the superior face of the centrum as compared with 89 per cent in *Sts* 14. The spine also differs in being orientated at right angles to the superior–inferior axis of the centrum, while those of modern man and of *Australopithecus* are swept downward at an appreciable angle. The neural canal is proportionately a little larger in the Swartkrans specimen: its width is 55 per cent of the maximum width of the superior face of the centrum and its antero-posterior height is 79 per cent, as compared with corresponding values of 50 per cent and 66 per cent in *Sts* 14.

The costal face on the base of the pedicle and the adjacent part of the centrum is very prominent in *SK* 3981*a*; it juts well away from the vertebra and faces quite strongly posteriad. That on the right side appears to be quite natural; that on the left side has the bone of the posterior portion of the facet thickened in an unusual way. In the *Australopithecus* specimen, the costal facets are well defined but are not nearly so protuberant as in *SK* 3981*a* and they face laterally. In both respects, *Sts* 14 resembles modern man considerably more than does *SK* 3981*a*. The transverse processes in *Sts* 14 are proportionately better developed than is the case in the Swartkrans specimen. In this respect also the Sterkfontein specimen resembles modern man more than does that from Swartkrans.

The superior and inferior faces of the centrum are rather more heart-shaped than are those of *Sts* 14, which are more rounded except for the concavity of the margin where it borders on the neural canal. In this feature the Swartkrans specimen is closer to modern man, but because there is some variability in this feature in modern man, larger samples of the fossil vertebrae might show much overlap.

From the larger size of the Swartkrans vertebra as a whole compared with the inferior–superior length of the centrum, and from the proportionately larger neural canal and spine, it seems fair to conclude that the individual from whom the Swartkrans vertebra came was appreciably more robust and muscular than the Sterkfontein individual, although the close similarity in length of the centrum in the two vertebrae suggests that the two had roughly similar statures. Put differently, one may say that the two vertebrae, both from adults, provide no evidence of difference in stature between the two individuals from whom they came but do provide evidence of difference in robustness. A single centrum (*Sts* 73) from Sterkfontein, which has the bases of the pedicles attached, is similar in superior–inferior length of the centrum to *SK* 3981*a*, but the cross-sectional area of the centrum is slightly greater in *Sts* 73. The individuals from whom these specimens came were therefore probably approximately similar with respect to both stature and robustness. From other evidence, such as femur size and robustness, it seems that the body of *Paranthropus* in South Africa was appreciably more robust than that of *Australopithecus*. If this was indeed so, then we may speculate that the *SK* 3981*a* specimen belonged to a female individual of *Paranthropus* and that there was overlap in body size between *Australopithecus* males and *Paranthropus* females.

Associated with *SK* 3981*a* was an incomplete last lumbar vertebra of an adult individual (*SK* 3981*b*). It consists of almost the entire centrum, the base of the right pedicle as well as much of the left pedicle and almost all of the transverse process. The specimen seems to belong to a higher primate and resembles most closely

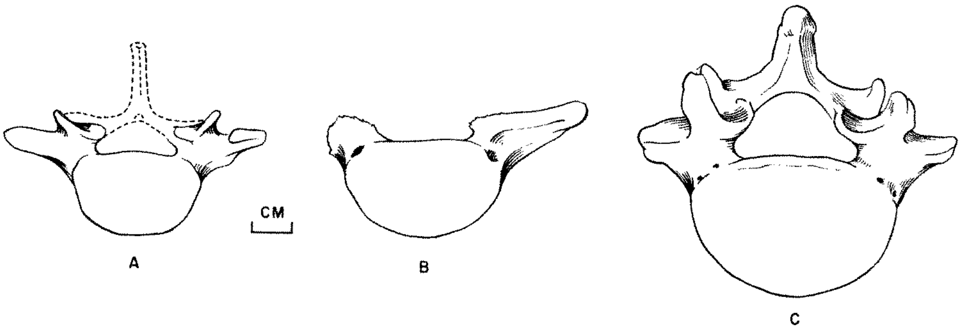


Fig. 2. Last lumbar vertebrae of (*A*), *Australopithecus* (*Sts* 14); (*B*), *Paranthropus* (*SK* 3981*b*); (*C*) *Homo sapiens* in superior view.

the one last lumbar vertebra of *Australopithecus* known (*Sts* 14).

The centrum is quite robust and the cross-sectional area of the superior face is appreciably greater than that of the inferior face. There is not much detail to be had from the specimen because all of the articular facets are gone as well as most of the neural arch and spine. The transverse process on the left side is very large compared with that of *Australopithecus* and is considerably larger even than that of modern man. As in the case of the latter two, the transverse process projects superiorly as well as laterally. In the Swartkrans specimen there is a tubercle on the inferior aspect of the transverse process which covers quite a large area (14·5 × 8·5 mm) but is not prominent. In the *Sts* 14 specimen of *Australopithecus*, this process is quite different and is very prominent, forming a very substantial part of the transverse process mass. In neither is the process like the usual condition in modern man, but that in *SK* 3981*b* is a little more like it than is that of *Sts* 14.

The neural canal in *SK* 3981*b* was wide (21·4 mm) but low, judging from what remains of it. The body not only has an appreciable disparity between the areas of the inferior and superior surfaces, but also is deeper anteriorly than it is posteriorly, that is, next to the neural canal (19·0 and 21·3 mm). This latter feature suggests a well developed promontory and lumbar lordosis. The former indicates that, as in *Australopithecus*, the body of the first sacral vertebral element was relatively small. The relatively great disparity between the inferior and superior surface areas of the body of the last lumbar vertebra seen in both the Sterkfontein and Swartkrans specimens is not typical of modern man, though there is a disparity of much smaller magnitude.

As in the case of *SK* 3981*a*, *SK* 3981*b* has a superior–inferior centrum length which is only slightly greater than that of *Sts* 14. But the cross-sectional area of the centrum is appreciably greater in the Swartkrans specimen, being 128 per cent as wide as that of *Sts* 14 in the case of the superior surface.

I conclude that both of the Swartkrans vertebrae belong to *Paranthropus*, and it also is likely that they came from a single individual. This conclusion is based on the rarity of hominid vertebral remains in the South African sites, hence two found directly associated are probably related, as well as the fact that the size relationship between the two closely matches that between the appropriate vertebrae in the Sterkfontein vertebral column *Sts* 14. Whether or not they came from a single individual, the Swartkrans specimens resemble the two known *Australopithecus* equivalents more closely than they do those of modern man. This material as a whole thus indicates that early hominids had vertebrae which are not only easily distinguishable from those of pongids but also are distinguishable from those of modern man. Furthermore, the material indicates that some differences existed between the *Australopithecus* and *Paranthropus* vertebrae. Because of the scantiness of this material, however, it is impossible to know whether adequate samples would reveal these as taxonomic differences or merely as individual variation. Because other, non-vertebral, evidence suggests appreciable differences between the two taxa, some differences could be expected also in the vertebrae. These vertebrae, for example, provide evidence of a difference in robustness —this is supported by other evidence. The evidence of these two vertebrae also makes it more likely now that, in spite of the difference in robustness, the population ranges for stature in the two taxa probably overlapped extensively. The robustness may have overlapped in such a way that *Paranthropus* females and *Australopithecus* males were similar in robustness as well as stature; but much more evidence is needed to prove or disprove this conclusion.

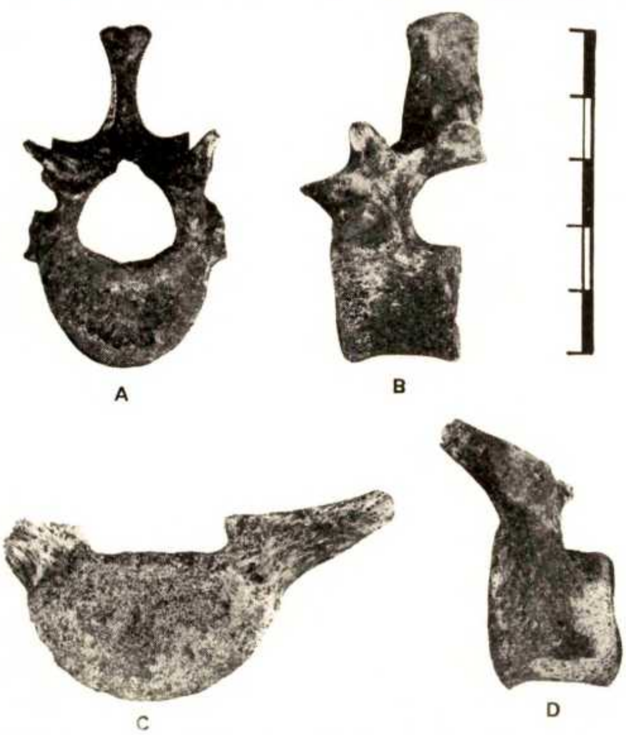


Fig. 3. *Paranthropus* vertebrae from Swartkrans. Last thoracic (*SK* 3981*a*), superior view (*A*), left lateral view (*B*); last lumbar (*SK* 3981*b*), superior view (*C*), left lateral view (*D*).

I thank Dr C. K. Brain of the Transvaal Museum, Pretoria, for allowing me to examine the Swartkrans material. This work was supported by the US National Science Foundation.

J. T. Robinson

University of Wisconsin,
Madison.

Received January 16, 1970.

[1] Brain, C. K., *Nature*, **225**, 1112 (1970).

More Evidence of an Advanced Hominid at Swartkrans

In July 1969, while two of us (R. J. C. and F. C. H.) were examining the australopithecine collections in the Transvaal Museum (Pretoria), one of us (R. J. C.) noticed that parts of a cranium (*SK* 847) from Swartkrans appeared to differ substantially from the robust australopithecine (*Paranthropus*) known in abundance from that site. Although the specimen had previously been regarded as *Paranthropus*, the possibility immediately came to mind that it might provide further evidence of the second hominid reported from that site by Broom and Robinson[1-3] in 1949 and then referred to *Telanthropus capensis*, but subsequently assigned by Robinson[4] to *Homo erectus*. Remains considered to represent this second hominid include a nearly complete mandible with most of the molar teeth (*SK* 15), an isolated worn left P_3 (*SK* 18*a*) thought to be from the same individual, a mandible fragment with two worn molar teeth (*SK* 45) and a maxilla fragment with worn and incomplete I^2, P^3 and P^4 (*SK* 80). A proximal radius (*SK* 18*b*), found closely associated with the type mandible (*SK* 15), has also been referred to the same hominid by Robinson[5].

After assembling portions of the *SK* 847 specimen, along with much of a left temporal (*SK* 846*b*), it was noted that there was a perfect join across the left side between the posterior palatal fragment of *SK* 847 and the previously known maxillary fragment *SK* 80. This perfect fit is clearly visible in Fig. 1.

The specimen now comprises most of the left side of the face, the left infratemporal fossa, and the left temporal region all in contact. The face includes the complete left

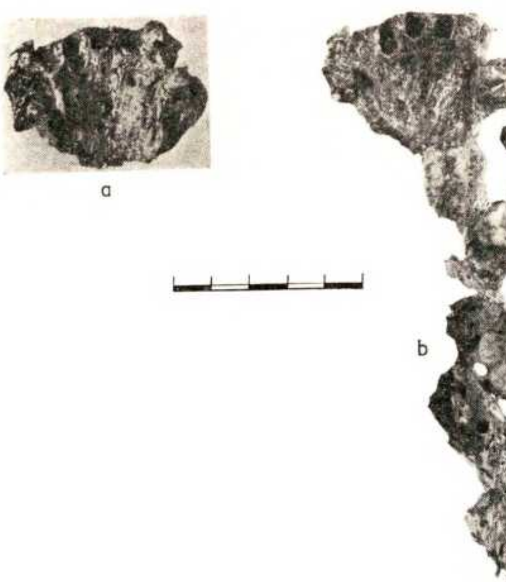


Fig. 1. (*a*) Palatal view of maxillary fragment (*SK* 80) as figured by Robinson[5]; (*b*) basal view of the assembled cranium (*SK* 847 and 846*b*) and the joined maxilla fragment, *SK* 80.

orbit and supraorbital torus and a bit of the adjacent frontal squama; the zygoma and anterior portion of the zygomatic arch; the complete nasal bones; the upper portion of the maxilla including the frontal process, orbital surface and the zygomatic process, as well as the posterior part of the palatal process with worn left M^3 in place and the distobuccal root of M^2; the anterior portion of the palate (*SK* 80) which is intact between the canine sockets, with worn left I^2, and bits of the roots of left and right P^3 (also an isolated lingual root of the right P^3 and a piece of the root and crown of the right P^4). The infratemporal region includes the left lateral pterygoid plate, a part of the greater wing of the sphenoid with infratemporal crest and foramen ovale. A substantial portion of the left temporal is present and comprises some of the squamous, tympanic and petro-mastoid elements, including the antero-inferior portion of the squamous part, the root of the zygomatic process, the preglenoid region, the mandibular fossa, the external auditory meatus, the inferior portion of the mastoid process (with exposure of the mastoid air cells) and the petrous temporal. A small fragment of the inferolateral border of the occipital is attached at the occipitomastoid suture. It should be noted that these skull parts are all attached and there is no crushing or distortion—the specimen having been prepared from its enclosing breccia by the acetic acid method—other than a slight lateral warping of the palate in relation to the midline of the face.

Other loose fragments from the same block of breccia and assumed to belong to the same individual include a small fragment of occipital with the left condyle, anterior condylar canal and jugular notch (847*j*), and a substantial fragment of the left orbital plate of the frontal adjoined to a fragment of the left lesser wing of the sphenoid with the optic foramen (847*d*). Also from this block of breccia are several other fragments which may or may not belong to the same skull. These are four thin intracranial fragments (847*k*, *l*, *m*, *n*), a large portion of thick left parietal, with sagittal and lambdoid sutures, and a well-delineated temporal line (847*e*), as well as two fragments of cranial vault (847*h* and *i*). Two other fragments labelled *SK* 847*f* and *g* do not belong to this specimen.

In his appraisal of the maxillary fragment *SK* 80, Robinson[5] stated that it differed from the Swartkrans robust australopithecine in the relatively great depth of the palate anteriorly, the shallow canine socket and the size and conformation of the incisive fossa and its immediate surroundings. He also noted that, in lateral view, the midline of this maxillary specimen is visible, whereas in the robust australopithecine this is not the case, because the canine roots and eminences are very robust, while the intercanine region of the maxilla is depressed. He also directed attention to the posterior situation in the nasal aperture of the anterior nasal spine and the lack of a well-differentiated sill or inferior margin of the nasal aperture in this australopithecine. By contrast, in the *SK* 80 specimen, the nasal spine is situated toward the front of the nasal aperture and there is a sharply defined sill-like inferior margin. He further mentioned that the vomer in the robust australopithecine is continuous with the back of the nasal spine, whereas in *SK* 80 there is a separation of 1 centimetre between the anterior nasal spine and the anterior point of the vomer.

The addition of a substantial portion of the upper facial skeleton and the lateral part of the cranial base to the maxillary fragment of *SK* 80 enables us to confirm that Robinson was quite correct in assigning this specimen to a hominid other than the robust australopithecine of Swartkrans. Moreover, it is now possible to demonstrate that the second hominid differed in several important features, not only from the robust, but also from the gracile form of australopithecine. In the following discussion, this new specimen (*SK* 847, *SK* 846*b*), together with the maxillary fragment (*SK* 80), will be treated as a unit and designated as *SK* 847.

In *SK* 847 the supraorbital torus is pronounced, being thickened both in its supraciliary (medial) and supraorbital (lateral) portions, the latter being rather markedly curved latero-inferiorly (Fig. 2). The specimen is broken in the midline, but the supraglabellar portion was evidently only slightly depressed between the adjacent supraciliary portions of the torus. This break exposes a substantial frontal sinus, extending laterally about 19 mm, distally about 22 mm, and superiorly about 16·5 mm. The postorbital constriction was only moderately developed at most. By contrast, in the robust australopithecine from Swartkrans, the supraorbital torus is thin, flattened and slopes smoothly backward into the frontal squama. The supraglabellar area is an extensive flattened planum with its postero-lateral margins defined by the postero-medially directed anterior temporal lines, which pass into a mid-sagittal crest in adult individuals. Postorbital constriction is markedly developed in robust australopithecines, both in South Africa and East Africa (*A. boisei*). In *Australopithecus africanus* (from Sterkfontein) the supraorbital torus is also thin and flattened, sloping posteriorly and gently superiorly into the frontal squama

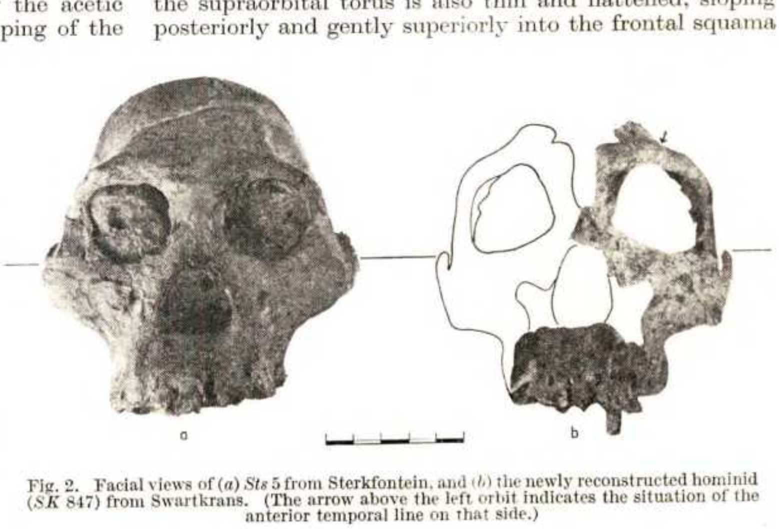


Fig. 2. Facial views of (*a*) *Sts* 5 from Sterkfontein, and (*b*) the newly reconstructed hominid (*SK* 847) from Swartkrans. (The arrow above the left orbit indicates the situation of the anterior temporal line on that side.)

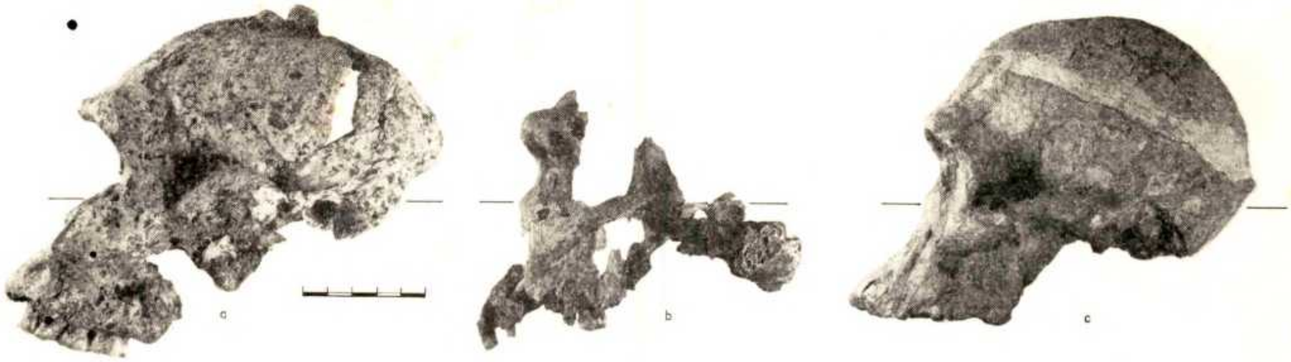


Fig. 3. Lateral views of (*a*) a robust australopithecine (*SK* 46) from Swartkrans; (*b*) the newly reconstructed specimen (*SK* 847)—a second hominid from Swartkrans; and (*c*) a gracile australopithecine (*Sts* 5) from Sterkfontein.

(Fig. 3). The supraglabellar planum is substantially less well developed and defined than in the robust australopithecines from Swartkrans. The degree of postorbital constriction is generally reduced in the gracile australopithecine from Sterkfontein, although it may be fairly substantial as in the *Sts* 17 individual; in any case, it differs from the characteristic robust australopithecine condition.

In *SK* 847, the frontal squama is undistorted and it slopes sharply upward behind the supraorbital torus. There is consequently a broad supratoral sulcus. The lower part of the frontal squama is obliquely fractured to expose the cancellous bone and its substantial thickness. In both the gracile australopithecine from Sterkfontein and the robust form from Swartkrans (Fig. 2), the suprafacial torus passes gently posteriorly into the squama, without well defined angulation between the squama and the torus. There is a complete absence in *SK* 847 of the very sharply defined posterior margin of the zygomatic process of the frontal, that is to say, the supraorbital element of the torus, which is so characteristic of the robust australopithecine from Swartkrans (for example, the individuals represented by *SK* 52, *SK* 48, *SK* 46 and *SK* 79). The frontal sector of the anterior temporal line is indicated in *SK* 847, but it is ill defined and not salient, and it is situated far laterally by comparison with its origin along the entire posterior margin of the supraorbital sector of the suprafacial torus in the robust australopithecines. This condition is less extreme in the gracile australopithecine from Sterkfontein (for example, *Sts* 5, *Sts* 71), but both the robust and gracile australopithecine condition contrasts markedly with that in the *SK* 847 individual.

In regard to the *SK* 80 maxillary fragment, Robinson[5] observed that from "the conformation of the immediate neighbourhood of the lower portion of the nasal aperture it seems probable that the bone surface on either side of the anterior nasal aperture was directed more nearly vertically than is the subnasal plane. The appearance of this general region must have been very similar to that in *Pithecanthropus* IV [Sangiran maxilla]. If this is the case there must have been a more definite projection of the [bony] nose in *Telanthropus* than is the case in *P. crassidens*". The new specimen (*SK* 847) now confirms Robinson's inference. The nasal bones are prominent, projecting well forward such that, in lateral view, they approach the plane linking the supraorbital torus with the anterior teeth. By contrast, in both robust and gracile australopithecines, the nasal bones are flattened, in approximately the same plane as the adjacent frontal process of the maxilla (the respective planes are different in the *SK* 847 specimen) and are situated behind or on a level with the plane of the zygoma.

The dental arcade of *SK* 847 is quite short (60 mm). The mandibular fossa is situated closer to the occlusal plane than in either robust or gracile australopithecines. This clearly indicates that the skull must have had a small mandible with a low ascending ramus. In describing the type mandible (*SK* 15) of the non-australopithecine hominid from Swartkrans, Robinson[5] noted that, although both mandibular condyles were missing, the break in the ascending ramus on the right side of the specimen occurred at that point where the ramus was thickening just below the condyle; on the left side, the break occurred in the same relative position. He therefore concluded that the ascending ramus was low and in his reconstruction of the specimen he gave it a condylar height of 55 mm. If the mandible of *SK* 15 is occluded with the skull of *SK* 847, the broken portion of the left ramus reaches about 18 mm below the articular eminence of the mandibular fossa. In this position the dental arcades match well (Fig. 4*c*). If allowance is made for breakage, and for some extension

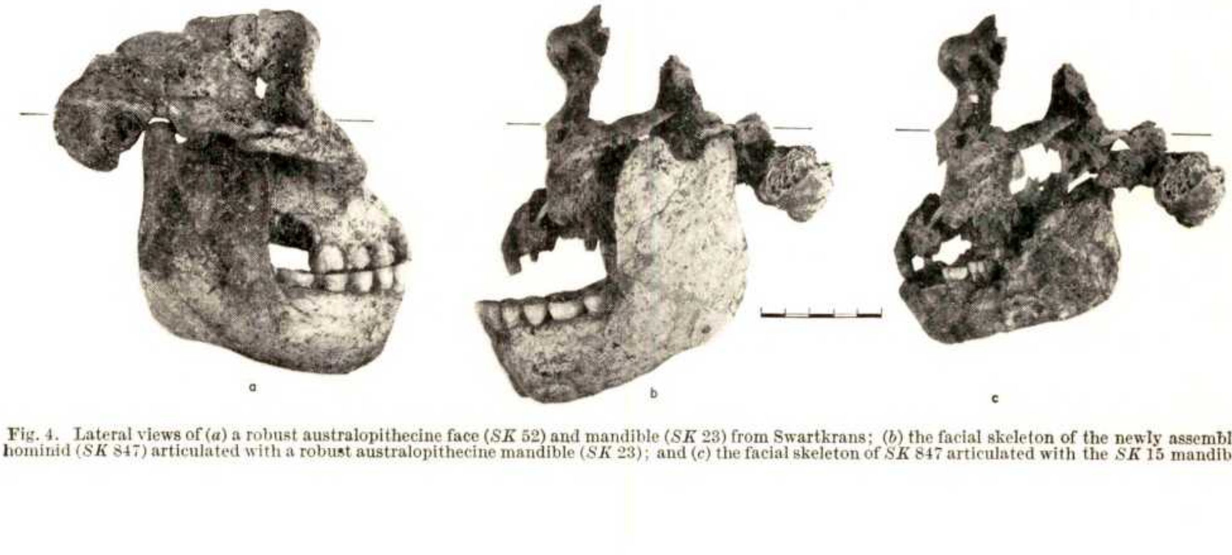


Fig. 4. Lateral views of (*a*) a robust australopithecine face (*SK* 52) and mandible (*SK* 23) from Swartkrans; (*b*) the facial skeleton of the newly assembled hominid (*SK* 847) articulated with a robust australopithecine mandible (*SK* 23); and (*c*) the facial skeleton of *SK* 847 articulated with the *SK* 15 mandible.

of the type mandible (*SK* 15) caused by crushing, then the measurement of the dental arcade would be very comparable with that of *SK* 847. The dental arcade of the type mandible in its distorted state is about 66 mm, but, when undistorted, would have approximated 60 mm. If, however, the condyle of a fairly "gracile" Swartkrans australopithecine mandible (*SK* 23) is fitted to the mandibular fossa of the newly assembled *SK* 847 specimen, the upper and lower teeth could not occlude, and the mandible projects far forward of the maxilla (Fig. 4*b*). On the other hand, the same mandible (*SK* 23) articulated nearly perfectly with the incomplete robust australopithecine basicranial fragment of *SK* 52 (Fig. 4*a*). It is clear that only a mandible of the size of *SK* 15, with a low ascending ramus, could have fitted this newly assembled skull of *SK* 847. The heavy wear on the M^3 of *SK* 847 shows that it could not, in fact, belong with the comparatively little worn mandible of *SK* 15, but the advanced hominid right mandible fragment, *SK* 45, with heavily worn M_1 and substantially worn M_2 could possibly represent the same individual as *SK* 847.

The buccolingual width of the left M^3 of *SK* 847 is 15·4 mm. There is a large mesial interproximal wear facet, so it is not possible to determine accurately its mesiodistal length. The actual minimum length measurement across this facet is 11·7 mm; its original length could have been as much as 12·7 mm.

The parietal fragment (*SK* 847*e*) is not complete. The length measurement on a chord, from the broken anterior edge along the sagittal suture to lambda, is 82·5 mm. The maximum thickness, taken on the sagittal suture 22 mm anterior to lambda, is 7·5 mm, but the maximum thickness on the posterior part of the temporal line is 8·5 mm. The distance of the temporal line below the sagittal suture is 37 mm (at lambda) and 39 mm at a maximum further forward. This indicates a pattern of temporal musculature origin which differs very markedly from that known to be characteristic of robust australopithecines.

The recognition of this specimen from Swartkrans removes it from the inventory of robust australopithecine individuals from this site[6]. The specimen confirms Robinson's view of the presence at that site of two distinct hominids. The most abundant, represented by at least sixty individuals, is a robust australopithecine variously designated as *Paranthropus robustus* or *Australopithecus robustus*. The second hominid, represented thus far by six catalogued specimens, clearly differs from that robust australopithecine and also from gracile australopithecines, as Robinson has long maintained. Its lower dentition and the structure of its facial skeleton and mandible indicate to us that it should be regarded as a species of the genus *Homo*. There are some resemblances to *Homo erectus*. It is preferable, however, until further fossil material is recovered as a consequence of renewed work at Swartkrans[7], to treat the remains as *Homo* sp. indet.

This work was made possible by a study grant to one of us (R. J. C.) from the Wenner-Gren Foundation for Anthropological Research and to the second author (F. C. H.) by a grant, in conjunction with the work of the Omo Research Expedition, from the US National Science Foundation.

R. J. CLARKE

Centre for Prehistory and
Palaeontology,
PO Box 30239,
Nairobi.

F. CLARK HOWELL

Department of Anthropology,
University of Chicago,
Illinois.

C. K. BRAIN

Transvaal Museum,
Pretoria.

Received January 16, 1970.

[1] Broom, R., and Robinson, J. T., *Nature*, **164**, 322 (1949).
[2] Broom, R., and Robinson, J. R., *Amer. J. Phys. Anthrop.*, **8**, 151 (1950).
[3] Broom, R., and Robinson, J. T., *Transvaal Mus. Mem.*, **6** (1952).
[4] Robinson, J. T., *S. Afric. J. Sci.*, **57**, 3 (1961).
[5] Robinson, J. T., *Amer. J. Phys. Anthrop.*, **11**, 445 (1953).
[6] Tobias, P. V., and Wells, L. H., in *Catalogue of Fossil Hominids* (edit. by Oakley, K. P., and Campbell, B. G.), 83 (British Museum (Natural History), 1967).
[7] Brain, C. K., *S. Afric. J. Sci.*, **63**, 378 (1967).

Stone Artefacts from Swartkrans

WHILE in South Africa on a visit to study the cultural material from the australopithecine sites, I was invited by Dr C. K. Brain to describe the artefacts he had obtained from Swartkrans[1]. Examination of this series and of the tools excavated from the Sterkfontein Extension Site has proved to be of particular interest on account of certain resemblances to developed Oldowan assemblages from Bed II, Olduvai Gorge, now being analysed for publication in volume three of the Olduvai series.

I have only taken into account specimens that are unquestionably derived from the pink breccia. The assemblage studied consists of twenty-seven artefacts, most of which still have patches of matrix adhering to them or else are specimens that have been removed from the breccia under Dr Brain's personal supervision. On the basis of the terminology used in describing the cultural material from Olduvai Gorge, the following tools, flakes and utilized material can be distinguished (Table 1).

Table 1

Side choppers	8	Heavy duty pick	1
End choppers	2	Heavy duty scrapers	2
Pointed chopper	1	Light duty scraper	1
Bifaces	3*		
Cleaver	1	Anvils	2
Discoid	1	Flakes	4
Sub-spheroids	2	Utilized cobbles	2
			30

* These bifaces were found after this paper had been written. It should be noted that they are proportionately less common than would appear, because other artefacts have also been found, which it has not been possible to describe here.

Quartzite, generally in the form of cobbles, is the most common raw material (twenty-one specimens), but one side chopper and the cleaver are made from diabase, the discoid from chert and another side chopper from vein quartz. With the exception of three diabase specimens and one massive quartzite side chopper, which are weathered, all are in sharp condition.

Tools

Side choppers. Three of the eight side choppers are unifacial (*SK* 3947, *SK* 3948 and *SK* 3964). Two are made from quartzite cobbles with a smooth cortex surface forming the butt ends and one from diabase. In one example, the working edge has been flaked only from the cortex surface and in two from a single negative flake scar (Fig. 1*b*, *SK* 3948). The working edges are usually more even than in the bifacial choppers, although secondary trimming scars are only visible on the figured specimen. In one example there is battering and damage on the butt end, indicating additional use as a hammerstone. In the diabase specimen, the butt is formed by a flat vertical face similar to that in the vein quartz chopper described below. (Measurements: 80 × 86 × 50 mm; 85 × 92 × 66 mm; and 69 × 76 × 43 mm. Working edge circumference ratios, 22 per cent, 29 per cent and 36 per cent.) The five bifacial side choppers are *SK* 3973, *SK* 3965, *SK* 3951, *SK* 3967 and *SK* 3971. *SK* 3973 is made from vein quartz (Fig. 1*d*). The working edge is jagged, chipped and partly blunted by use and the butt is formed by a flat vertical surface. The nature of the material makes accurate determination of flake scars difficult, but this specimen appears to have been flaked over the upper and

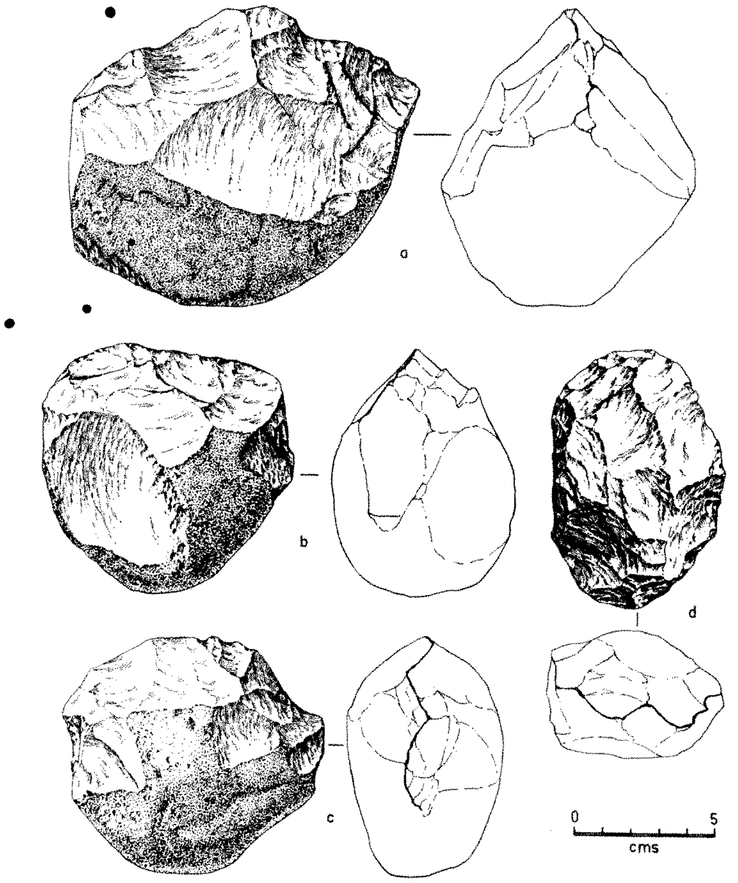


Fig. 1. Stone artefacts from Swartkrans: side choppers, *a*, *SK* 3951; *b*, *SK* 3948; *c*, *SK* 3967; and *d*, *SK* 3973.

lower faces, as well as along the working edge and butt. (Measurements: 50 × 92 × 41 mm, working edge/circumference ratio 36 per cent.) *SK* 3965 and *SK* 3951 are two massive specimens, both made from quartzite and weighing 3 pounds 2 ounces and 3 pounds 0 ounces respectively. The butts are formed by natural weathered surface or rounded cortex. The working edges are jagged and sharp but chipped by use and in one example also partially blunted. In the second specimen (Fig. 1*a*) projecting areas on the butt have also been battered and damaged. (Measurements: 108 × 117 × 88 mm and 106 × 131 × 88 mm, working edge/circumference ratios 55 per cent and 36 per cent respectively.) *SK* 3967 (Fig. 1*c*) is a particularly well made example of a "classic" chopper, made from a quartzite cobble, with multiple bifacial flaking of the working edge and a rounded butt formed by cortex surface. It is in mint condition with only a minimum of chipping on the working edge, including a crushed notch 15 mm wide. (Measurements: 85 × 96 × 57 mm, working edge/circumference ratio 56 per cent.) *SK* 3971 is a small, oblong specimen, made from a quartzite cobble. One edge is almost vertically flaked and the intersection of this surface with the upper and lower faces forms two working edges along the same side, both of which show some bifacial flaking. The butt is formed by a rounded cortex surface. (Measurements: 52 × 72 × 37 mm.)

End choppers (*SK* 3950 and *SK* 3949). Both examples are made from quartzite cobbles and both are unifacial. In one the working edge is trimmed from a single negative scar and the other, which exhibits only a minimum of flaking, has been trimmed from the cortex surface. The specimen is made on an oblong cobblestone flaked transversely across one end. In the second example, the working edge is at the wide end of the cobble, which tapers towards the butt end. Both specimens are in sharp condition, although the working edges have been considerably chipped by use. (Measurements: 108 × 60 mm and 123 × 74 × 55 mm. Working edge/circumference ratios 26 per cent and 27 per cent.)

Pointed chopper (*SK* 3966). Made from a quartzite cobblestone with a small area of cortex surface retained at the butt and on one face. This specimen is diamond-shaped in cross-section, towards the tip, with a marked longitudinal ridge on both the upper and lower faces, formed by the intersection of the trimming scars on either edge. The tip is bluntly pointed and the edges on either side are chipped by use. In mint condition. (Measurements: 71 × 65 × 56 mm.)

Bifaces. Two of the three bifaces are considerably weathered. In *SK* 3979, made of quartzite, the ridges intervening between the flake scars are abraded and rounded, so that the specimen appears to be rolled (Fig. 2*c*). In *SK* 3980, made of diabase, the edges are relatively sharp, but the whole surface is pitted and damaged by weathering (Fig. 2*b*). *SK* 7879, made of dolerite, is in mint condition (Fig. 2*a*). *SK* 3979 (98 × 58 × 38 mm) is asymmetrical, with an oblique butt which is relatively thick in relation to the pointed end. A longitudinal dorsal heel is present on one face (opposite to that illustrated) and the flaking is simple, with large, deeply indented scars. The edge is sinuous and there is some irregular chipping which may have been caused by use. *SK* 3980 (122 × 87 × 49 mm) might be described as a crude ovate. It is difficult to determine the nature of The trimming scars on account of the extensive weathering, but they appear to be flatter and more accurately directed than in the previous specimen. The edge is relatively straight and the cross-section is biconvex and symmetrical. *SK* 7879 (137 × 95 × 32 mm) is made on a side-struck flake in which the bulb has been trimmed away. The edges are almost straight and the tip is sharply pointed. The butt is also pointed, but is formed by the intersection of an oblique natural fracture with a flake scar on the primary surface. Trimming of the edge is confined to the dorsal aspect and extends round the circumference with the exception of an area near the butt.

Cleaver (*SK* 3962). This is made on a relatively thin side-struck flake, approximately quadrilateral in shape (Fig. 2*d*). Both lateral edges are trimmed by means of shallow well-controlled flaking and the bulb has also been trimmed off. The tool is square-ended and the edges at both extremities are thin and sharp and are formed in typical cleaver fashion by the intersection of broad flat scars with the primary flake surface. Slight chipping is evident at the broader end. This specimen is extensively weathered, but would fit comfortably within a middle or upper Acheulean assemblage. (Measurements: 124 × 86 × 25 mm.)

Heavy duty pick (*SK* 3955). A crude, asymmetrical specimen, made from a cobble of coarse-grained quartzite (Fig. 3*a*). Cortex is present on one face and on the butt end. The tip is bluntly pointed and quadrilateral in cross-section, with marked longitudinal ridges on the upper and lower faces, at the intersection of the trimming scars from either edge. One lateral edge is also bifacially flaked for the greater part of its length. At the butt end, the surface has been battered and pitted along a projecting ridge. (Measurements: 150 × 95 × 68 mm.)

Discoid (*SK* 3959). A flat, relatively thin specimen made from chert (Fig. 3*c*). The circumference is uneven and the whole tool is somewhat irregular in shape. Radial trimming is evident on one face, but the obverse appears to consist largely of a natural fracture surface. Chipped utilization is present along part of the edge. (Measurements: 70 × 69 × 26 mm.)

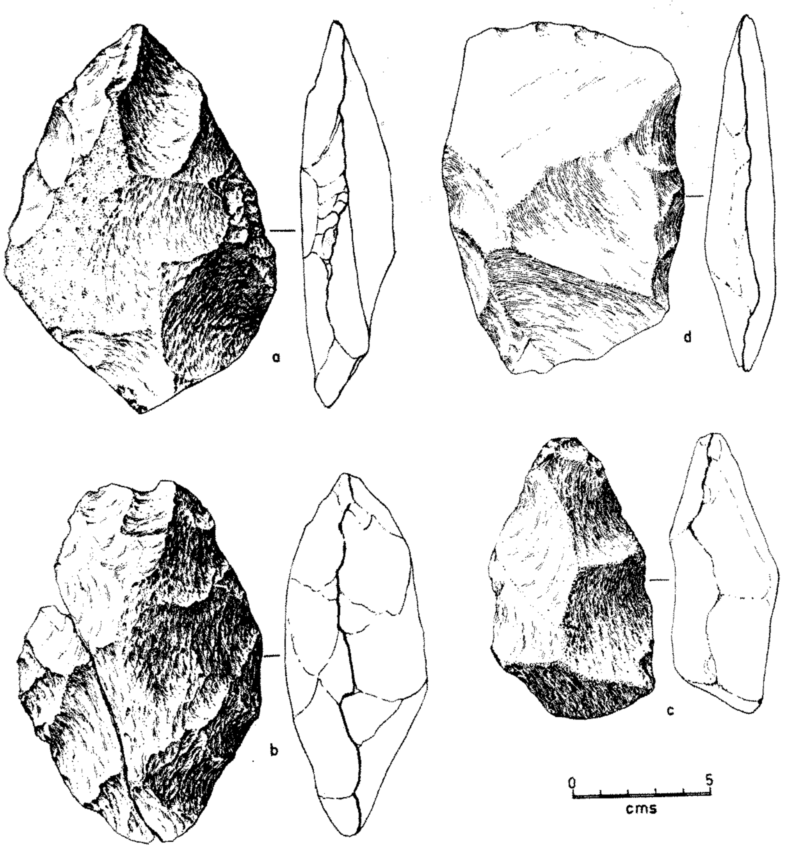


Fig. 2. Stone artefacts from Swartkrans: bifaces (*a*) *SK* 7879; (*b*) *SK* 3980; (*c*) *SK* 3979; and cleaver (*d*) *SK* 3962.

Sub-spheroids (*SK* 3956 and *SK* 3957). Both these specimens are of quartzite and both are faceted although the greater part of the surfaces have been battered, so that projecting ridges are blunted and partly obliterated. One example is oblong, but shows identical flaking and wear to that on the more spherical specimens. (Measurements: 85 × 72 × 68 mm and 88 × 66 × 58 mm.)

Heavy duty scrapers (*SK* 3963 and *SK* 3960). One example is made on a quartzite flake 84 mm long and 88 mm wide, struck from a cobblestone. Approximately 50 per cent of the circumference has been steeply but irregularly trimmed. The edge is chipped and blunted by use on the dorsal aspect and there is also a crushed notch 12 mm wide and approximately 4 mm deep. The second specimen is made on a sub-spherical quartzite cobble with cortex remaining on the greater part of the exterior (Fig. 3*b*). The working edge has been steeply and evenly trimmed from a single negative scar on the under-surface and the edge is considerably blunted by use. In many respects this specimen resembles the unifacial chopper *SK* 3948 except for the angle of the working edge, which is approximately a right angle instead of being acute. (Measurements: 96 × 80 × 60 mm.)

Light duty scraper (*SK* 3970). A small "nosed" scraper made on a fragment of quartzite 34 mm long and 24 mm wide. The working edge is steeply trimmed with a notch on either side of a small, rounded scraper edge.

Anvils (*SK* 3958 and *SK* 3952). Neither of these specimens can be considered as typical, but they have been classed as anvils because they more closely resemble these tools than any other category. *SK* 3958 consists of a large oblong quartzite cobble with a longitudinal flake removed from one face. Further flakes have been struck from both lateral edges of this flake scar, forming approximately right-angled edges which have been battered and step-flaked in the manner seen on more typical anvils. The edges of some subsidiary scars on one side of the main flake scar also show similar utilization. (Measurements: 160 × 87 × 85 mm.) The second example is a sub-spherical quartzite cobble, flaked on one face to a blunt edge. In some respects it resembles a chopper, except for the angle of the edge, which exceeds 90°. Blunting and battering are evident on the edge. (Measurements: 77 × 52 × 60 mm.)

Flakes. These consist of three whole and one broken quartzite flakes. The complete flakes are end-struck, and one has been slightly chipped along the edges. The largest specimen is a cortical flake struck from a cobblestone. (Measurements: 73 × 70 mm, 70 × 36 mm and 38 × 27 mm respectively.)

To sum up the characteristics of the industry which I have described, choppers can be said to constitute 37 per cent of the tools, while other forms are scantily represented. Polyhedrons do not occur at all. The outstanding tool is the diabase cleaver which seems to be too evolved to be compatible with the remainder of the series. The nature of the material, which permitted well controlled flaking, may, however, be the cause of this. (The existence of a cleaver in such a context would scarcely have been accepted until recent years, when several specimens have come to light at Olduvai Gorge in similar assemblages.)

Comparisons with Sterkfontein Artefacts

In order that these notes on the collection of artefacts from Swartkrans may be more meaningful, it is useful to compare them briefly with the much larger assemblage recovered by Dr J. T. Robinson from the pink breccia at the Sterkfontein Extension Site which probably does not antedate the Swartkrans material by any significant interval of time*.

As a result of examining the Sterkfontein material in the light of familiarity with developed Oldowan assemblages from East Africa, I do not agree with Mason's classification published in 1962 (ref. 2). None of the material from Olduvai was then available for study, and Dr Mason himself would now probably find reason for modifying his classification if he had the opportunity to examine it. He lists a large number of cores, only two choppers, one whole and one broken biface. He also states that polyhedrons are not present. In my view, there are only two or three specimens which can be classed as cores and I would term the greater part of Dr Mason's "cores" as either choppers or polyhedrons. I also consider that there are not two but three complete and five broken bifaces. Leaving aside the utilized material, manuports and debitage (of which there is singularly little), I would prefer to re-classify the tools as shown in Table 2.

Table 2

Side choppers	19
End choppers	4
Bifaces (3 complete, 5 broken)	8
Polyhedrons	23
Discoids	4
Spheroid	1
Sub-spheroids	3
Scrapers, heavy duty	10
Scraper, light duty	1

The principal difference between my classification and that published by Dr Mason lies in my rejection of his "cores" and the allocation of these specimens either to polyhedrons (the term polyhedron has been defined elsewhere as referring to specimens with three or more cutting edges) or choppers. This is because I am reluctant to class artefacts as cores unless there is evidence to indicate that they were by-products of tool-making and jettisoned as debitage when the required flakes had been obtained from them. This is not the case at Sterkfontein, where flake tools are almost absent. Added to this, cores in the strict sense should show little or no utilization whereas the Sterkfontein specimens are often considerably chipped by use and blunted on the edges, as indeed was noted by Dr Mason. I consider therefore that they should rank rather as end-products or tools, in common with choppers, discoids or spheroids, which are, in fact, also "cores" in the sense that they are nuclei from which flakes have been detached.

---

* The material from the pink breccia at Makapansgat was kindly put at my disposal by Professor R. Dart and Mr B. Maguire, but, although it included a number of quartzite flakes and utilized pieces, there do not seem to be recognizable tool forms among the *in situ* specimens.

In connexion with the lack of flakes, it may be noted that at sites in Olduvai Gorge when similar assemblages occur, including choppers and polyhedrons and where the heavy duty tools are usually made from lava, there are likewise insufficient flakes for the number of tools present, had they been made on the spot, although quartz and quartzite flakes are often abundant. It would therefore seem that heavy duty tools were usually made away from the living sites, wherever the raw materials were obtained. Sterkfontein should almost certainly be considered as a domestic site in view of the localized concentration of artefacts in the area of the West Pit and Sterkfontein Extension Site and their virtual absence nearby at the same horizon. The position at Swartkrans is still not clear and the conditions under which the artefacts occur will only be known when the detailed excavations planned by Dr Brain have been carried out.

Comparisons with Olduvai Artefacts

One of the most striking features in the assemblages from Swartkrans and Sterkfontein is the relatively large size of the tools when compared with similar specimens from Olduvai, although a high proportion at both sites are made from cobbles. This is clearly demonstrated by the figures for the average mean diameters of side choppers, a tool class which is numerically strong at all three sites (Table 3).

Table 3

Swart-krans	Sterkfon-tein	Olduvai Lower Bed I (*DKI*)	Olduvai Upper Bed I (*FLK* N)	Olduvai Middle Bed II (*MNK*)	Olduvai Upper Bed II (*TK*)
79 mm	71 mm	65 mm	65 mm	64 mm	61 mm

The collection from Swartkrans is unfortunately too limited for quantitative analysis, but a comparison of the Sterkfontein assemblage with assemblages from developed Oldowan sites in Bed II, Olduvai, is shown in Table 4.

Table 4

	Sterk-fontein	*MNK* II (Mid Bed II)	*SHK* II (Upper Bed II)	*TK* II (Top Bed II)	*BK* II (Top Bed II)
Choppers	37·0%	17·5%	17·5%	9·9%	15·8%
Bifaces	10·0	2·0	7·0	8·6	5·1
Polyhedrons	29·0	—	2·3	1·0	1·3
Discoids	5·0	5·3	6·3	3·1	3·9
Spheroids and sub-spheroids	5·1	36·3	34·6	26·5	27·9
Scrapers, heavy duty	12·6	5·1	5·2	4·5	2·5
Scrapers, light duty	1·3	10·8	8·0	26·5	14·1
Other heavy duty tools	—	19·7	14·0	10·6	12·1
Other light duty tools	—	3·3	5·0	9·2	17·2

It will be seen that choppers and polyhedrons are proportionately far more numerous at Sterkfontein, with 37 per cent and 29·0 per cent respectively as against 17·5 per cent and 2·3 per cent, which are the highest figures for these tools at Olduvai. Bifaces are also slightly more numerous, although the difference between the highest Olduvai figure of 8·6 per cent at *TK* and 10·1 per cent at Sterkfontein is probably not of great significance. Spheroids and sub-spheroids are very much scarcer at Sterkfontein than at any of the Olduvai sites, where they are always plentiful in developed Oldowan assemblages. At Sterkfontein, too, heavy duty scrapers outnumber light duty specimens, whereas at Olduvai the latter are nearly always more plentiful.

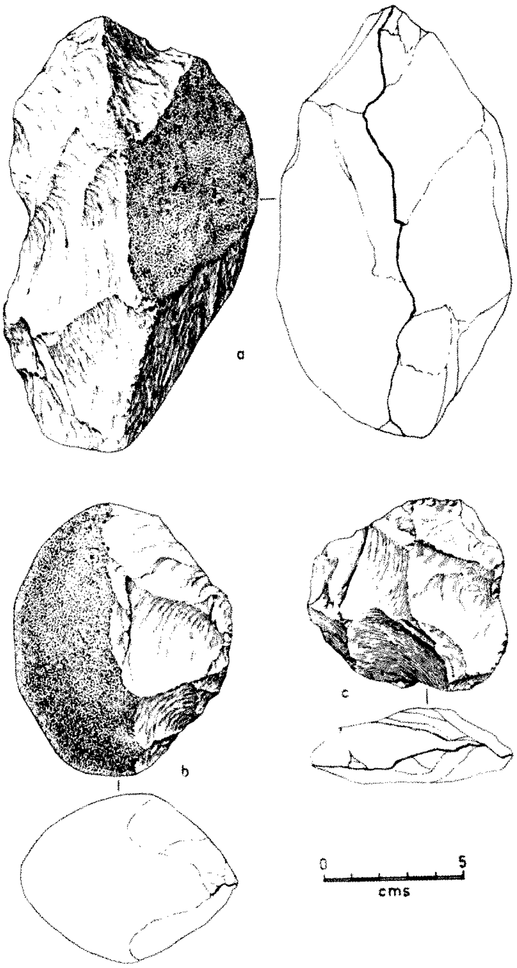


Fig. 3. Stone artefacts from Swartkrans: (*a*) heavy duty pick *SK* 3955; (*b*) heavy duty scraper *SK* 3960; (*c*) discoid *SK* 3959.

In spite of the difference in the relative proportions of the various tool forms, it must be considered significant that the forms which occur at Swartkrans and Sterkfontein are represented in all the developed Oldowan assemblages, including the somewhat crude bifaces, the cleaver and the heavy duty pick. The assemblages from these sites, however, include a wider range of tools, particularly those in Upper Bed II, where modified and battered blocks and nodules are a common form and where burins, awls and *outils écaillés* also occur. In fact, in spite of the distance separating the two areas, there seems to be a similarity between the industries in South and East Africa. When the planned excavations of the implement-bearing breccias at Swartkrans and Sterkfontein have been carried out, so that a more comprehensive series of artefacts is available, much more detailed comparisons should be possible.

I thank Professor and Mrs R. Dart for their kindness and hospitality during my stay in Johannesburg, and Dr C. K. Brain, Mr B. Maguire and Mr A. Hughes for so generously giving their time to show me the sites and material and also for helping me in every way possible. Dr R. Mason also very kindly invited me to re-examine the Sterkfontein material.

M. D. Leakey

Centre for Palaeontology and Prehistory,
PO Box 30239, Nairobi.

Received January 16, 1970.

[1] Brain, C. K., *Nature*, **225**, 1112 (1970).

[2] Mason, R. J., *S. Afric. Archaeol. Bull.*, **17**, 109 (1962).

Conceptual Model of the Bahamian Platform for the Last 135 Million Years

by
GEORGE W. LYNTS
Department of Geology and Marine Laboratory,
Duke University,
Durham, North Carolina 27708

The subsidence history of the Bahamian Platform points to a non-uniform rate of sea-floor spreading since the beginning of the Cretaceous. Lower Cretaceous rates of perhaps 5 cm/yr led to an active trench system at the boundary between the American and Caribbean lithosphere plates, and the rapid subsidence of the continental margin produced graben faults on the Bahamian Platform and normal faults in Cuba.

THE Hess–Dietz hypothesis of sea-floor spreading[1,2] has been tested over the past decade. The lithosphere plate–global tectonic model[3–5] requires an interrelationship between sea floors and continents and among continents because of interacting lithosphere plates. This model provided a relatively simple basis for formulating and testing hypotheses about the Earth, and Dott[6] therefore suggested that the study of well known land areas should "provide tests and refinements of postulated spreading histories".

Dietz *et al.*[7] and Lynts and Stehman[8] have suggested that the origin and history of the Bahamian region (Fig. 1) might be understood in terms of sea-floor spreading. I therefore propose to look at the sedimentary and tectonic history of the Bahamian Platform to see what it can reveal about the spreading history over the last 135 m.y.

A 4,445 m test well that bottomed out within the lower Cretaceous[9] was drilled on Andros Island (Figs. 1 and 2). Only shallow water carbonates and evaporites were encountered in the well[9,13], implying that the Bahamian Platform has subsided 4,445 m since lower Cretaceous time. The rate of subsidence of the Bahamian Platform has not been uniform over the past 120 m.y. (Fig. 3). Higher rates of subsidence prevailed during the lower Cretaceous and Palaeocene–Eocene, while lower rates occurred during the other epochs.

Vogt and Ostenso[14] suggest that continental margin subsidence seems to be related to the subsidence of oceanic crust after it leaves the crestal region of a mid-oceanic ridge. If so, then rates of continental margin subsidence should be related to rates of sea-floor spreading. Continental margins adjoining the Bahamian Platform should therefore record parallel subsidence histories. Fig. 3 shows that the subsidence history of the Florida Keys and Cape Hatteras is similar to that found on the Bahamian Platform.

Parallel subsidence histories on the Bahamian Platform and south-eastern North American continental margin support the validity of the Vogt–Ostenso[14] hypothesis and suggest that sea-floor spreading has not been steady state over the last 120 m.y. This history of sea-floor spreading seems to contradict the apparently steady state spreading history indicated by data from ocean basins. Heirtzler *et al.*[15] summarized magnetic anomaly data for all major ocean basins and pointed out that the anomalies suggest a steady state spreading history for the past 70 m.y. Percival[16] discussed the palaeontological evidence from drill holes in the South Atlantic and concluded that they supported a steady state spreading history for the Caenozoic. Data from continental margins in the western North Atlantic and circum-Pacific belt[6] suggest a non-steady state spreading history, while data from magnetic anomalies and deep sea drill holes in ocean basins support a steady state spreading history. Which of these data records the correct spreading history?

Schneider and Vogt[17] reviewed lines of evidence that may be used to recognize non-steady state sea-floor spreading. Phillips[18] and Vogt[19] noted that magnetic anomalies record a non-uniform spreading history for part of the Mid-Atlantic Ridge in, respectively, the North Atlantic and a region east of the Reykjanes Ridge. Le Pichon *et al.*[20], Van Andel *et al.*[21] and Fox *et al.*[22] reported that fracture zones in the Atlantic were not consistent with a steady state spreading history. Langseth *et al.*[23] suggested that the total heat released over the Mid-Atlantic Ridge seemed to be considerably lower than would be expected in a steady state model. Ewing and Ewing[24] described abrupt changes in sediment thickness between crests and flanks of mid-oceanic ridges that may record an episodic spreading history. Finally, Le Pichon[4] computed various lithosphere plate models for different magnetic anomalies that supported a non-steady state spreading history.

There seems to be sufficient evidence to reject the steady state model of sea-floor spreading and accept a non-uniform model. What, then, do the ocean basin magnetic anomalies[15] and deep sea drill holes[16] data imply? They seem to provide evidence against any long quiescent interval discussed by Ewing and Ewing[24], Schneider and Vogt[17] and Le Pichon[4].

The lithosphere plate model requires the rate of spreading to vary as the sine of the distance from pole of rotation[4].

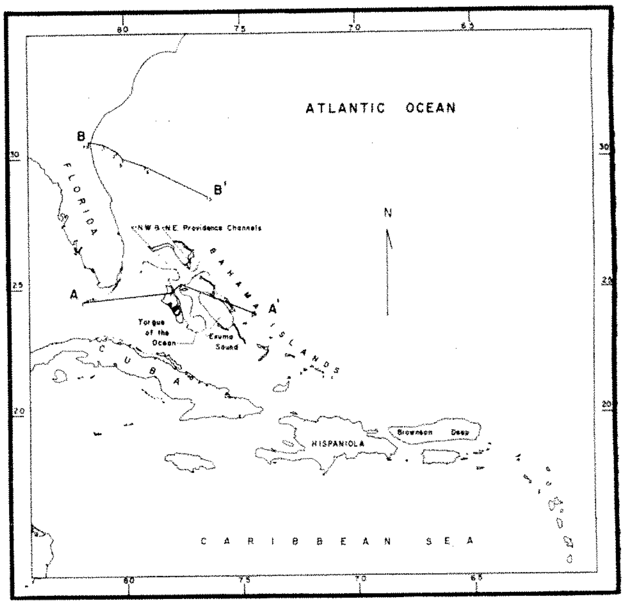


Fig. 1. Index map of Bahamian–Caribbean region. A–A′ and B–B′ are locations of cross-sections shown in Fig. 2.

The Vogt–Ostenso[14] hypothesis would result in a corresponding variation in continental margin subsidence with distance from lithosphere plate rotational pole. The North Atlantic model locates the lithosphere plate rotational pole in the vicinity of Greenland for the last 9 m.y.[3,4,22]. This would mean that the rates of spreading —and therefore the amount of subsidence—should decrease northward from the Bahamian Platform. Fig. 3 shows that there is less subsidence in the vicinity of Cape Hatteras than on the Bahamian Platform. Figs. 2 and 3 also show that there has been less subsidence in the western part of the continental margin than in its eastern extension.

The subsidence history of the western North Atlantic continental margin fits a lithosphere plate model of the Earth. It also provides further evidence that sea-floor accretion has been continuous, but not uniform over the past 135 m.y.

Le Pichon[4] constructed a model of the Atlantic at the time of magnetic anomaly 31 (Palaeocene). He argued that if most Cretaceous drift occurred during the lower Cretaceous (Bahamian subsidence history (Fig. 3) tends to support this interpretation), spreading half rates may have been as high as 6–8 cm/yr. He reported that spreading half rates greater than 5 cm/yr now cause thrusting, while half rates less than 5 cm/yr cause folding. What effects, if any, did spreading rates of this magnitude have on the region ?

The Puerto Rico Trench[25] (Brownson Deep, Fig. 1) is south-east of the Bahamian Platform. It is an anomalous trench in that it occurs in a region with spreading half rates of less than 5 cm/yr[4,18]. Seismic data of Drake *et al.*[26] imply that the mantle east of the Bahamas has a depth and configuration similar to that found beneath the Puerto Rico Trench. This may represent either a separate trench or an extension of the Puerto Rico Trench that has filled with sediment. The Puerto Rico Trench does not seem to be as active seismically as its Pacific counterparts, and the Bahamian Platform is not seismically active[27]. This suggests that the Puerto Rico Trench is a relict feature.

Deep entrenchments[28–31] dissect the Bahama Banks (Fig. 1). Gravity and seismic data suggest that these entrenchments have a tectonic origin[31,32], and Talwani *et al.*[31] called on graben faulting for their origin. Many workers have suggested that the Florida Straits also have a graben fault origin[33–37]. Because this region is not now tectonically active[27] and the entrenchments have been deep water features since at least the early Tertiary[8,38], they may have originated during either the Cretaceous or early Tertiary. Caenozoic subaqueous erosion and deposition have modified them to their present topographic expression[36,38,39].

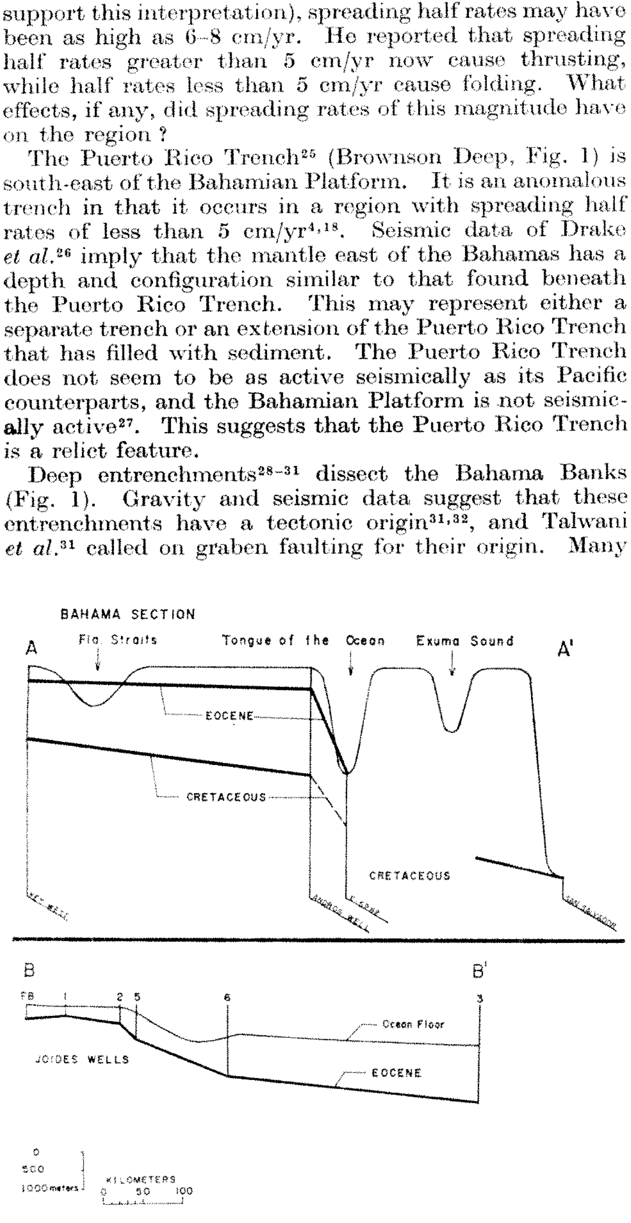


Fig. 2. Diagrammatic cross-sections of traverses from Florida Keys to eastern Bahamian region[8–10] (A–A') and JOIDES[11] drill holes on the Florida continental margin (B–B').

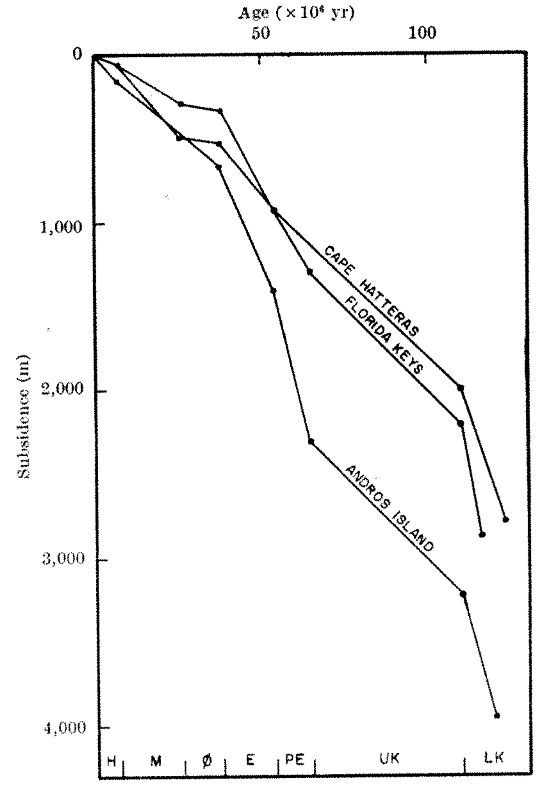


Fig. 3. Subsidence plotted against time. Curves represent record of subsidence through time. Andros Island data from Spencer[9]. Florida Keys data from Maher and Applin[10]. Cape Hatteras data from Swain[13]. H, Holocene–Pliocene; M, Miocene; Ø, Oligocene; E, Eocene; P, Palaeocene; UK, upper Cretaceous; LK, lower Cretaceous.

Goodell and Garman[12] proposed a causal relationship between Cuban orogenic activity and Bahamian Platform tectonic-subsidence history. G. W. L. and Stehman[8] also recognized this relationship, but rather than calling it causal they suggested a parallel relationship resulting from sea-floor spreading. Orogenic activity in Cuba was most intense during the lower Cretaceous and late Cretaceous to middle Eocene[40–42]. Most faults in Cuba are of a high angle[40]. Volcanic activity began, and was most intense, during the early Cretaceous, but continued into the Eocene[40]. Most of the volcanics were basic, but during the upper Cretaceous ultrabasics (harzburgite, lherzolite, dunite and anorthosite) were intruded[40]. Engel and Fisher[43] suggested that lherzolite may be an upper mantle rock exposed at the Earth's surface by faulting.

Thus although the Bahamian region is not now tectonically active, it may have been active during the late Mesozoic and early Caenozoic. Tectonic activity was probably greatest during the lower Cretaceous and Palaeocene–Eocene.

The tectonic framework discussed above leaves us with the apparently anomalous association of compressional (trenches) and tensional (graben and normal faults) features that may be dynamically related. I believe that a model of the Bahamian region may be developed to explain this dynamic relationship logically.

The JOIDES deep sea drilling programme data[44] and quiet magnetic zone[45] suggest that the history of the Bahamian Platform extends back into the Jurassic, and

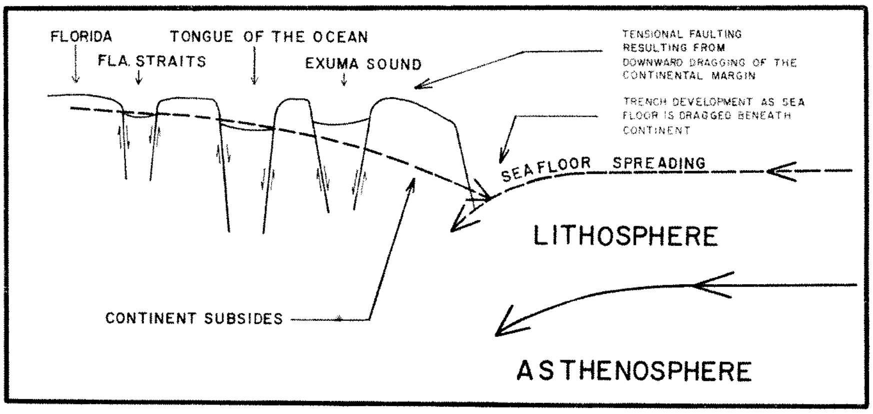

FLORIDA
TONGUE OF THE OCEAN
FLA. STRAITS
EXUMA SOUND
TENSIONAL FAULTING RESULTING FROM DOWNWARD DRAGGING OF THE CONTINENTAL MARGIN
TRENCH DEVELOPMENT AS SEA FLOOR IS DRAGGED BENEATH CONTINENT
SEA FLOOR SPREADING
LITHOSPHERE
CONTINENT SUBSIDES
ASTHENOSPHERE

Fig. 4. Dynamic model of Bahamian Platform region during lower Cretaceous illustrating genetic relationship of compressional (trench) and tensional (graben faults) structures to sea-floor spreading.

possibly into the Palaeozoic. Present data provide direct evidence of the tectonic-subsidence history of this region back into the Cretaceous. The model will therefore only be applicable back to 135 m.y.

The subsidence history of the Bahamian Platform (Fig. 3) and models constructed by Le Pichon[4] indicate a higher rate of sea-floor spreading during the lower Cretaceous than at present (6–8 cm/yr). As a result, the Puerto Rico Trench and the proposed trench east of the Bahamas would be areas of active thrusting of the American lithosphere plate under the Caribbean plate (Fig. 4). The region west of the trench system, the Bahamian Platform, was subsiding rapidly and the easternmost part of the platform was being dragged downward into the mantle, giving rise to tension to the west (Fig. 4). The tension resulted in the development of graben faults on the Bahamian Platform and normal faults in Cuba. This may be visualized as analogous to the formation of crevasses in a glacier. The normal faults created avenues along which basic lavas rose. The volcanic and associated orogenic activity resulted in the Antillean island arc system.

The rate of sea-floor spreading decreased during the upper Cretaceous (Fig. 3) and the trench system became inactive; the postulated trench east of the Bahamas began to fill with carbonate sediments. During the Palaeocene and Eocene the spreading rate increased, but the rate was probably not high enough to reactivate the trench system. Rates of drift decreased markedly during the Oligocene but increased during the Miocene to half rates[18] of about 1·7 cm/yr. There was another decrease in spreading during the Pliocene (6 m.y. ago) to its present half rate of 1·25–1·4 cm/yr[18]. This rate of spreading is now expressed by deformed sediments on the Barbados Ridge[19,46] and "horizon A" outcrop area[47].

The model implies that all the tectonic-subsidence history of the Bahamian Platform–Cuba–South Florida region can be explained in terms of a lithosphere plate-global tectonic model of the Earth's crust. Included in the model is the dynamic relationship of compressional and tensional structures (Fig. 4). The dynamic relationship of trenches and normal faults may explain the development of volcanic island arc systems landward of continental margin-trench systems[19].

This study was partially supported by the US National Science Foundation. C. F. Stehman and L. K. Glover assisted in drafting the figures, and I thank M. M. Ball, R. S. Dietz, J. Dorman, B. C. Heezen, T. Saito and R. E. Sheridan for valuable discussions.

Received January 5; revised January 23, 1970.

[1] Hess, H. H., *Petrologic Studies: A Volume in Honor of A. F. Buddington*, 599 (Geol. Soc. Amer., New York, 1962).

[2] Dietz, R. S., *Nature*, **190**, 854 (1961).

[3] Morgan, J., *J. Geophys. Res.*, **73**, 1959 (1968).

[4] Le Pichon, X., *J. Geophys. Res.*, **73**, 3661 (1968).

[5] Isacks, B., Oliver, J., and Sykes, L. R., *J. Geophys. Res.*, **73**, 5855 (1968).

[6] Dott, jun., R. H., *Science*, **166**, 874 (1969).

[7] Dietz, R. S., Holden, J. C., and Sproll, W. P., *Abstracts with Programs for 1969*, **7**, 48 (Geol. Soc. Amer., 1969).

[8] Lynts, G. W., and Stehman, C. F., *Abstracts with Programs for 1969*, **7**, 281 (Geol. Soc. Amer., 1969).

[9] Spencer, M., *Bull. Amer. Assoc. Petrol. Geol.*, **51**, 263 (1967).

[10] Maher, J. C., and Applin, E. R., *Amer. Assoc. Petrol. Geol. Cross Sec. Publ.*, 6 (1968).

[11] JOIDES, *Science*, **150**, 709 (1965).

[12] Goodell, H. G., and Garman, R. K., *Bull. Amer. Assoc. Petrol. Geol.*, **53**, 513 (1969).

[13] Swain, F. M., *Bull. Amer. Assoc. Petrol. Geol.*, **31**, 2054 (1947).

[14] Vogt, P. R., and Ostenso, N. A., *Nature*, **215**, 810 (1967).

[15] Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pitman, W. C., III, and Le Pichon, X., *J. Geophys. Res.*, **73**, 2119 (1968).

[16] Percival, jun., S. G., *Abstracts with Programs for 1969*, **7**, 171 (Geol. Soc. Amer., 1969).

[17] Schneider, E. D., and Vogt, P. R., *Nature*, **217**, 1212 (1968).

[18] Phillips, J. D., *Science*, **157**, 920 (1967).

[19] Vogt, P. R., Schneider, E. D., and Johnson, G. L., *Geophys. Mon.*, **13**, 556 (1969).

[20] Le Pichon, X., Eittreim, S. L., and Ludwig, W. J., *Phys. Chem. Earth*, **8**, 2 (1968).

[21] Van Andel, T. H., Phillips, J. D., and Von Herzon, R. P., *Earth Plan. Sci. Lett.*, **5**, 269 (1969).

[22] Fox, P. J., Pitman, W. C., III, and Shephard, F., *Science*, **165**, 487 (1969).

[23] Langseth, M. G., Le Pichon, X., and Ewing, M., *J. Geophys. Res.*, **71**, 5321 (1966).

[24] Ewing, J., and Ewing, M., *Science*, **156**, 1590 (1967).

[25] Worzel J. L., *Submarine Geology and Geophysics*, 335 (Butterworths, London, 1965).

[26] Drake, C. L., Ewing, J. I., and Stockard, H., *Canad. J. Earth Sci.*, **5**, 933 (1968).

[27] Barazangi, M., and Dorman, J., *Bull. Seism. Soc. Amer.*, **59**, 369 (1969).

[28] Hess, H. H., *Navy-Princeton Gravity Expedition to the West Indies in 1932* (US Hydrog. Office, 1933).

[29] Ericson, D. B., Ewing, M., and Heezen, B. C., *Bull. Amer. Assoc. Petrol. Geol.*, **36**, 489 (1952).

[30] Newell, N. D., and Rigby, J. K., *Soc. Econ. Paleont. Mineral. Sp. Publ.*, **5**, 15 (1957).

[31] Talwani, M., Worzel, J. L., and Ewing, M., *Trans. Second Caribbean Geol. Conf., 1959*, 156 (1960).

[32] Ewing, M., Worzel, J. L., Beall, A. O., Berggren, W. A., Burky, D., Burk, C. A., Fischer, A. G., and Pessagno, E. A., *Initial Rep. Deep Sea Drilling Project*, **1**, 179 (1969).

[33] Pressler, E. D., *Bull. Amer. Assoc. Petrol. Geol.*, **31**, 1851 (1947).

[34] Jordan, G. F., Malloy, R. J., and Kofoed, J. W., *Mar. Geol.*, **1**, 259 (1964).

[35] Kofoed, J. W., *Southeast Geol.*, **6**, 159 (1965).

[36] Uchupi, E., *Science*, **153**, 529 (1966).

[37] Maher, J. C., *Geological Framework and Petroleum of the Atlantic Coastal Plain and Continental Shelf* (US Geol. Surv. Open-File Rep., 1967).

[38] Sheridan, R. E., Drake, C. L., Nafe, J. E., and Hennion, J., *Bull. Amer. Assoc. Petrol. Geol.*, **50**, 1972 (1966).

[39] Athearn, W. D., *Bull. Mar. Sci. Gulf Caribbean*, **13**, 365 (1963).

[40] Khudoley, K. M., *Bull. Amer. Assoc. Petrol. Geol.*, **51**, 668, 789 (1967).

[41] Hatten, C. W., *Bull. Amer. Assoc. Petrol. Geol.*, **51**, 780 (1967).

[42] Škvor, V., *Bull. Geol. Soc. Amer.*, **80**, 961 (1969).

[43] Engel, C. G., and Fisher, R. L., *Science*, **166**, 1136 (1969).

[44] Ewing, M., Worzel, J. L., and Burk, C. A., *Initial Rep. Deep Sea Drilling Project*, **1**, 624 (1969).

[45] Heirtzler, J. R., and Hays, D. E., *Science*, **157**, 185 (1967).

[46] Schneider, E. D., *Abstracts with Programs for 1969*, **7**, 291 (Geol. Soc. Amer., 1969).

[47] Ewing, M., Worzel, J. L., and Burk, C. A., *Initial Rep. Deep Sea Drilling Project*, **1**, 3 (1969).

LETTERS TO THE EDITOR

PHYSICAL SCIENCES

Origin of the Ultra-short Period Binary WZ Sagittae

THE recent discovery of several binaries with periods in the range of a few hours has raised the problem of their past evolution. Three eclipsing binaries are in that group: the old nova WZ Sge, EX Hya and VV Pup. I shall confine myself to the case of WZ Sge, for which the largest amount of data is available. Krzeminski and Kraft[1] have shown that the system is composed of a DA white dwarf, for which they estimate a mass $M=0{\cdot}59\ M_\odot$, and a secondary of mass $m=0{\cdot}03\ M_\odot$. The radii are $R\simeq 0{\cdot}87\times10^9$ cm and $r\simeq 7\times10^9$ cm, and the separation $a=3{\cdot}7\times10^{10}$ cm.

If we assume that the secondary is a completely degenerate star and use the mass–radius relation recently calculated by Zapolsky and Salpeter[2] for such stars, we find that the star is composed of almost pure hydrogen. This indicates that, in its past, it has never been massive enough to burn hydrogen.

The primary is a white dwarf of mass $M=0{\cdot}59\ M_\odot$ and current ideas on stellar evolution assume that it is the remnant of a more massive star which once burned hydrogen and became a red giant with a radius greater than, say, 100 $R_\odot$. It lost a considerable amount of mass and left a remnant that is the present white dwarf.

Let us take the initial separation of the two stars to be less than the radius of the primary in its giant phase. The secondary was then, for a time, surrounded by a giant envelope. It accreted matter at a rate estimated by Bondi and Hoyle[3] as

$$\dot{m}=2\pi\alpha G^2m^2V^{-3}\rho \tag{1}$$

where $\alpha=1{\cdot}25$ and V is the orbital velocity of the secondary. If we assume that the matter collected has, on the average, no angular momentum, we have

$$L=mvr=1{\cdot}023\times10^{50} \tag{2}$$

I finally assume, for the sake of simplicity of calculation, that the secondary moves under the gravitational attraction of a mass equal to the actual mass of the white dwarf. That gives $V=(GM/r)^{1/2}$. These three relations give for the change in radius

$$\frac{1}{r}-\frac{1}{r_0}=\frac{4\pi\alpha L}{M^2}\rho t$$

where r_0 is the initial and r the present separation of the stars. In the extreme case $r_0=\infty$, we have

$$t(\text{yr})=\frac{0{\cdot}74\times10^{-3}}{\rho}$$

If we take as a typical value of the density $\rho=10^{-6}$ we get $t\simeq10^3$ yr. This time is much shorter than the lifetime of a giant star. If the secondary had been, for a substantial length of time, inside the envelope of the giant, it would have accreted matter, become closer to the primary and would eventually have been captured. We know this did not happen.

The secondary was then, almost at all times, outside the envelope of the primary. Let us calculate the time required for gravitational waves to change their separation by a substantial amount. If that separation were at one time, say, a hundred times its actual value, by Kepler's third law we would have $a^3P^{-2}=4\pi^2(M+m)$ and the period would be a thousand times the actual period. The time required for a substantial change in r is of the order of magnitude of the binary collapse time, given by Paczynski[4]

$$T_0(\text{yr})=3{\cdot}22\times10^{-3}\,\frac{(M+m)^{1/3}}{Mm}\,P_0^{8/3}$$

where P_0 is the period in seconds and M and m are in solar masses. The value of T_0 for the present configuration is $1{\cdot}12\times10^9$ yr. The collapse time from a position where $P=1{,}000\ P_0$ is $10^8\ T_0$, much longer than the age of the universe.

There is another way to place the secondary in its present position. Suppose the giant primary ejected matter with very low velocity until only the present mass remained. Part of this matter was accreted by the secondary and the mutual separation decreased, but the secondary was always outside the envelope of the primary.

Let us evaluate the changes in mass and separation of both stars. The secondary mass will be given by equation (1), and the constancy of angular momentum by equation (2). We take a very simple model for mass loss in which the rate is given by

$$R=4\pi r^2\rho c \tag{3}$$

Here ρ is the density of matter at distance r and c is its velocity, taken to be constant.

Finally, the orbital velocity of the secondary is given by

$$V=\left[\frac{G(M_0-Rt)}{r}\right]^{1/2} \tag{4}$$

where M_0 is the mass of the primary at $t=0$.

Elimination of V and ρ using equations (3) and (4) gives

$$\dot{m}=\frac{\alpha RG}{2cL}\ \frac{m^3}{(M_0-Rt)} \tag{5}$$

and

$$r=\frac{L^2}{m^2G(M_0-Rt)} \tag{6}$$

Let us assume that the mass loss starts with $M=M_0$, $m=m_0$ and $r=r_0$ and ends with $M=M_1=0{\cdot}59\ M_\odot$, $m=m_1=0{\cdot}03\ M_\odot$ and $r=r_1=3{\cdot}7\times10^{10}$ cm. Integration of equations (5) and (6) between these values gives

$$\frac{1}{m_0^2}=\frac{1}{m_1^2}+\frac{\alpha G}{cL}\ln\left(\frac{M_0}{M_1}\right) \tag{7}$$

and

$$r_0=\frac{L^2}{GM_0}\left[\frac{1}{m_1^2}+\frac{\alpha G}{cL}\ln\left(\frac{M_0}{M_1}\right)\right] \tag{8}$$

It is interesting to note that equations (7) and (8) are independent of the duration of the mass loss.

The ratio of initial to final separation is

$$\frac{r_0}{r_1}=\left(\frac{M_1}{M_0}\right)\left[1+\frac{m_1^2\alpha G}{cL}\ln\left(\frac{M_0}{M_1}\right)\right] \tag{9}$$

If we substitute the actual values $r_1 = 3{\cdot}7\times 10^{10}$, $L = 1{\cdot}015\times 10^{50}$ and $m_1 = 5{\cdot}967\times 10^{31}$ and if we take $\alpha = 1{\cdot}25$ and $M_0/M_1 = 5$, we have

$$\frac{r_0}{r_1} = 0{\cdot}20\left[1+\frac{4{\cdot}7\times 10^6}{c}\right]$$

which for $c \gg 4{\cdot}7\times 10^6$ reduces to $\dfrac{r_0}{r_1} \simeq \dfrac{10^6}{c}$

If $c \simeq 10^3$ cm s^{-1}, we have $r_0 \simeq 10^3\ r_1$. That places the secondary well outside the region to be occupied by the envelope of the primary. Equation (9), then, gives for the initial secondary mass $m_0 = 0{\cdot}87\times 10^{30}$ g.

These values for the initial separation and mass are comparable to the values for Jupiter and the Sun. If the Sun becomes a giant star and loses a considerable fraction of its mass at very low flow velocity, part of it would be collected on Jupiter, which would then move close to the Sun. Their final configuration would probably not be very different from the actual one for the binary star SW Sge.

SAMUEL C. VILA

University of Pennsylvania,
Philadelphia.

Received February 10, 1970.

[1] Krzeminski, W., and Kraft, R. P., *Astrophys. J.*, **140**, 921 (1964).
[2] Zapolsky, H. S., and Salpeter, E. E., *Astrophys. J.*, **158**, 809 (1969).
[3] Bondi, H., and Hoyle, F., *Mon. Not. Roy. Astron. Soc.*, **104**, 273 (1944).
[4] Paczynski, B., *Acta Astron.*, **17**, 287 (1967).

Bremsstrahlung Radiation in an Intense Magnetic Field and Emission from Pulsars

Simon and Strange recently argued[1] that one of the processes (bremsstrahlung in the 0→0 transition in a magnetic field) which we proposed[2] as the chief mechanism of emission from pulsars cannot give rise to a negative absorption coefficient. They based their argument on an existing formula for the absorption coefficient valid only for synchrotron radiation. Further, their expression for the emissivity was found by us to be applicable only to the high quantum number case. When the correct formula for the absorption coefficient was used in conjunction with the emissivity valid for the 0→0 transition, we found that the absorption coefficient can become negative and amplification of electromagnetic waves is possible from the 0→0 bremsstrahlung process.

First we shall show that the result of Simon and Strange, equation (3) of their paper,

$$\alpha_\omega = \frac{8\pi^3 c}{\omega^2}\ \frac{n(\theta)\cos\theta}{n_r^2}\int_{-\infty}^{\infty} f(p_z)\,\frac{\mathrm{d}Q_\omega}{\mathrm{d}p_z}\,\mathrm{d}p_z$$

cannot be correct as it gives a zero absorption coefficient for a gas in equilibrium, independent of any absorption process. (α_ω = absorption coefficient, ω = frequency, p_z = electron momentum parallel to the field, $f(p_z)$ = electron distribution function, θ = angle of emission with respect to the field, Q_ω = rate at which energy is emitted spontaneously by the electron with momentum between p_z and $p_z + \mathrm{d}p_z$ at the frequency ω, per $\mathrm{d}\omega$, per unit solid angle, in the direction θ, and $n(\theta)$ and n_r are refractive indices.) Q_ω depends on the absolute value of p_z and is therefore an even function of p_z. Consequently $\mathrm{d}Q_\omega/\mathrm{d}p_z$ is an odd function of p_z. In equilibrium $f(p_z)$ is an even function and hence α_ω in equation (3) of ref. 1 vanishes identically, independent of any absorption process. This clearly violates basic laws of thermodynamics.

The origin of this inconsistency can be traced to Simon and Strange's improper application of equation (2.54) of ref. 3 of their paper[3]. This result is valid only for classical synchrotron radiation, derived from the master equation (2.36) after using equations (2.46) and (2.50) which are applicable only to synchrotron radiation and not to the bremsstrahlung process. Equation (2.50) describes the momentum conservation condition which is absent in the bremsstrahlung case, for the nucleus can absorb momentum without absorbing energy. The improper application of equation (2.54) leads to the result of zero absorption coefficient.

In order to derive the correct equation, we refer to the master equation (equation (2.36) of ref. 3) modified for a one-dimensional gas

$$\alpha_\omega = \frac{8\pi^3 c^2}{n_r^2\hbar\omega^2}\int_{-\infty}^{\infty} Q_\omega(p')[f(p)-f(p')]\,\mathrm{d}p' \qquad (1)$$

where p and p' are electron momenta before and after absorbing the photon $\hbar\omega$. The energy conservation law requires that

$$\hbar\omega + \frac{p_z^2}{2m} = \frac{p_z'^2}{2m} \qquad (2)$$

Because $\hbar\omega \ll p_z^2/2m$, we can expand $(f(p_z)-f(p_z'))$ and write

$$f(p_z)-f(p_z') = \frac{\partial f(p_z')}{\partial p_z'}\,\Delta p_z \qquad (3)$$

$$\Delta p_z = p_z - p_z' = -\frac{2m\hbar\omega}{p_z+p_z'}$$

Therefore

$$\alpha_\omega = \frac{8\pi^3 c^2}{n_r^2\,\omega^2}\cdot 2m\int_{-\infty}^{\infty}\frac{Q_\omega(p_z')}{p_z+p_z'}\ \frac{\partial f(p_z')}{\partial p_z'}\,\mathrm{d}p_z' \qquad (4)$$

By partial integration and rearranging terms, we find the correct form of equation (3) of Simon and Strange's paper

$$\alpha_\omega = \frac{8\pi^3 c^2}{n_r^2\omega^2}\ 2m\int_0^{\infty}\left[f(p_z')\frac{\partial}{\partial p_z'}\left(\frac{Q_\omega(p_z')}{p_z'-p_z}\right)+\right.$$
$$\left. f(-p_z')\frac{\partial}{\partial p_z'}\left(\frac{Q_\omega(p_z')}{p_z'-p_z}\right)\right]\mathrm{d}p_z' \qquad (5)$$

which no longer vanishes when $f(p_z) = f(-p_z)$. The criterion for a negative absorption coefficient in the case $f(-p_z) \ll f(p_z)$ (which corresponds to a population inversion in terms of a coherent streaming motion with respect to the medium in the $+p_z$ direction) is

$$\frac{\partial}{\partial p_z'}\left(\frac{Q_\omega(p_z')}{p_z'+p_z}\right) < 0 \qquad (6)$$

or $Q_\omega(p_z)$ must not rise faster than the first power of p_z.

The absorption coefficient quoted by Simon and Strange has been found by us recently to be correct only in the high quantum number regime and is totally inapplicable to the 0→0 transition. The correct expression for the transition probability in the 0→0 transition is:

$$w(p_{z1}\omega) \simeq C\,\frac{n_r}{\omega|p_z|}\quad g(\omega) \qquad (7)$$

where $g(\omega)$ is a slowly varying function and C is a constant. The presence of p_z in the denominator is purely the effect of the density of the final state of a one-dimensional particle. As is well known, the density of states of a one-dimensional particle, $\rho(\varepsilon)$, is

$$\rho(\varepsilon) = \frac{\mathrm{d}p}{\mathrm{d}\varepsilon} \propto \frac{1}{p} \qquad (8)$$

Accordingly

$$\frac{\mathrm{d}}{\mathrm{d}p_z}\left(\frac{Q_\omega(p_z)}{p_z}\right)$$

is negative and the absorption coefficient can be negative. Laser amplification of radiation can therefore take place.

We have applied equation (7) to electromagnetic wave amplification in our model and found that the spectrum of the resultant coherent radiation is proportional to $\nu^{-\alpha}$, where α is of the order unity but α is a function of various parameters associated with the neutron star. This type of spectrum agrees roughly with that observed. A full account of this theory is being prepared for publication.

One of us (V. C.) is an NAS–NRC senior postdoctoral resident research associate.

HONG-YEE CHIU*
VITTORIO CANUTO

Institute for Space Studies,
Goddard Space Flight Center, NASA,
New York, New York 10025.

Received December 15, 1969.

---

* Also with Physics Department and Earth and Space Sciences Department of the State University of New York at Stony Brook, and Physics Department of the City University of New York.

[1] Simon, M., and Strange, D. L. P., *Nature*, **244**, 49 (1969).
[2] Chiu, H.-Y., and Canuto, V., *Phys. Rev. Lett.*, **22**, 415 (1969).
[3] Behefi, G., *Radiation Processes in Plasmas*, 52 (John Wiley, New York, 1966).

Possibility of a Terrestrial Component in the Doppler Shifted Zodiacal Light

LIGHT scattered off particles in circum-terrestrial space may make an appreciable contribution to the zodiacal glow. Just how significant this contribution might be unfortunately is still unknown. The information we have comes directly from the infrared observations by Petersen[1] and McQueen[2]. These show that an appreciable portion of the dust must be in circum-solar orbits, some of it quite near the Sun. Direct observations on the circum-terrestrial components, however, are still ambiguous, despite extensive particle detection experiments from space probes[3]. Such experiments have been largely sensitive to particles larger than those thought responsible for the zodiacal glow.

For this reason, the recent observations of Reay and Ring[4] are welcome, and though they are not specific enough, as yet, to permit conclusions about the circum-terrestrial particles, they indicate that the spectroscopic method is intrinsically sensitive enough to permit an early decisive test.

Our aim in this letter is first to show that the use of Mie theory does not appreciably change the conclusions already drawn by Bandermann and Wolstencroft[5] and by Reay[6], so that an appreciably better fit to the experimental data could not be expected even if quite unusual particle properties or orbits were taken seriously. This leads to our second point, which assumes a certain circum-terrestrial dust component and then predicts that the low velocity Doppler shifts observed at high elongations will be confirmed by more detailed observations and will, in fact, be present both in the morning and evening zodiacal glow.

The difference between a circum-terrestrial dust containing model and the pure interplanetary dust model, for which James[7] has computed characteristic Doppler shift curves, is large enough so that present techniques seem capable of making a distinction.

If an appreciable fraction of the dust is concentrated near the Earth, one would expect the value of the wavelength shift to be below the theoretical curve at elongations of the order of 70°, while, as James has shown, the wavelength shift for an entirely circum-solar cloud exhibits no such drop.

Like the previous authors[5,6] we assume an effective Doppler shift

$$<\delta\lambda> = \frac{\int \delta\lambda(B)\,\mathrm{d}B}{\int \mathrm{d}B}$$

where $\delta\lambda(B)$ is the wavelength shift and $\mathrm{d}B$ the surface brightness due to individual volume elements along a line of sight through the zodiacal dust cloud. $<\delta\lambda>$ is the expected averaged shift obtained from direct observations. Ingham[8] as well as Reay[6] considered that the phase function which determines $\mathrm{d}B(\theta)$ for phase angle θ is the sum of phase functions for reflected and diffracted light. Such a function, however, is not identical with that obtained from Mie theory, and we therefore consider whether appreciable changes in results are to be expected on that basis. We take a phase function

$$F(\theta,\, x,\, m) = \tfrac{1}{2}\, i_1\,(\theta,\, x,\, m) + i_2\,(\theta,\, x,\, m)$$

where $x = 2\pi a/\lambda$ is the size parameter, dependent on particle radius a, and m is the refractive index. If $C\,(x,\, m)$ is chosen as the scattering cross-section and $\varphi\,(x)$ represents the particle size distribution function, the phase function for the polydisperse, optically thin cloud becomes

$$\Psi(\theta) = \frac{\lambda^2 \int_{x_1}^{x_2} F(\theta,\, x,\, m)\;\varphi\,(x)\;\mathrm{d}x}{\pi \int_{x_2}^{x_1} C\,(x,\, m)\;\varphi\,(x)\;\mathrm{d}x}$$

The phase function $\Psi(\theta)$ has a characteristic minimum at $\theta \sim 90°$, which is very pronounced for dielectric particles. At elongations ε of about 90°, particles relatively distant from the Earth therefore contribute appreciably to the Doppler shift, because $\theta \gg 90°$ for these grains. This makes the value of $<\delta\lambda>$ practically independent of the physical properties assumed for the scatterers.

In Fig. 1 we show the Doppler shift as a function of elongation, computed for circular orbits about the Sun.

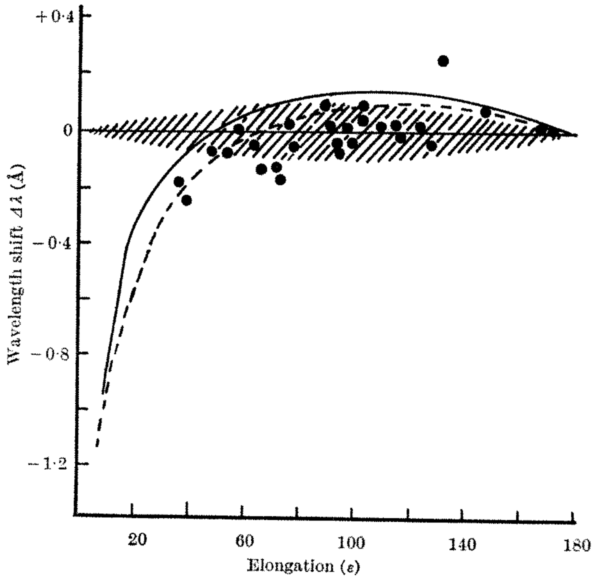


Fig. 1. Wavelength shift $\Delta\lambda$ at the evening zodiacal light as a function of elongation ε in degrees. The dashed curve represents James's results for isotropically scattered particles. The full line represents our results for Mie scatterers. The hatched area represents circum-terrestrial grains with peak elongations somewhat arbitrarily chosen as ±6 km s^{-1}. The full points are data gathered by Reay and Ring.

The assumed space density distribution varied as r^{-2} with increasing distance r from the Sun. We took $\varphi(x) = (x/x_0)^{-4}$ with size limits $x_1 = 0{\cdot}8$ and $x_2 = 10$. The refractive index was that of silicate-like particles with small absorption, $m = 1{\cdot}50 - 0{\cdot}1\,i$. This gives the full curve, while the broken curve represents the results of James for isotropic scattering. Both these curves are drawn for the evening zodiacal light, and they do not seem to be capable of explaining the negative value of $<\delta\lambda>$ obtained by observations[4]. Those are shown by the dots in Fig. 1.

If we assume that the observations are correct, then the values obtained at elongations in excess of $\varepsilon > 70°$ may represent the dominance of dust near the Earth. This would be the case if an areal density of scatterers were assumed such that

$$\frac{1}{4\pi}\int_0^R S\,\sigma\,N(s)\,\mathrm{d}s \sim 3\times10^{-10}\,\mathrm{W\ cm^{-2}\ sr^{-1}}$$

This expression merely states that if $N(s)$ is the number of particles per unit volume, at distance s from the Earth, and R is the distance from the Earth beyond which contributions can be neglected, then the total observed flux, given on the right-hand side of the equation, should equal the scattered sunlight integrated along a column out to distance R. We have called the particle cross-section σ and assumed that they scatter isotropically[9]. S is the solar constant $\sim 0{\cdot}14$ W cm^{-2}.

If we assume $N(s)$ to be constant out to distance R, we obtain the simple relation

$$N\sigma \sim 3\times10^{-8}\,R^{-1}\ \mathrm{cm^2\ cm^{-3}}$$

Because absorption of radiation and predominance of forward scattering have been neglected, the actual value of the areal density may be half an order of magnitude or so higher. Even so, if we choose R to have a value of order 10^{10} to 10^{11} cm, we obtain $N\sigma$ values in the range $10^{-17\cdot5}$ to $10^{-18\cdot5}$ cm. These do not seem to be ruled out by present observations.

We may also compute the mass infall rate from such dust, if the circum-terrestrial component reached as far as the Moon. The mass accreted through action of the Poynting–Robertson drag would then amount to roughly 10^3 kg each day for micron sized grains and less for submicron sized particles. This is conservative with respect to figures quoted by Hawkins[3] for infall rates onto the Earth.

With such a circum-terrestrial concentration, the plot of wavelength shift against elongation would take on the form of the shaded portion in Fig. 1. This region can be expected to be wide because both prograde and retrograde circum-terrestrial orbits are likely. As Bandermann and Wolstencroft have pointed out, these have a sin ε dependence for velocity shifts as a function of elongation. The spread in velocities due to this effect would be of the order of a few kilometres per second for typical grains in the space between Earth and Moon.

One of us (M. H.) thanks Dr L. Perek, director of the Astronomical Institute of the Czechoslovak Academy of Science, for hospitality and acknowledges support from the Czechoslovak and the US Academies of Science. Both of us thank Mr J. F. James for interesting comments.

V. VANÝSEK

Charles University,
Prague.

M. HARWIT*

Astronomical Institute,
Czechoslovak Academy of Science,
Prague.

Received January 19, 1970.

* On leave of absence from Cornell University, Ithaca, New York.

[1] Petersen, A. W., *Astrophys. J. Lett.*, **148**, L37 (1967).
[2] McQueen, R. M., thesis, Johns Hopkins Univ. (1967).
[3] Hawkins, G. S., *Ann. Rev. Astron. Astrophys.*, **2**, 150 (1964).
[4] Reay, N. K., and Ring, J., *Nature*, **219**, 710 (1968).
[5] Bandermann, L. W., and Wolstencroft, R. D., *Nature*, **221**, 251 (1969).
[6] Reay, N. K., *Nature*, **224**, 54 (1969).
[7] James, J. F., *Mon. Not. Roy. Astron. Soc.*, **142**, 45 (1969).
[8] Ingham, M. F., *Mon. Not. Roy. Astron. Soc.*, **122**, 159 (1961).
[9] Harwit, M., *Cong. Coll. Univ. Liège*, **26**, 506 (1964).

The 11 Canis Minorids—a New Meteor Stream probably associated with Comet Mellish 1917 I

WE have detected a new meteor stream complex, active between solar longitudes $\lambda_\odot = 257°$ and $262°$, with a true radiant some 3° north-west of 11 Canis Minor. The stream was first recorded on the morning of December 10, 1964. During visual watches with a richest field 5-inch telescope, positional data were recorded for meteors in the range from magnitude +6 to +12 (ref. 1). Five meteors, originally classified as sporadic, were found to be radiating from an observed centre at right ascension $(\alpha) = 115{\cdot}8 \pm 1{\cdot}2°$, declination $(\delta) = +13{\cdot}0 \pm 0{\cdot}8°$. Statistical tests indicate that the probability of this radiant being due to the chance lining up of sporadic meteors is only 1 in 10^4. No velocity measurements were available and so orbital elements were computed using Porter's method[2], initially assuming parabolic motion. Corrections to the observed radiant for diurnal aberration and zenithal attraction were small, the resulting parabolic orbit being highly inclined with a remarkably small perihelion distance. The true radiant and preliminary orbit are given in Table 1.

Table 1. TRUE RADIANT AND PRELIMINARY ORBIT

R.A.	$115{\cdot}1 \pm 1{\cdot}2°$	
Dec.	$+12{\cdot}4 \pm 0{\cdot}8°$	
i	$89 \pm 17°$	1950·0
ω	$158{\cdot}1 \pm 1{\cdot}4°$	
Ω	$78{\cdot}476 \pm 0{\cdot}002°$	
q	$0{\cdot}036 \pm 0{\cdot}009$	

The longitude of the true radiant is close to the anti-helion point making the computed orbital elements, particularly the orbital inclination i and to a lesser extent the longitude of the perihelion ω, extremely sensitive to changes in radiant position. The computed orbit is hence relatively indeterminate in i despite an accurate radiant determination.

Published catalogues of photographic meteors[3-9] have been searched for possible stream members, yielding the four meteors in Table 2. These were the only meteors recorded with radiants near the expected area from $\lambda_\odot = 250°–270°$. In computing mean characteristics, the more accurate meteor No. 4 has been weighted × 2. The mean orbit of these photographic meteors bears a considerable resemblance to the telescopic orbit and, like the latter, is also relatively indeterminate in i. Using the mean semi-major axis from the photographic meteors $a = 1{\cdot}90$, the observed telescopic radiant has been used to compute an ellipse, shown in Table 3 as solution 1, together with the photographic ellipse. Reducing the value of the assumed telescopic meteor velocity in this way drastically reduces i and increases the value of q. A further possible solution is to reduce this velocity further to a value producing $i = 32{\cdot}9°$, the mean photographic inclination. This telescopic meteor orbit is also shown in Table 3, as solution 2, with q now near the photographic value, but with $a = 1{\cdot}17$.

Such an arrangement, with the less massive telescopic meteors having an orbital period shorter than the photographic meteors, is consistent with the operation of the Poynting–Robertson effect for some time on an initially compact stream with larger period. We regard this second telescopic solution as being the most likely and conclude

Table 2. FOUR METEORS FROM PUBLISHED CATALOGUES

Meteor No.	Date	Radiant R.A.	Radiant Dec.	Heliocentric velocity km s^{-1}	a	e	q	1950·0 i	1950·0 ω	1950·0 Ω	Reference
1	Dec. 9.2, 1952	111	+11	34·0	1·38	0·94	0·08	39	154	77	4
2	Dec. 12.4, 1953	109	+13	39·6	3·79	0·98	0·08	36	149	80	4
3	Dec. 12.4, 1952	110	+11	35·1	1·55	0·93	0·11	30	147	80	4
4	Dec. 14.8, 1958	113·4	+15·2	37·2	2·13	0·971	0·06	26·4	154·7	82	8
Mean		110·9 ±1·5	+12·6 ±1·5	36·5 ±1·9	1·90 ±0·75	0·955 ±0·021	0·078 ±0·014	32·9 ±4·7	151·2 ±3·2	80·2 ±1·5	

Table 3. ASSOCIATION WITH THE COMET

		Telescopic meteors Elliptical orbits Solution 1	Telescopic meteors Elliptical orbits Solution 2	Photographic meteors Elliptical orbit	Orbit of Comet Mellish 1917 I
1950·0	i	59 ± 19°	32·9° assumed	32·9 ± 4·7°	32·68°
	ω	160·7 ± 1·6°	157·9 ± 1·6°	151·2 ± 3·2°	121·31°
	Ω	78·476 ± 0·002°	78·476 ± 0·002°	80·2 ± 1·5°	87·98° (*81·9°)
	q	0·038 ± 0·011	0·069 ± 0·012	0·078 ± 0·014	0·1902
	a	1·90 assumed	1·17 ± 0·09	1·90 ± 0·75	27·9
	e	0·976 ± 0·022	0·940 ± 0·023	0·955 ± 0·021	0·993

* Longitude of closest approach.

that the photographic and telescopic orbits represent the same meteor stream, the photographic meteors confirming the existence of the stream observed visually.

The 11 Canis Minorid meteor stream is remarkable in being only the second case of a stream with $q < 0·1$ AU, the other case being the δ Aquarids ($q = 0·061$ AU) (ref. 10). Orbits of this type do not possess long term stability. Because of dispersive and erosive forces, this new stream has a future lifetime of at most 10^5 yr. A full discussion of these effects will appear elsewhere[11].

The catalogue of known cometary orbits[12] reveals one comet with an orbit similar to the 11 Canis Minorids—Comet Mellish 1917 I. This is one of the nineteen comets with elliptical orbits passing within 0·1 AU of the Earth's orbit[13]. The comet orbit is quoted in Table 3 with an ascending node $\Omega = 88·0°$. This is near the meteor nodes $\Omega = 78·5°$ and 80·2°, but the longitude of the comet's closest approach to the Earth's orbit, where one would expect to be able to detect meteor activity, is $\lambda = 81·9°$, very close to the observed meteor orbit nodes. The separation of the orbits of the comet and the Earth at this closest approach is only 0·061 AU. The orbital inclinations of the comet and meteor orbits are almost identical, but the stream perihelion averages $q = 0·074$ AU, less than half of the comet's $q = 0·190$ AU, making the difference in ω as high as 36°. But a stream with such a small q has elements which are very sensitive to perturbations and dispersive and erosive forces. We believe the differences between the comet and meteor orbits may be explicable on these grounds and that the evidence is good for the 11 Canis Minorids being associated with Comet Mellish 1917 I. This suggested association is similar in many respects to those suggested for the Orionids with Halley's Comet[14-16] and the Ursids with Comet Tuttle 1939 X (refs. 17 and 18).

We request groups and individuals who record meteors using either photographic or radar methods to look for evidence of this shower.

We thank Dr P. J. Message and Mr H. B. Ridley for discussions and the Science Research Council, Professor J. M. Cassels and Dr J. Read (Oliver Lodge Laboratories, University of Liverpool) for the use of the IBM 360/65 computer.

KEITH B. HINDLEY

The Robert Robinson Laboratories,
University of Liverpool,
Liverpool L69 3BX.

MICHAEL A. HOULDEN

The Oliver Lodge Laboratories,
University of Liverpool,
Liverpool L69 3BX.

Received January 28, 1970.

[1] Hindley, K. B., *J. Brit. Astron. Assoc.*, **79** (2), 138 (1969).

[2] Porter, J. G., *Comets and Meteor Streams*, 81 (Chapman and Hall, London, 1952).

[3] Hawkins, G. S., and Southworth, R. B., *Smithsonian Contr. Astrophys.*, **2** (11), 349 (1958).

[4] McCrosky, R. E., and Posen, A., *Smithsonian Contr. Astrophys.*, **4 (2)**, 15 (1961).

[5] Jacchia, L. G., and Whipple, F. L., *Smithsonian Contr. Astrophys.*, **4 (4)**, 97 (1961).

[6] Babadzhanov, P. B., and Kramer, E. N., *Smithsonian Contr. Astrophys.*, **11**, 67 (1967).

[7] Jacchia, L. G., Verniani, F., and Briggs, R. E., *Smithsonian Contr. Astrophys.*, **10** (1), 1 (1967).

[8] Babadzhanov, P. B., *Smithsonian Contr. Astrophys.*, **7**, 287 (1963).

[9] McCrosky, R. E., in *Physics and Dynamics of Meteors* (edit. by Kresak, L., and Millman, P. M.), 265 (D. Reidel, Dordrecht, Holland, 1968).

[10] Whipple, F. L., Wright, F. W., and Jacchia, L. G., *Astron. J.*, **59**, 400 (1954).

[11] Hindley, K. B., and Houlden, M. A., *J. Brit. Astron. Assoc.* (in the press).

[12] Porter, J. G., *Mem. Brit. Astron. Assoc.*, **39**, No. 3 (1961).

[13] Porter, J. G., *Comets and Meteor Streams*, 92 (Chapman and Hall, London, 1952).

[14] Olivier, C. P., *Trans. Amer. Phil. Soc.*, **22**, Part 1 (1911).

[15] Svoboda, J., *Astr. Nach.*, **197**, 203 (1913).

[16] Hoffmeister, C., *Meteorstrome, Ch. 8: Die Meteore* (Leipzig, 1937).

[17] Hawkins, G. S., and Almond, M., *J. Brit. Astron. Assoc.*, **60**, 251 (1950).

[18] Ceplecha, Z., *Bull. Centr. Astron. Inst. Czech.*, **11**, 156 (1951).

Quantization of Wheeler–Feynman Electrodynamics

IN a recent letter, Katz, Ornan and Schwartz[1] (KOS) have argued that our treatment of spontaneous emission[2] deviates from the classical Wheeler–Feynman (WF) prescription[3]. They base their argument on the form of the interaction term

$$e_b \int A^{(a)}_{\mathrm{ret}}[b(t)] \cdot \dot{b}(t)\mathrm{d}t \qquad (1)$$

where $A^{(a)}_{\mathrm{ret}}$ is the retarded potential arising from the motion of the source particle a, and $b(t)$ is the path of a typical absorber particle b. Written in this form (1) denotes the retarded effect of particle a on particle b. KOS, however, rewrite (1) in the form

$$\begin{aligned} e_b \int A^{(a)}_{\mathrm{ret}}[b(t)] \cdot \dot{b}(t)\mathrm{d}t &= 2e_a e_b \iint \delta(S^2_{\mathrm{AB}})\dot{a}(t') \cdot \dot{b}(t)\mathrm{d}t\mathrm{d}t' \\ &= e_a \int A^{(b)}_{\mathrm{adv}}[a(t')] \cdot \dot{a}(t')\mathrm{d}t' \qquad (2) \end{aligned}$$

and argue that (2) represents the advanced effect of b on a.

This argument would have been correct if we were dealing with a two-particle system. This, however, is not the case. In (1), $A^{(a)}_{\mathrm{ret}}[b(t)]$ represents not just the direct retarded effect of a, but also the retarded effect produced by other particles, $c, d, \ldots$, in the future absorber which have been excited by the retarded potential of a. The use of the simple delta function in (2) is therefore not valid. The effect of other particles in the future absorber is to attenuate and change the phase of the original retarded disturbance emanating from a. All these effects cannot be included in an equality of the type (2).

We now come to the connexion between the quantum and classical versions of the theory of direct interparticle action. Quantum theory is necessarily richer than the classical theory. The interference of probability amplitudes in the former leads to results that have no analogues in the latter theory. When $\hbar \to 0$, however, the quantum theory must lead to the classical theory. The following remarks show that this happens in the treatments given in refs. 2 and 3.

The total field acting on particle a at time $t=0$ in the classical theory is given by

$$\tfrac{1}{2}\sum_{b\neq a}(F^{(b)}_{\text{ret}}+F^{(b)}_{\text{adv}})=\tfrac{1}{2}\sum_{\substack{b\neq a\\ t<0}}(F^{(b)}_{\text{ret}}+F^{(b)}_{\text{adv}})+R^{(a)} \qquad (3)$$

In (3) the left hand side denotes the field exerted by all particles $b\neq a$. Each particle acts on a via retarded and advanced fields. The first term on the right hand side denotes the part due to the past half of the universe ($t<0$), whereas $R^{(a)}$ denotes the part due to the future half ($t>0$). In a perfect future absorber $R^{(a)}$ is given by

$$R^{(a)}=\tfrac{1}{2}\sum_{\substack{b\\ t<0}}(F^{(b)}_{\text{ret}}-F^{(b)}_{\text{adv}}) \qquad (4)$$

$R^{(a)}$ is the classical response of the universe. The term $b=a$ in (4) leads to radiative damping. This is the classical WF theory.

In the quantum calculation of ref. 2, a path integral approach is followed. We can no longer calculate the unique path followed by a, we can only calculate probabilities for different paths. This necessarily leads to the squaring of the amplitude and hence to an influence functional $F[a,a']$ involving paths and conjugate paths (compare ref. 2). The quantum analogue of (4) expressed in terms of potentials is

$$R^{(a)}=\tfrac{1}{2}\sum_{\substack{b\\ t<0}}[A^{(b)}_{\text{ret}+}-A^{(b)}_{\text{adv}+}]+\tfrac{1}{2}\sum_{\substack{b\\ t<0}}[A'^{(b)}_{\text{ret}-}-A'^{(b)}_{\text{adv}-}] \qquad (5)$$

where $A^{(b)}_{\text{ret}+}$ denotes the positive frequency part of $A^{(b)}_{\text{ret}}$. The other quantities in (5) are similarly defined. The primed quantities arise from conjugate paths. The important point is that in a cold environment (provided by the expanding universe) paths contribute positive frequencies and conjugate paths negative frequencies. The appearance of paths and conjugate paths is an indication of the richness of quantum theory in comparison with the classical one, and is responsible for the variety of phenomena in quantum electrodynamics.

In the limit $\hbar\to 0$, paths and conjugate paths coincide and we arrive at (4). Thus the classical Wheeler–Feynman radiation reaction formula follows from the quantum calculation in the limit $\hbar\to 0$.

F. HOYLE
J. V. NARLIKAR

Institute of Theoretical Astronomy,
University of Cambridge,
Cambridge.

Received March 2, 1970.

[1] Katz, A., Ornan, E., and Schwartz, M., *Nature*, **225**, 534 (1970).
[2] Hoyle, F., and Narlikar, J. V., *Ann. Phys.*, **54**, 207 (1969).
[3] Wheeler, J. A., and Feynman, R. P., *Rev. Mod. Phys.*, **17**, 157 (1945).

Desert Stream Channels resembling Lunar Sinuous Rilles

SOME of the sinuous rilles on the Moon seem to have been formed by fluid flow, but what the fluid was is unknown. Lava[1,2], ash[3], water[4,5], or combinations such as mud, or water under or over permafrost (refs. 6–8 and unpublished work of Hapke and Goldberg), have been suggested (Fig. 1).

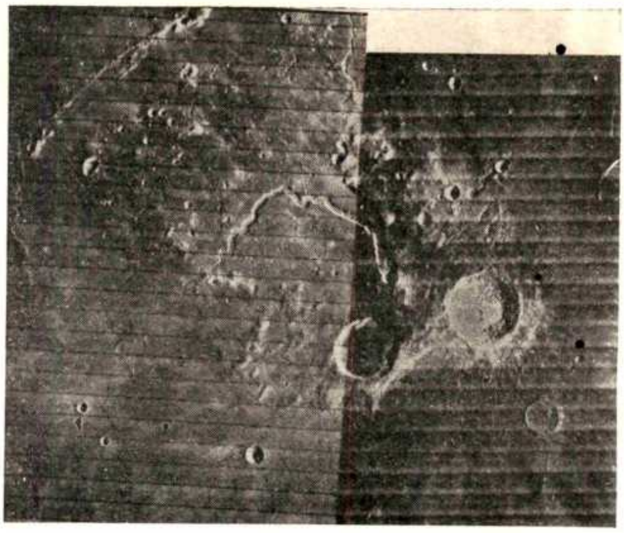


Fig. 1. Aristarchus region of Moon. Main channel of Schröter's Valley is 3 to 5 km wide. Lunar Orbiter IV, Frames H-150 and H-157.

Air photographs of the Mojave Desert geomorphic province of southern California show several stream channels that resemble certain lunar sinuous rilles. We have examined two of these in the Deadman Lake Quadrangle approximately 12 miles north of Twentynine Palms, to find how they are formed. The desert rilles are on alluvial slopes where windblown sand is present so that the intermittent streams emerging from canyons are forced to transport fine sand in addition to their normal burden of alluvium. The meandering channels are then partly covered by more windblown sand, much of it resting at the angle of repose typical for desert dunes. Thus all shapes are gently rounded and the channel banks are hidden except for occasional outcrops of loose or weakly consolidated sand, gravel and angular cobbles. These stream beds look quite unlike ordinary desert washes, but are strikingly similar to lunar rilles.

Fig. 2 shows the desert rilles, and Fig. 3 some examples of normal desert stream beds. The California rilles are about the same size as the smallest lunar rilles identifiable; they are 3–5 km long and up to 50 m deep at the upper end. Rilles on both the Earth and Moon differ from ordinary alluvial stream beds in that they lack tributaries, branches or braiding, and they taper away to nothing at the downstream end with no evidence of a delta. Some parts of the desert rilles (Fig. 4) have the same kind of channel banks as an ordinary wash or arroyo, including vertical walls where the alluvium is sufficiently con-

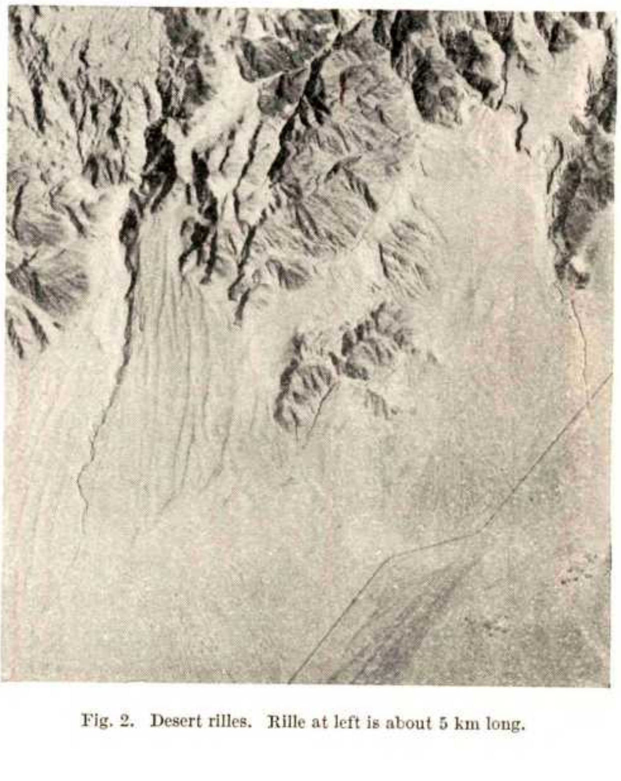


Fig. 2. Desert rilles. Rille at left is about 5 km long.

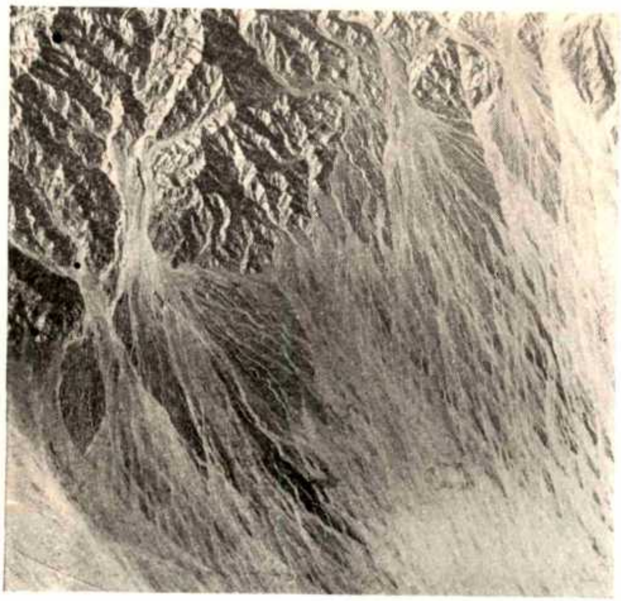


Fig. 3. Typical desert stream patterns on alluvial fans.

solidated, but usually these banks are hidden by windblown sand.

The highlands are the chief catchment area for runoff water and are massive granitic rocks made up of quartz, potassic feldspar and plagioclase. Some samples contain considerable biotite and/or horneblende, desert varnish and brown iron staining are common, and the average composition and texture are of quartz monzonite. The rilles are cut into old alluvial fans perhaps 150 m thick and composed of moderately sorted cobbles and pebbles in coarse arkosic sand. There are cross-bedded lenses, sections of micaceous siltstone, and cobble-boulder deposits. Windblown sand forms a veneer over the older alluvium and in some places has drifted into 10 m thick dunes. The sand consists of loose, fine-grained quartz showing frosting and rounding of individual grains from wind action. Silt and clay particles have been deflated from this material.

The site has been affected by several erosion cycles brought about by either climatic changes or changes in local base level. The oldest cycle affecting the rilles was of erosion that prepared the area for deposition of the older alluvium, which rests unconformably on the igneous rocks. The most recent cycles have again been erosion in which the older alluvium was cut. There are at least two terrace levels along the stream channels and one of these seems to have had a channel about five times the present width of the stream bed. The meander belt of the present stream is usually contained within the terrace scar of this older stream bed.

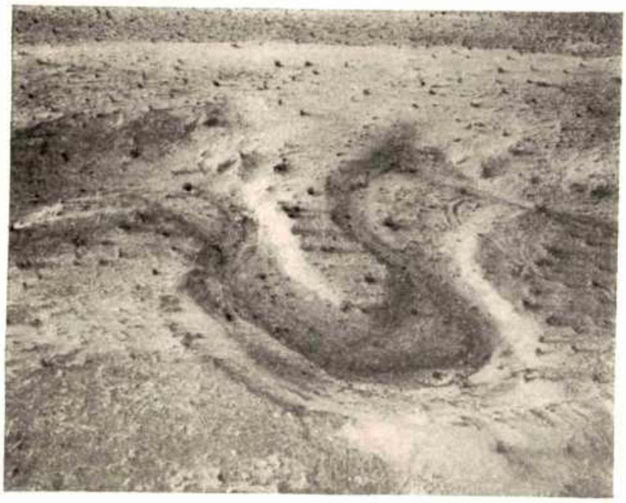


Fig. 4. Meanders in desert rille. Vehicle tracks give scale.

There are no indications of possible tilting or faulting of the older alluvium, but regional structure suggests that the western flanks of the igneous highlands are faulted. This igneous block forms a horst with a graben centred to the west in Deadman Lake. The older alluvium was built as a fan between the uplifted fault block and the Deadman playa, and drainage of the streams is dictated by this slope.

In the highland area the streams have typically youthful channels, but in the alluvium they are more mature. Because of the permeability of the older alluvium, the streams are more mature and incised near their origin than near their terminus. We tried to detect a subdued delta by examining the downstream ends of the desert rilles at low Sun angles. Some of the aerial photographs show what might be interpreted as accumulations of debris from the eroded stream channels, but ground observation suggests that most of the volume of material that should be present has been redistributed over a larger area by the combined action of wind and the wandering of the stream channels. The former courses are now hidden by the drifting sands.

Lunar rilles such as those trending down the flanks of the elevated area around Aristarchus resemble the California desert rilles in the following ways: (1) single sinuous channel, growing smaller from top end to lower end; (2) small inner channel meandering between terraces in a larger valley; (3) no braided channels, tributaries or deltas; (4) tendency to detour around obstacles and to parallel adjacent headlands; (5) cross-sectional profiles which are softly rounded with signs of slumping and soil creep features; (6) occasional subdued outcrops, with loose rocks on slopes and bottom of valley.

There are several differences, however. Lunar rilles tend to start in circular pits. In the desert this is simulated by the abrupt change from rock to sandy alluvium at the exit of the mountain canyon. Lunar rille slopes have a "tree bark" appearance which is probably a soil creep feature peculiar to the lunar environment. The desert rilles have a gradient of at least 30 m per km, and the gradient of the lunar rilles[5] is probably lower than this in most cases. The lunar rilles extend to much larger sizes.

We conclude that the difference between desert rilles and "normal" stream beds on the alluvial slopes has to do with the presence or absence, respectively, of a blanket of windblown sand. The desert rilles have ordinary water-cut channel banks of bedded alluvium near their upper ends, but these are often hidden by the windblown sand. It seems that the relationship between slope and transport capacity for the medium flowing in the rilles on both the Earth and Moon is such as to cause meandering, even to the point of incipient oxbow cutoff (see Figs. 1 and 4). On both Earth and Moon the presence of terraces is strong evidence for a change in either the base level or the supply of fluid at different times in the past.

We have two more speculative points. (1) The windblown sand may have had a primary and a secondary effect. The primary effect was to change the balance between erosion and transport for the stream, relative to what it would normally be at the point of emergence onto the alluvial pediment. The secondary effect was the softening of most channel topography by deposits of windblown sand (on the Moon, the mechanism was different presumably, meteorite redistribution or soil creep for example, but the result is similar). (2) As suggested by Lingenfelter *et al.*[6], the material in which the lunar rilles were eroded was unconsolidated during the flow, and may still be. It is not essential to postulate that a large quantity of lunar volatiles, such as ice, had to escape to account for the existence of a voluminous valley with no apparent delta. The volume of the desert rilles must be distributed at their downstream ends by the combined action of water and wind in such a way that all channels except the most recent are hidden. On the Moon, therefore, either the volume vanished by evaporation or there was a distribution mechanism which smoothed out the deposits as they were delivered at the downstream end.

We thank Colonel D. J. Burger, First Lieutenant Carlton Crenshaw and Gunner Sergeant R. E. Bingham, USMC, for their help, and Mr W. Sjogren of JPL, Dr George Ulrich of the US Geological Survey, and Mr R. Bryson of NASA for discussions. The aerial photographs were provided by the USGS and by G. Edwards and J. Gregoire of JPL. This study was supported by NASA.

J. D. Burke
R. G. Brereton
P. M. Muller

Jet Propulsion Laboratory,
California Institute of Technology,
Pasadena, California.

Received December 23, 1969.

[1] Kuiper, G. P., Strom, R. G., and LePoole, R. S., *Interpretation of the Ranger Records, Rangers VIII and IX, JPL TR 32-800, Part II*, 35 (1966).
[2] Green, J., photograph and comment in article by Hunter, G. S., *Aviation Week*, 43 (April 22, 1968).
[3] Cameron, W. S., *J. Geophys. Res.*, **69**, 2423 (1964).
[4] Urey, H. C., *Nature*, **216**, 1094 (1967).
[5] Gilvarry, J. J., *Nature*, **221**, 533 (1969).
[6] Lingenfelter, R. E., Peale, S. J., and Schubert, G., *Science*, **161**, 266 (1968).
[7] Peale, S. J., Schubert, G., and Lingenfelter, R. E., *Nature*, **220**, 1222 (1968).
[8] Gold, T., in *The Origin and Evolution of Atmospheres and Oceans*, 249 (Wiley, New York, 1964).

Sunrise and Moonrise at Stonehenge

A few years ago Hawkins[1-3] demonstrated that Stonehenge had more astronomical significance than previously recognized, but serious criticisms have been raised against parts of his theory. In particular, Atkinson[4,5] has challenged Hawkins's basic proposition that the Heel Stone indicated the direction of sunrise on midsummer morning. In this report I offer an improvement to Hawkins's theory, which accommodates this objection, and at the same time I propose an explanation of why the Heel Stone was asymmetrically situated, south of the centre line of the Avenue (see Fig. 1).

It is usually agreed[1,4,6] that when Stonehenge was in use an observer at its centre saw first light of the midsummer Sun somewhat to the left of the Heel Stone—in a direction indicated, at least approximately, by the axis of the structure and Avenue. Then the Sun rose upward and to the right; when directly above the Heel Stone, it hung just over the horizon, with its lower limb at an altitude of one-half degree.

Hawkins[1,3,7] argued that originally the Heel Stone extended one-half degree above the observer's apparent horizon, so midsummer sunrise was indicated by the Sun's standing tangent on the Heel Stone's tip; thus the Heel Stone fitted into the grand pattern of the astronomical theory, in which many other stones and archways similarly indicated most of the key rising and setting points of the Sun and Moon. Atkinson[4,5,7] argued that the Heel Stone never did extend above the apparent horizon, that the proper direction of midsummer sunrise was the direction of its first gleam, and that the Heel Stone's failure to mark midsummer sunrise cast significant doubt on Hawkins's entire theory. I wondered whether Hawkins and Atkinson might each be partly right.

If the Heel Stone and the axis had both designated the same celestial event, Stonehenge would have involved an unseemly redundance. I therefore sought another function for the Heel Stone and found that the Heel Stone accurately marked the rising point of the midwinter full Moon, if it happened to be precisely centred on the northern solstice point of the ecliptic. This direction of rising differed slightly from the direction of midsummer sunrise because of the geocentric parallax of the Moon, and it represented a set of rising points associated with the danger of a midwinter, lunar eclipse.

Some aspects of these ideas were originated by Hawkins, who explained[2,3] why the rising of the midwinter full Moon "over" the Heel Stone could have warned Stonehenge man of an impending eclipse. But considering Hawkins's concept of "moonrise" (lower limb tangent), his explanation involved another sizable error in altitude. Furthermore, Hawkins's reason for the off-axis location[1] of the Heel Stone had nothing to do with the Moon, and he continued to designate the Heel Stone as a sunrise marker[3]. I suggest that the Heel Stone was used exclusively as a winter eclipse moonrise marker, while the axis and Avenue were oriented toward midsummer sunrise. That would explain functionally why the Heel Stone was set slightly south of the centre line of the Avenue.

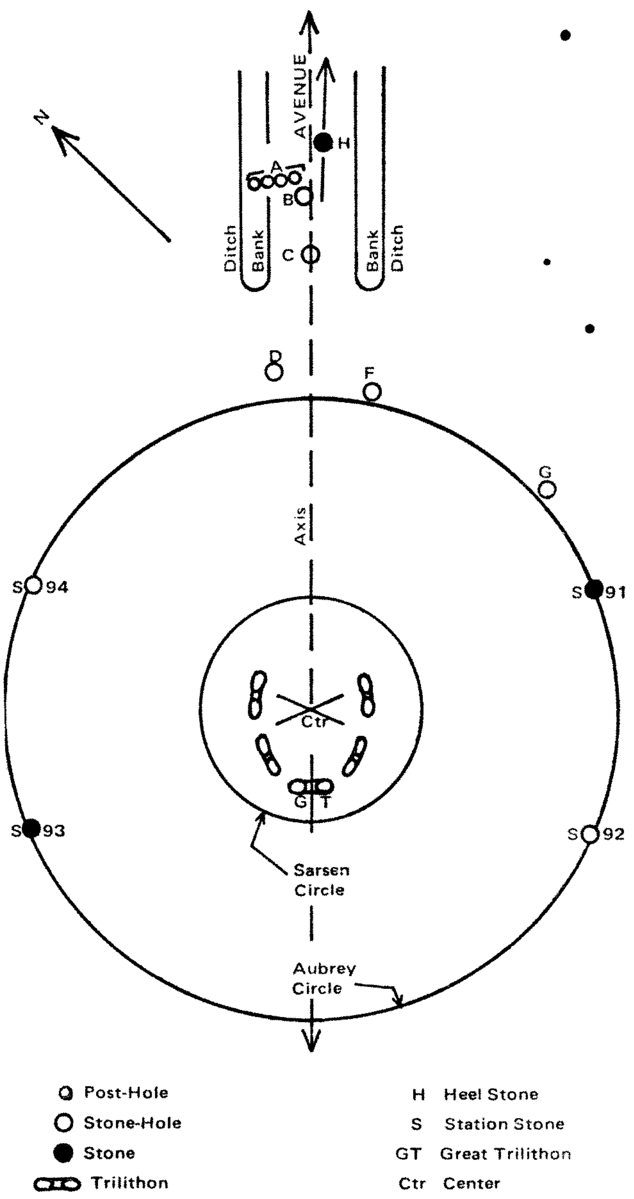


Fig. 1. Plan of Stonehenge.

The magnitude of the parallax effect provides a simple check that does not require any initial assumption about Stonehenge man's concept of "sunrise" and "moonrise". Celestial bodies rising beyond the Avenue and Heel Stone follow slanting paths, making an angle with the horizon of about 32°. Due to the Moon's proximity to the Earth, it has to rise 0·95° higher than the Sun does before it becomes visible on the apparent horizon (because the Moon's parallax is 0·95°, and the Sun's parallax is practically negligible). In the process of rising this extra 0·95°, the Moon moves 1·5° to the right. Thus if there was a winter solstice moonrise marker as well as a summer solstice sunrise marker on the Avenue, their azimuths should have differed by 1·5°.

Actually, there are two prominent stone-holes in the Avenue near the Heel Stone; one of them—stone-hole C—is well centred between the Avenue banks, and could have been the site of a precise Avenue axis marker. Measurements on a map of Stonehenge (Fig. 8 in ref. 4) showed that the azimuth of stone-hole C differed from that of the Heel Stone by 1·5°, as expected.

As an initial step of a second test, I calculated the altitude of the Sun—to see if it was indeed just beginning to rise—when at the azimuth of stone-hole C on mid-summer mornings around 1700 BC. The result was that the upper limb was just barely above the apparent horizon, by 0° 4′ of arc.

The accuracy of this result may have been fortuitous because the azimuth of stone-hole C had to be determined from measurements on a map of uncertain accuracy, but another similar calculation showed that on midwinter evenings, when the setting Sun was at the azimuth indicated by the Great Trilithon, its upper limb was only 0° 2′ from the horizon.

Furthermore, it followed that if the Heel Stone similarly marked winter eclipse moonrise, then the Moon at northern solstice should have been showing its upper limb just over the north-east horizon when at the Heel Stone's azimuth. Calculation confirmed that prediction, with a discrepancy of only 0° 5′.

Some additional implications seem worth mentioning:

(1) Atkinson was right in judging that the Stonehenge people watched for first gleam or last gleam of the Sun and Moon. (This was the same conclusion that C. A. Newham[6] reached by a different method.)

(2) Hawkins was correct in asserting great astronomical significance of Stonehenge, including some capability for predicting eclipses.

(3) The Heel Stone and other markers indicated directions only; altitudes were observed with respect to the distant horizon.

(4) Perhaps the astronomical theory of Stonehenge could be further improved, by reduction of other alignment "errors", if some of the other stones and archways were associated with the Moon rather than the Sun.

But one new question is how the Stonehenge people knew where the Heel Stone should be placed in order to indicate potential eclipse of the midwinter full Moon.

I suggest that the Stonehenge people first noted the passage of the seasons, by placing markers to indicate rising and setting points of the Sun on midsummer and midwinter days. That would have been a natural and easy thing for them to do empirically. Later they discovered that if the midwinter full Moon rose a bit south of the midsummer sunrise marker, then a lunar eclipse was likely. But if the midwinter full Moon rose much to the north or much to the south of the summer sunrise marker, then it was never eclipsed. So they just observed the north and south limits of winter full moonrise, and placed the Heel Stone midway between those limits.

My subsequent calculations showed that the full Moon closest to midwinter could have risen as far north as azimuth 41·1° or as far south as 61·4°; and the mean of these values was within 0·1° of the actual azimuth of the Heel Stone. The north and south limits of winter full moonrise as actually observed from Stonehenge were probably at azimuths somewhat greater than 41·1° and less than 61·4°, because each theoretical limit required conditions that almost never occurred simultaneously. Nevertheless, the direction midway between observed limits should have led to accurate placement of the Heel Stone, due to small imperfections counteracting each other.

My calculations were based, whenever possible, on the data presented by Hawkins in ref. 3; and my equations were essentially the same as his. Yet my numerical results were somewhat different, for various reasons: use of the first-gleam concept of sunrise and moonrise; use of 5·00° for inclination of the orbit of the Moon during non-eclipse seasons (instead of the average value 5·15°); and modification of Hawkins's table of atmospheric refraction versus geometric altitude—to include the effect of observer's height above sea level, as well as the effect of winter cold and summer heat (±20° F).

JACK H. ROBINSON

University of South Florida,
Tampa, Florida.

Received August 20; revised December 29, 1969.

---

[1] Hawkins, G. S., *Nature*, **200**, 306 (1963).

[2] Hawkins, G. S., *Nature*, **202**, 1258 (1964).

[3] Hawkins, G. S., *Vistas in Astronomy*, **10** (Pergamon Press, London, 1968).

[4] Atkinson, R. J. C., *Stonehenge* (Hamish Hamilton, London, 1956).

[5] Atkinson, R. J. C., *Antiquity*, **40**, 212 (1966).

[6] Newham, C. A., *The Enigma of Stonehenge* (private publication, 1964).

[7] CBS Television, *Mystery of Stonehenge* (McGraw-Hill, Film Department; New York, 1965).

Earthquakes and Explosions: Comment on Molnar *et al.*

It is incorrect of Molnar *et al.*[1] to say that the shape of the surface wave spectra for discriminating between earthquakes and explosions had been hypothesized but not tested.

The spectral differences were demonstrated some two years ago as a result of P. D. Marshall's work in the Seismological Research Group of AWRE at Blacknest. These early results had been widely discussed here among colleagues from the USA[2] and the USSR. I presented this work, together with A. Douglas's theoretical studies on the same problem, to the Seismic Study Group sponsored by SIPRI in Stockholm. Marshall's experimental data, which include those of explosions at the American, French and Russian test sites, are published in *Seismic Methods for Monitoring Underground Explosions* (SIPRI, Stockholm, 1968). So far as I know, they are the only teleseismic explosion versus earthquake surface wave spectra published to date.

Molnar's contribution to this problem vividly demonstrates the value of broadening the recording band for this purpose, provided this can be done without raising the detection threshold (m_b 4·5 explosions) which, if necessary, could be achieved by narrow band long period systems. They demonstrate that it is possible to eliminate environmental noise at the bottom of a deep mine, and thereby operate at magnifications which are comparable with a narrow band system.

The broader interests of seismologists have been well served by this painstaking instrumental development. In the more restricted field of detecting underground explosions, it remains to be seen whether a world-wide network of instruments in near-surface vaults will be capable of detecting surface waves of small distant explosions. On Molnar's evidence, the detection threshold for explosion generated surface waves for the Ogdensburg mine wide band system lies between m_b 5 and m_b 5·3 at a distance of 3,500 km.

H. I. S. THIRLAWAY

UKAEA, Blacknest,
Brimpton, Reading RG7 4RS,
Berkshire.

Received January 26, 1970.

---

[1] Molnar, P., Savino, J., Sykes, L. R., Liebermann, R. C., Hade, G., and Pomeroy, P. W., *Nature*, **224**, 1268 (1969).

[2] Liebermann, R. C., and Pomeroy, P. W., *J. Geophys. Res.*, **74**, 1575 (1969).

Reply to Thirlaway

WE apologize to Thirlaway and his colleagues for stating categorically that the shape of the surface wave spectra for discriminating between earthquakes and explosions was suggested prior to our work[1] but not tested. The two figures presented by him in the SIPRI report[2] certainly suggest such spectral differences for explosions and earthquakes. For his data, however, it is not possible to attribute these differences unambiguously to the two kinds of sources because earthquake and explosion data were not shown for the same region. Because Liebermann and Pomeroy[3] and others demonstrate large regional differences in M_S–m_b populations, sceptics could question the interpretation of these differences. Also, the two figures are presented with practically no discussion; we strongly urge publication of the results through established journals. His explosion data do not extend to periods greater than 25 s for two events and about 33 s for the third.

Our explosion and earthquake data[1], however, were obtained for nearly identical source regions and propagation paths, and they extend to periods of about 50 s. Thus we can unambiguously state that differences in the source spectra for the set of explosions and earthquakes that we studied must be attributed largely to differences in the two different types of source time functions and not to differences in focal depth, propagation path, radiation pattern or source dimensions.

We agree that it remains to be seen whether instruments (particularly long period horizontal seismometers) in near-surface vaults will give results comparable with those obtained in the New Jersey mine. Nevertheless, we are optimistic that this can be done. Our location in an active zinc mine is, in fact, quite noisy for long period pressure fluctuations. We successfully eliminated pressure changes as a major contributor to the seismic background noise. Amplitudes of microseismic noise for periods near 20 s in the mine are comparable with those reported[2] for high-quality sites on the surface. Because our instruments are limited by microseismic noise with periods near 20 s, detection capability is not sacrificed by broadening the recording band in comparison with existing narrow band instruments peaked near 20 s.

We stated[1] that we did not have adequate data for m_b (USCGS) less than 5·0 to identify the lower threshold for detection and discrimination of explosions and earthquakes. Also, we noted that body-wave magnitude, m_b, as determined by the USCGS for events in western North America, tends to be larger than determinations made at great distance (which form the basis for most comparisons of detection and discrimination levels[2]). Thus Thirlaway's assessment of our detection threshold probably is a pessimistic interpretation of our results.

In our analysis, a comparison of 50 s Rayleigh waves with m_b seems to be the best discriminant for underground explosions and earthquakes. The amplitudes of 50 s waves for the smallest explosions detected (m_b 5·3) are about twenty times smaller than those for earthquakes of the same m_b. The near parallelism of the explosion and earthquake populations makes us optimistic that the discrimination threshold can be lowered by similar study of additional earthquakes and explosions with $4·0 < m_b < 5·0$ and by more sophisticated data processing.

PETER MOLNAR
JOHN SAVINO
LYNN R. SYKES
ROBERT C. LIEBERMANN
GEORGE HADE

Lamont-Doherty Geological Observatory of
Columbia University,
Palisades, New York 10964.

PAUL W. POMEROY

Seismological Observatory,
University of Michigan,
Ann Arbor, Michigan 48104.

Received March 4, 1970.

---

[1] Molnar, P., Savino, J., Sykes, L. R., Liebermann, R. C., Hade, G., and Pomeroy, P. W., *Nature*, **224**, 1268 (1969).

[2] *Seismic Methods for Monitoring Underground Nuclear Explosions—An Assessment of the Status and Outlook* (International Institute for Peace and Conflict Research (SIPRI), Stockholm, 1968).

[3] Liebermann, R. C., and Pomeroy, P. W., *J. Geophys. Res.*, **74**, 1575 (1969).

Measurements on Cloud Chamber Tracks

RECENT work reported from Sydney[1-5], on tracks in cloud chamber photographs of particles near the axis of extensive air showers, might be interpreted in terms of particles of subunit charge. It is important, however, to examine the conditions in which normal tracks might possess the reported features. This report draws attention to some specific effects.

If the images counted in this work are indeed drops, data reported by Ghosh, Jones and Wilson[6], from a study of the logarithmic rise of ionization with increasing energy for muons, are relevant. These include two distributions of actual measurements for segments, of 1 cm and about 15 cm respectively, of drops from field-separated tracks near the minimum of ionization in the fully condensed positive ion column. They refer to oxygen at standard pressure and exclude ion clusters arising from energy transfers of about 1 keV upwards. The actual number of drops condensed on the ions of an unseparated plateau track in the Sydney work probably lies in the range 100–160 drops per centimetre, according to the completeness of condensation and the criterion adopted for the exclusion of large energy transfers (delta-rays). Accordingly, this implies that, if condensation were complete, the reported drop counts refer to those in about 1·5 cm of track and the fluctuations would be comparable with those in about two centimetres of the tracks measured by Ghosh *et al*. These fluctuations would therefore be more like those of Fig. 2 than of Fig. 3 of that paper. If condensation were incomplete, the counts would refer to a greater length of track, but an additional fluctuation, caused by actual condensation taking place would be introduced. In either case, the extreme fluctuations would certainly be large and the reported measurements would fall near to them.

Fluctuations of numbers of single drops, although these must occur, are most unlikely to represent the whole situation correctly. The workers in Sydney have sent me direct contact prints of their negatives (from and onto 35 mm film), and it seems that the number of objects counted per centimetre of track is of the order of 30–40 and certainly nothing like 100–160. The Sydney tracks are said to be about 0·17 cm wide, and the magnification for the plane of the tracks is likely to be about 1/13. The smallest objects counted were about 20 μm in diameter, and objects larger than this may be irregular. They do not cover more than about one-fifth of the whole track area. The most likely interpretation is that detectable objects in the track are derived from superpositions of drop images, and that in these photographic conditions the image of a single isolated drop does not produce a recognizable blackening of the emulsion. This statement is probably not correct for all the Sydney photographs nor necessarily for the whole area of some others, but it does seem to apply in the region of the particular photograph used by McCusker and Cairns[1].

The nature of superposition involved in the formation of these objects can be deduced from the fact that they are not concentrated exclusively along the central line of the track. Indeed, their spread from this line forms the basis for measuring the track breadth. The significance of this feature is apparent from Plates I and II of Ghosh *et al.*[6]. The middle point of the image of an energetic delta-ray may be displaced from the central line of the track (Plate II), but those of smaller drop clusters (Plate I) are not. Superpositions which do not lie on the central line are not usually caused by the drops having a common origin in still smaller clusters. While primary ionization will have been spaced on average about 0·03 cm along the line of track, the distance of "random walk", along which many ions must have moved to reach the points where they contribute to an image, must have been several times greater than this value. Accordingly, the drops can be considered to be independent in origin. This suggestion about what is in fact photographed should lead to tracks rather less diffused than those expected from the reported time delay of photography, because the ions which diffused furthest would reach relative isolation and would probably be unrecorded. This effect should be examined in detail from the primary data, acceptance criteria and techniques. But a rough indication, using reasonable figures, is worthwhile.

One centimetre of track is 0·17 cm² in area and gives an image area of about $1 \cdot 0 \times 10^{-3}$ cm². Suppose the effective size of a single drop image is comparable with that of the smallest visible objects in the pictures (4×10^{-6} cm², or 4×10^{-3} of the area of the image of 1 cm of track) and that effective overlapping leads to a visible object in the photograph if the centre of one drop image falls anywhere within the 4×10^{-6} cm² area of another image. A rough calculation indicates that for a track density of 150 drops/cm, there will then be seventy-nine non-overlapping drops, twenty-five pairs of overlapping drops and seven examples of multiple (three or greater) superposition. Thirty-two objects will therefore be visible if the images of isolated drops are not seen and 111 objects if the images of single drops are visible. For a track density of 100 drops per centimetre, the corresponding numbers of objects in the image are sixteen and eighty-two respectively. These figures, although derived from a very rough analysis, are comparable and the change from 100 drops/cm to 150 drops/cm, a ratio of 1·5, is similar to the difference between the minimum of ionization and the relativistic plateau.

If all drops and drop overlaps were recorded, the observed ratio would not be 1·5, but 1·35, and there would also be about 110 objects/cm in the measured plateau track. If only superpositions yielded recordable objects in the photograph, the corresponding ratio would be 2·0 and there would be about thirty objects per centimetre. These ratios, so strikingly different, even before any consideration of fluctuation has been introduced, emphasize the need for an exact analysis based explicitly on the Sydney work and the material used in it.

This discussion has been concerned with features which do not seem to exclude the possibility of the tracks being normal. The most difficult characteristic of the published data to relate to normal tracks, however, is the relative lack of delta-rays. This should not be sensitive to the nature and energy of the initiating particle but should depend strongly on its charge. There is therefore a good chance that selection could be made of delta-rays which would apply in different parts of a single photograph and for many photographs. It is true that the relatively small number of delta-rays make large fluctuations on a limited length of track probable, but sufficient delta-ray counts might be made to establish whether or not the number counted on tracks such as that published[1] can possibly lie in the tail of a general distribution.

J. G. Wilson

Department of Physics,
University of Leeds.

Received December 1, 1969; revised March 6, 1970.

[1] McCusker, C. B. A., and Cairns, I., *Phys. Rev. Lett.*, **23**, 658 (1969).

[2] Cairns, I., McCusker, C. B. R., Peak, L. S., and Woolcott, R. L. S., *Phys. Rev.*, **186**, 1394 (1969).

[3] Adair, R. K., and Kasha, H., *Phys. Rev. Lett.*, **23**, 1355 (1969).

[4] Frauenfelder, H., Kruse, V. E., and Gard, R. D., *Phys. Rev. Lett.*, **24**, 33 (1970).

[5] Rahm, D. C., and Louttit, R. I., *Phys. Rev. Lett.*, **24**, 279 (1970).

[6] Ghosh, S. K., Jones, G. M. D. B., and Wilson, J. G., *Proc. Phys. Soc.*, A**67**, 331 (1954).

Frequency Mixing in the Scattering of Light by Harmonically Bound Electrons

Classical electrodynamics predicts harmonic generation[1-3] and frequency mixing[4] in the scattering of light by free electrons. Quantum theory confirms these results[3-7]. Several authors (for example, refs. 8 and 9) have used the classical anharmonic oscillator model to give a qualitative account of non-linear phenomena in dielectrics. In the absence of an anharmonic restoring force, non-linear effects, up to the lowest order in external electric field, are in general quite small[10]. Calculations[11] stress this point in the case of second harmonic generation in the scattering of light. Here we extend these calculations to study the scattering of light when the incident beam contains two frequencies. As expected, we find that the combination frequencies also exist in the scattered radiation besides the fundamentals and their harmonics, and that the non-linear cross-sections become large for resonant denominators. Further, a proper choice of the two incident frequencies can yield an appreciable increase in the non-linear scattering cross-sections even without any precise resonance.

The equation of motion of the electron bound by a restoring force $m\omega_0^2\,\mathbf{z}$ when the incident electromagnetic beam contains two frequencies ω_a with wave vectors K_a $(a=1,\,2)$ can be written as

$$v_0[\ddot{\mathbf{z}}+v^2\,(\ddot{\mathbf{z}}\cdot\dot{\mathbf{z}})\,\dot{\mathbf{z}}]+\omega_0^2\mathbf{z} = (e/m)\sum_a\{\mathbf{E}_a+[\dot{\mathbf{z}},\,\mathbf{H}_a]\}\cos\,(\omega_a t-\mathbf{k}_a\cdot\mathbf{z}+\delta_a) \qquad (1)$$

where $\mathbf{z}$ is the position of the electron, δ_a are constants and $v_0=(1-\dot{\mathbf{z}}^2)^{-1/2}$.

This equation can be solved by making a perturbation expansion in power of E_a $(|\mathbf{E}_a|=|\mathbf{H}_a|=E_a)$. The radiation field can then be evaluated by a method described in detail in ref. 1. It contains a complete spectrum of combination frequencies as well as the fundamentals and their harmonics. If terms are retained up to the third order in the amplitude of the two incident frequencies, scattering cross-sections are produced which correspond to sum and beat frequencies, along with those for fundamentals and their harmonics. Explicit expressions for the total cross-sections for scattering into ω_1, $2\omega_1$ and $\omega_1+\omega_2$ are given by

$$\begin{aligned}\sigma\omega_1 = {} & (I_1/I_0)\sigma_T\omega_1{}^4\,g_1{}^2\,[1+(1/20)\,q_1{}^2\,g_1\,\omega_1{}^4\,(10\,g_{1+1}-g_1+ \\ & 15\omega_1{}^2\,g_1{}^2)+(1/40)\,q_2{}^2\,\omega_2{}^2\,\{10\omega_1{}^2\,g_1\,(g_{1+2}+g_{1-2})+ \\ & 10\omega_1\omega_2\,g_2\,(g_{1+2}-g_{1-2})-\omega_1{}^2\,g_2{}^2+30\,\omega_1{}^2\,\omega_2{}^2\,g_1\,g_2{}^2\}]\end{aligned} \qquad (2a)$$

$$\sigma_2\omega_1=(32\pi e^2/5m^2)\,(I_1{}^2/I_0)\,\sigma_T\,\omega_1{}^6\,g_1{}^2\,(5g_{1+1}{}^2+g_1{}^2) \qquad (2b)$$

$$\begin{aligned}\sigma\omega_1+\omega_2 = {} & (2\pi e^2/5m^2)\,(I_1I_2/I_0)\sigma_T(\omega_1+\omega_2)^4 \\ & \times[5(\omega_1\,g_1+\omega_2\,g_2)^2\,g_{1+2}{}^2+(\omega_1+\omega_2)^2\,g_1{}^2\,g_2{}^2]\end{aligned} \qquad (2c)$$

where

$$g_{a\pm b}=(\omega_0{}^2-\omega^2{}_{a\pm b})^{-1}, \quad q_a=e\,E_a/m\,\omega_a,$$

$\sigma_T=(8\pi/3)_{r_0}{}^2$ is the Thomson scattering cross-section, $I_a=E_a{}^2/8\pi$ is the intensity of the components of the incident wave, and $I_0=I_1+I_2$.

Total cross-sections for scattering into ω_2, $2\omega_2$ and $\omega_1-\omega_2$ can be obtained from those for ω_1, $2\omega_1$ and $\omega_1+\omega_2$ respectively by interchanging the suffixes 1 and 2 in the first two cases and changing the sign of ω_2 in the third. In evaluating cross-sections for the fundamental frequency we have excluded the case $\omega_1=\omega_2$, for its inclusion would unnecessarily complicate the calculations.

We may note that the expressions for $\sigma\omega_1\pm\omega_2$ obtained here agree in the limit $\omega_0/\omega_a\rightarrow 0$ with those given by Fried and Frank[7] and do not agree with those given by Prakash and Vachaspati[4]. Expression 2*c*, however, is consistent with 2*b*, as can be seen by putting $\omega_1=\omega_2$.

Expression 2*b* is in general quite small, except for resonant denominators (see equation 7*d* of ref. 11). The same is true for 2*c*, and for the intensity dependent part of expression 2*a*. It may, however, be noted that the cross-section for the fundamental frequency, ω_1, depends not only on ω_1 but also on ω_2. The change in the cross-section of the fundamental frequency due to the presence of ω_2 is easily obtained from the expression under the curly bracket in 2*a*. It is determined by the relative positions of ω_1, ω_2 and ω_0 in addition to the intensity of ω_2. Simple numerical analysis shows that this change is too small to be detectable when neither ω_1 nor ω_2 $(\sim\omega_1)$ is in resonance with ω_0. When $\omega_1\gg\omega_2$, ω_0 an interesting situation arises. It is clear that the cross-section for sum and difference frequencies should in this case be added to that of the fundamental. The total increase in $\sigma\omega_1$, denoted by $\Delta\sigma\omega_1$, can then be seen to be $\sim(3\pi/5)\,(e^2/m^2c^3)I_2\,\sigma_T\,\omega_1{}^2\,g_2{}^2\sim 1{\cdot}4\times 10^{-3}\times(\lambda_2{}^3/\lambda_1 d)^2 I_2\,\sigma_T$, $\lambda=2\pi c/\omega$, if ω_0 and ω_2 are of the same order and $d(=\lambda_0\sim\lambda_2)$ is expressed in Å. It can further be seen that $\Delta\sigma\omega_1$ is larger than $\sigma_2\omega_1$ or $\sigma\omega_{1\pm}\omega_2$ $(\omega_1\sim\omega_2)$ for a large d.

The expression for $\Delta\sigma\omega_1$ given above suggests that the non-linear increase in scattering could become large. This could happen also for those incident frequencies that are far away from the resonance frequency of the scattering body which consists of harmonically bound electrons. As an illustration we consider a simple experiment consisting of the scattering of two incident light beams, one of which is ruby laser light ($\lambda_2\sim 7\times 10^{-5}$ cm, $I_2\sim 10^7$ W/cm², say), by sodium vapour ($\lambda_0\sim 6\times 10^{-5}$ cm, $d\sim 10^3$ Å). For this case we find the non-linear increase in scattering to be $\Delta\sigma\omega_1\sim 1{\cdot}7\times 10^{-20}\times\lambda_1{}^{-2}\,\sigma_T$, which is appreciable for high frequencies and seems to be experimentally detectable for λ_1 in the X-ray region. Instead of sodium vapour as scatterer and ruby laser light as one of the incident beams, one can choose other scatterers and incident frequencies. With the advent of dye lasers one can have a fairly intense coherent radiation over a wide range of frequencies. This would allow for a suitable selection of λ_2, and hence of d, for a given λ_0, keeping in view the availability of I_2 and λ_1.

We thank Professor E. H. Hutten for his interest in this work, and the Royal Holloway College and the Science Research Council for their financial support.

D. ANDREOU
L. M. BALI

Department of Physics,
Royal Holloway College,
University of London,
Englefield Green,
Surrey.

Received November 25, 1969; revised January 20, 1970.

[1] Vachaspati, *Phys. Rev.*, **128**, 664 (1962); **130**, 2598 (E) (1963).
[2] Vachaspati, and Punhani, S. L., *Proc. Nat. Inst. Sci. Ind.* (*A*), **29**, 129 (1963).
[3] Brown, L. S., and Kibble, T. W. B., *Phys. Rev.*, **133**, A705 (1964).
[4] Prakash, H., and Vachaspati, *Nuovo Cimento*, **53B**, 24, 34 (1968).
[5] Fried, Z., *Nuovo Cimento*, **22**, 1303 (1961).
[6] Prakash, H., and Vachaspati, *Ind. J. Pure Appl. Phys.*, **5**, 21 (1967).
[7] Fried, Z., and Frank, W. M., *Nuovo Cimento*, **27**, 218 (1963).
[8] Bloembergen, N., *Non-linear Optics* (Benjamin, New York, 1965).
[9] Garret, C. G. B., and Robinson, F. N. J., *J. Quant. Electron.*, QE-**2**, 328 (1966).
[10] Adler, E., *Phys. Rev.*, **134**, A728 (1964).
[11] Bali, L. M., and Dutt, J., *Nuovo Cimento*, **35**, 805 (1965).

Measurements of Electrical Potential for detecting Crack Extension in COD Tests

THE crack opening displacement (COD) test is currently being used by a large number of research establishments throughout Britain and the USA to establish the fracture characteristics of high toughness–low strength steels.

When a pre-cracked fracture-toughness specimen breaks in a rising load test, the crack tip opening displacement at fracture is frequently deduced from displacement measurements made on the surface of the specimen using a clip gauge. If the specimen size is such that fracture occurs under plane stress or mixed plane stress–plane strain conditions, final rupture may be preceded by a period of stable crack growth. Slow growth may be an essential prelude to fast fracture, so the COD at which it starts can in one sense be regarded as a critical value. Unfortunately, this information cannot be deduced from the conventional force-displacement record of a fracture-toughness test because COD and crack growth affect the surface displacement similarly. This type of behaviour is retarding the development of the COD test, but we believe that we have successfully circumvented this obstacle to the use of the COD measurements for fracture toughness assessment by using the potential drop method of measuring cracks developed by Gilbey and Pearson for fatigue work[1].

Tests were conducted on full-plate-thickness mild steel specimens (256 MNm⁻² 0·2 per cent proof stress at 10° C) of the following composition: 0·2 per cent carbon, 0·05 per cent silicon, 0·03 per cent sulphur, 0·01 per cent phosphorus, 0·69 per cent manganese.

The specimens of the single-edge-notched configuration were tested in three-point-bending. Their dimensions

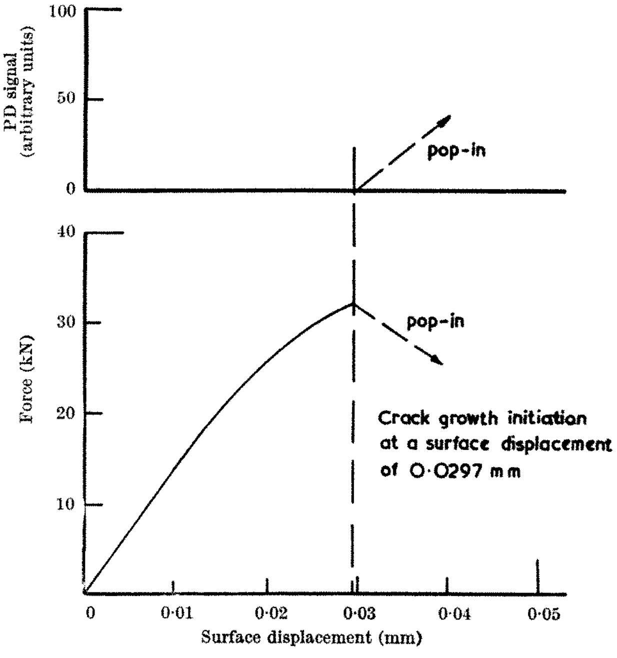


Fig. 1. Type 1 test record indicating an absence of slow crack growth before pop-in.

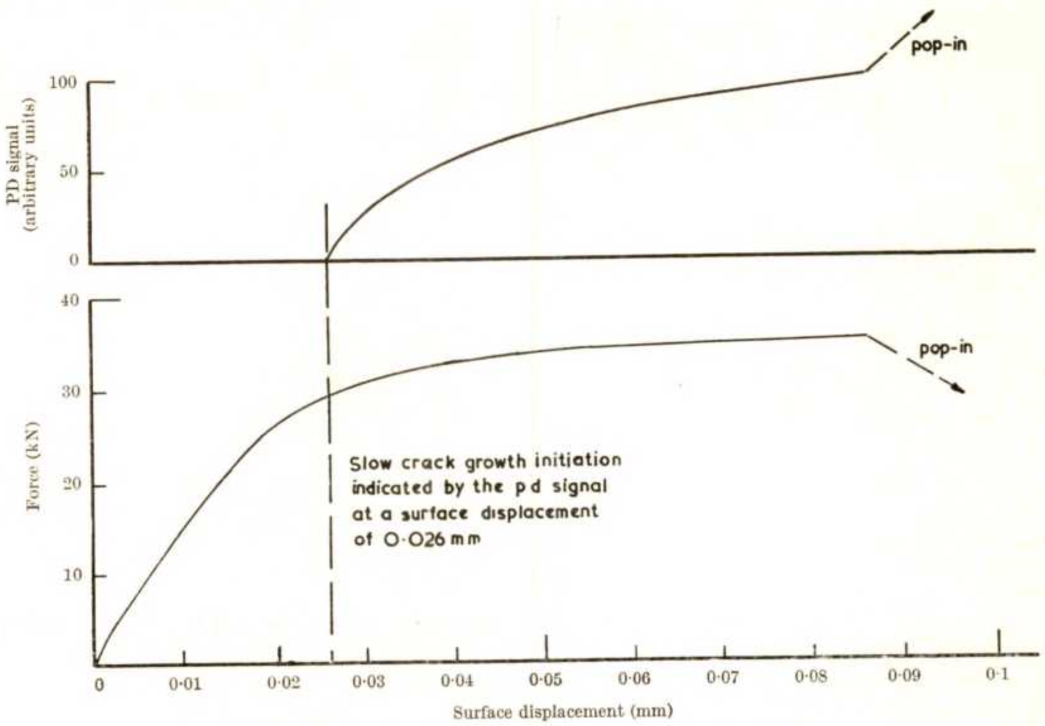


Fig. 2. Type 2 test record indicating slow crack growth before pop-in.

(American Society for Testing and Materials notation) were $B = 28$ mm, $W = 62$ mm, $a = 31$ mm. The tests were conducted in accordance with the proposed British Standard for fracture toughness testing, surface displacement[2], measured using a clip gauge being autographically recorded as a function of the force applied to the specimen. In addition, nichrome wire probes of 0·2 mm diameter were capacitor-discharge-welded to the specimen at points 6·2 mm (0·1 W) on either side of the centre-line of the notch at a distance 15·5 mm (0·25 W) from the notched face of the specimen. A constant direct current of 10 A was passed through the specimen, being introduced at points remote from the notch. Crack extension was detected by monitoring the potential difference between the nichrome probes, this being a "function" of crack length in constant current conditions. The probe signal, after amplification, was recorded on an ultraviolet recorder together with the load cell signal.

In tests conducted at 10° C, two types of behaviour were observed. In the first type of test (Fig. 1) the probe signal, replotted here as a function of surface displacement, did not increase before pop-in. The specimen's fracture face was later examined using a Stereoscan scanning electron microscope and this confirmed that no ductile tearing preceded brittle fracture (Fig. 3*a*). In the second type of test (Fig. 2) the probe potential rose before pop-in, indicating that stable crack growth was occurring. This was subsequently confirmed by Stereoscan observations (Fig. 3*b*).

Concerning the test records, it may well be significant that both brittle fracture and slow crack growth appear to have initiated at similar CODs in these specimens. At this stage, however, comments will be restricted to the potential drop technique itself. It provides a simple yet sensitive method for detecting crack extension and moreover might be made quantitative by previous calibration of the specimen.

Experimentally, application of the method presents few problems, but the following points should be noted. In the vicinity of the crack tip the measured potential is very sensitive to the position of the probes. It is therefore inadvisable to place them in this region because the slight displacement associated with the opening of the crack will affect the signal. They are best positioned on the notched face of the specimen if this is compatible with the clip gauge mounting. In this position the probe signal is unaffected by COD but remains adequately sensitive to crack extension. Second, a small thermo-electric e.m.f. may be superimposed on the probe signal. For qualitative work this is not important provided that it remains constant. Ideally the thermo-electric e.m.f. should be eliminated by using probes of the same material as the specimen. Alternatively the basic potential signal can be

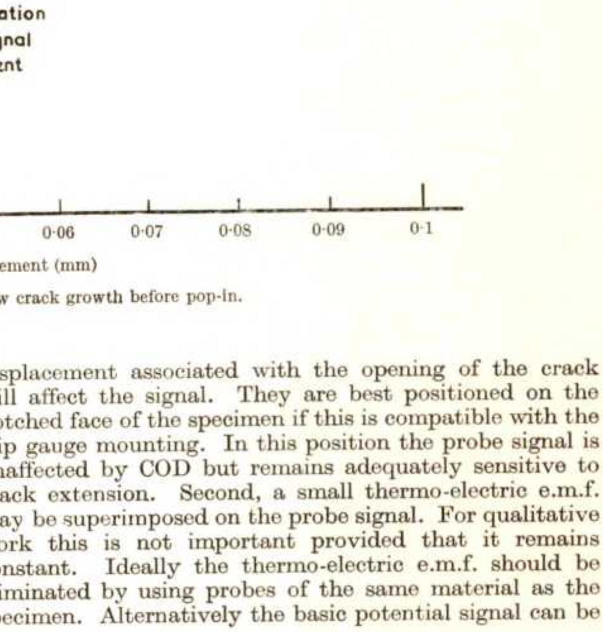


Fig. 3. Scanning electron fractographs showing (*a*) the absence of ductile tearing associated with type 1 behaviour and (*b*) the ductile tearing associated with type 2 behaviour. X, Fatigue area; Y, ductile tearing; Z, brittle fracture (× 187).

increased (by increasing the current) so that the thermal contribution is negligible.

The method is less suitable for use on very large specimens, first because the size of the constant current source required becomes excessive and second because the change in potential is dependent on the change in (a/W) and hence becomes less sensitive to absolute crack extension as W increases.

We believe that additional instrumentation of this or a similar nature is essential if progress is to be made in the development of the COD approach to fracture toughness assessment. It must be accepted that as general yielding is approached in the regime of mixed mode or plane-stress failure, the simple instrumentation used for plane-strain fracture toughness testing is no longer adequate.

We thank Dr G. S. Lane, BR Engineering Research Division, for performing the fractographic work.

J. DAVIES
D. F. CANNON
R. J. ALLEN

British Railways Board Research Department,
Engineering Division,
London Road,
Derby.

Received November 17; revised December 5, 1969.

[1] Gilbey, D. M., and Pearson, S., *RAE Tech. Rep. 66402* (December 1966).
[2] *BISRA Indust. Rep., MG/EB*/350/68 (December 1968).

Occurrence of Indium in Seawater and some Marine Sediments

LITTLE is known about the occurrence of indium in the marine environment. Shaw[1] succeeded in detecting it at concentrations between 0·06 and 0·28 p.p.m. in four of the eleven marine sediments that he examined. No figures seem to have been published yet for the occurrence of the element in seawater, although Baric and Branica[2] have deduced from polarographic studies of saturated solutions of indium in seawater medium that its concentration should be 10^{-10} M. We have determined indium at four depths in the water column at Discovery Station 6917 (23° 00′ N, 18° 42′ W, depth 3,100 m). 'Millipore' filtration (0·45 μm) and preliminary concentration of the indium (by means of chelating ion exchange[3]) were carried out at sea immediately after the samples had been collected. The recovered indium was purified by anion exchange using 'Dowex *AG*2-X8' and then irradiated with thermal neutrons at a flux of 5×10^{12} neutrons cm^{-2} s^{-1} for four weeks. The irradiated sample was taken up in concentrated hydrochloric acid, equilibrated with 15 mg of inert indium as carrier and submitted to a twelve stage radiochemical separation to free the ^{114}In from other radioactive species. Indium was converted to its oxinate for determination of the chemical yield (~60 per cent) and for β-counting. The identity of the separated ^{114}In activity was confirmed by measurement of the half-life, plotting an aluminium absorption curve, and by γ-spectrometry. The concentrations of indium found at depths of 2, 500, 1,000 and 2,000 m were 0·31 ± 0·02, 0·12 ± 0·01, 0·10 ± 0·01 and 0·11 ± 0·01 ng/l. respectively. These concentrations are of the order of a hundred times less than those predicted by Baric and Branica[2]. Neutron activation analysis was also used to determine indium in three deep sea sediments (Table 1). The concentration of indium found in the argillaceous sediment is identical with that found by Shaw[1] in a red clay from the Equatorial Pacific. He was, however, unable to detect indium in a siliceous ooze or in a calcareous ooze.

Table 1. OCCURRENCE OF INDIUM IN DEEP SEA SEDIMENTS

Sediment type	Sample No.	Lat.	Long.	Depth (m)	Indium p.p.m.
Argillaceous	Lamont A172-19	24° 35′ N	64° 02′ W	5,395	0·074
Calcareous ooze	Navado J.4	37° 22′ N	38° 47′ W	3,721	0·023
Diatom ooze	Discovery WS203	62° 56′ S	59° 50′ W	949	0·12

A. D. MATTHEWS
J. P. RILEY

Department of Oceanography,
University of Liverpool,
PO Box 147, Liverpool L69 3BX.

Received January 19, 1970.

[1] Shaw, D. M., *Geochim. Cosmochim. Acta*, **2**, 185 (1952).
[2] Baric, A., and Branica, M., *Limnol. Oceanog.*, **14**, 796 (1969).
[3] Riley, J. P., and Taylor, D., *Anal. Chim. Acta*, **40**, 479 (1968).

Late Maastrichtian Nannoplankton Provinces

IT has been known for some time that there was a sharp differentiation of late Maastrichtian faunal provinces with respect to planktonic foraminifera. Axelrod[1] has presented evidence that this was also the case with some terrestrial megaphyta, for he found a rather abrupt southward shift for the coniferous vegetation of the northern hemisphere during this time. Before this provincialism was discovered, serious problems were encountered in long-range correlation by planktonic foraminifera, because the two late Maastrichtian species, *Abathomphalus mayaroensis* and *Racemiguembelina fructicosa*, are both restricted to the tropics (Fig. 1: *A. mayaroensis* is extremely rare in the two northernmost occurrences (Denmark and Sweden) shown).

Associated with the planktonic foraminifera in marine sediments are skeletal remains of minute planktonic calcareous algae, usually referred to as the calcareous nannoplankton, which are used for age determination of the sediments—especially in small deep sea samples—and on which a detailed zonation is based.

It was at first thought that late Maastrichtian nannofossils were more cosmopolitan than the foraminifera and could eventually be used for correlation in areas

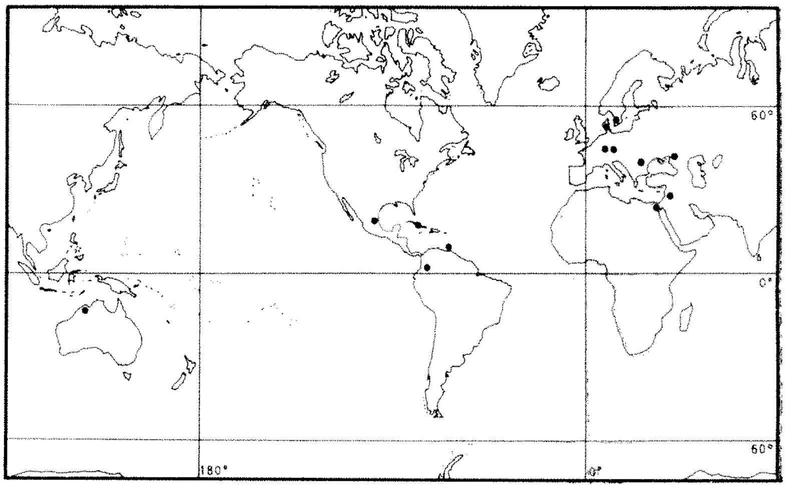


Fig. 1. Occurrences of *Abathomphalus mayaroensis* and/or *Racemiguembelina fructicosa* in the Upper Maastrichtian.

where the planktonic foraminifera were faunally restricted, but evidence has come to light that this is not entirely the case. It seems now that two of the latest Maastrichtian nannofossil index species, *Tetralithus murus* and *Nephrolithus frequens* (that is, *N. frequens* Gorka and closely related forms), were climatically restricted. Fortunately, *N. frequens* occurs toward the poles while *T. murus* is tropical, unlike their foraminiferal counterparts *A. mayaroensis* and *R. fructicosa*, both of which are tropical. It can be seen from Table 1 that *T. murus* and *N. frequens* have been found to occur together only in south-west France[2]. The occurrence of the diagnostic late Maastrichtian planktonic foraminifer *A. mayaroensis* and the belemnite *Belemnella casimirovensis* is also plotted on this table for comparative purposes.

It is highly significant that while *T. murus* is always restricted to the latest Maastrichtian, *N. frequens* is a latest Maastrichtian guide fossil only towards the limits of its province[2]. In south-west France, where both are found together, their known stratigraphic range is approximately ten metres. In the United States, the two have not been found together but have been reported from within a few hundred miles of each other. In Alabama, *N. frequens* is restricted to the uppermost two metres of the Maastrichtian. It is absent in Texas and Arkansas, but *T. murus* has been identified from a single sample from Arkansas[2]. This distribution pattern fits remarkably well with that of the planktonic foraminifera. *A. mayaroensis* has not been found in the Gulf Coastal Plain north of the Tampico Embayment in Mexico, and *R. fructicosa* has been reported from Texas but no farther east, according to Čepek *et al.*[14]. Fig. 2 shows the distribution of occurrences of *T. murus* and *N. frequens* along with the known thickness of the sequence in which they occur for each locality. It is evident that the stratigraphic ranges of both these species seem to be a function of latitude, with *T. murus* thinning toward the poles and *N. frequens* thinning toward the equator.

If *N. frequens* and *T. murus* are indeed provincial, it becomes possible to predict where the most likely additional occurrences of each are likely to be found. *N. frequens*, to our knowledge, has been reported from all the known high-latitude calcareous Upper Maastrichtian sections. It seems probable, however, that a careful search of the Upper Maastrichtian strata containing *A. mayaroensis* and/or *R. fructicosa* might yield *T. murus*. Especially promising areas for search are the Tampico Embayment and western Texas.

As more pelagic sediments of Maastrichtian age are recovered, we cannot refrain from speculating that neither *N. frequens* nor *T. murus* will be found in them because of a rapid upward migration of compensation level in the deep sea during the late Maastrichtian as worldwide climates changed. Should either ever be found, however, one could predict that it would be *T. murus* because it will have been deposited nearer to the late Maastrichtian equator where the compensation depth was probably closer to the bottom in the deep ocean basins, as shown by present conditions[16], and where increased productivity overbalanced the increased rate of solution of calcium carbonate[17].

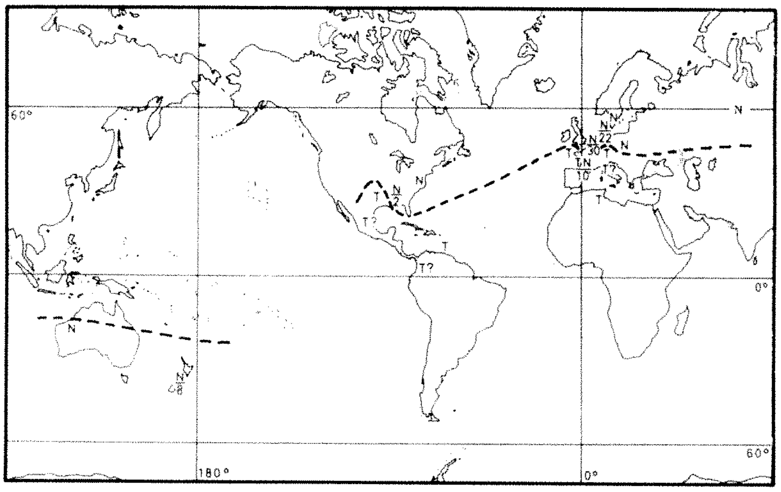


Fig. 2. Late Maastrichtian nannoplankton provinces (**N**, ***Nephrolithus frequens*** **and closely related species**; T, ***Tetralithus murus***; T ?, suggested occurrence of *T. murus*; numbers, if shown, indicate minimum thickness in metres of strata in which one or both species occur).

Table 1. DISTRIBUTION OF THE NANNOFOSSILS *Nephrolithus frequens* AND *Tetralithus murus* IN THE LATE MAASTRICHTIAN, AND COMPARISON WITH THE OCCURRENCES OF THE FORAMINIFER *Abathomphalus mayaroensis* AND THE BELEMNITE *Belemnella casimirovensis*

	Nephrolithus frequens (see text)	*Tetralithus murus*	*Abathomphalus mayaroensis**	*Belemnella casimirovensis*†
Sweden	+[3]	–	(+)	+
Denmark	+[4]	R ‡	(+)	+
Netherlands	+[2]	–	–	+
Poland	+[5,6]	–		+
Siberia (W)	+[7]	–		
New Jersey	+	–	–	
Alabama	+[2]	–	–	
English Channel (W)	–	+[15]	–	
France (SW)	+[2]	+[2,8]	–	
Austria		+[11]	+	
Italy		?		+
Tunisia	–	+[2]		
Arkansas	–	+[2]	–	
Mexico		?	+	
Trinidad	–	+[2,10]	+	
New Zealand	+[9]	–		

+, Present; –, absent; ?, supposed occurrence; R, reworked; parentheses, extremely rare; blank, no data.

* *A. mayaroensis* also occurs in the Caucasus, Columbia, Cuba, Bavaria, Rumania, Egypt and Australia, according to ref. 12.

† *B. casimirovensis* also occurs in the Caucasus, Crimea and South Kazakhstan, according to ref. 13.

‡ Rare reworked specimens in the fish-clay of the lowest Danian, according to ref. 10.

T. WORSLEY

Department of Geology,
University of Washington,
Seattle,
Washington 98105,
USA.

E. MARTINI

Geologisch-Paläontologisches Institut der
Universität Frankfurt a. Main,
Senckenberg-Anlage 32,
Germany.

Received October 24; revised December 12, 1969.

[1] Axelrod, D. I., and Bailey, H.P., *Evolution*, **22**, 595 (1968).
[2] Bramlette, M. N., and Martini, E., *Micropaleontology*, **10**, 291 (1964).
[3] Åberg, M., *Stockholm Contr. Geol.*, **13**, 63 (1966).
[4] Perch-Nielsen, K., *Biol. Skr.*, **16**, 3 (1968).
[5] Gorka, H., *Acta Palaeont. Pol.*, **2**, 236 (1957).
[6] Reinhardt, P., and Gorka, H., *Neues Jb. Geol. Paläont. Abh.*, **129**, 240 (1967).
[7] Vekshina, V. N., *Trudy̆ Sib. Nauchno-Issled. Inst. Geol. Geofiz. Miner. Syr'ya*, **2**, 56 (1959).
[8] Martini, E., *Senckenberg. Leth.*, **42**, 1 (1961).
[9] Edwards, A. R., *NZ J. Geol. Geophys.*, **9**, 481 (1966).
[10] Martini, E., *Senckenberg. Leth.*, **50**, 117 (1969).
[11] Stradner, H., and Papp, A., *Jb. Geol. Bundesanst.*, **7**, 1 (1961).
[12] Berggren, W. A., *Stockholm Contr. Geol.*, **9**, 1 (1962).
[13] Jeletzky, J., *Beih. Geol. Jb.*, **1**, 3 (1951).
[14] Čepek, P., Hay, W. W., Masters, B. A., and Worsley, T. R., *Guidebk. Sixth Ann. Fieldtrip Alabama Geol. Soc.*, 27 (1968).
[15] Curry, D., Hersey, J. B., Martini, E., and Whittard, W. F., *Phil. Trans. Roy. Soc.*, B, **248**, 315 (1965).
[16] Holmes, R. W., *Mem. Geol. Soc. Amer.*, **67**, 109 (1957).
[17] Revelle, R., and Fairbridge, R., *Mem. Geol. Soc. Amer.*, **67**, 239 (1957).

BIOLOGICAL SCIENCES

Derivation of Partial Sequence of Human β Chain Messenger RNA from Mutation Data

THREE general approaches can be used to determine the base sequence of natural messenger ribonucleic acids (mRNAs): (1) the molecular species in question can be purified and sequenced by chemical methods[1]; (2) purified aminoacyl-tRNAs (which are codon-specific) can be used to deliver labelled amino-acids to particular sites in a protein of known sequence[2,3]; and (3) the mRNA sequence can be inferred from the pattern of amino-acid substitutions in mutant proteins of known sequence[4-6]. It has been possible to determine indirectly the RNA sequence which codes for a carboxy-terminal segment of lysozyme[4], but no intensive effort seems to have been made to apply the large amount of sequence data[7] accumulated on homologous proteins to similar ends.

If the universality of the genetic code is assumed[8], examination of amino-acid substitutions in a series of proteins shown to have a common ancestral origin should reduce considerably and sometimes uniquely specify the probable codons for certain amino-acids. These data could be of great assistance to those determining the sequences of mRNAs directly and might provide information on codon frequencies not easily obtainable by any other method.

Because the mRNA for the β chain of haemoglobin has been considerably purified[9] and the nucleotide sequence may soon be known, that protein was chosen for study. Realizing that in most cases distinction could not be made between "wobble" codons[10], fifty-eight human β chain variants[9] and the amino-acid sequences from β chains of thirty-six animal species[7] were compared with the normal human β chain and the codons compatible with each amino-acid deduced. For example, residue 67, which is normally occupied by valine in human β chains, is known to have mutated to glutamic acid in human haemoglobin M Milwaukee, and is occupied by isoleucine in lamprey globin. If it is assumed that the minimum number of base changes should be invoked to explain each difference in amino-acid sequence, then the observations are most compatible with an assignment of the GUA codon to residue 67 in the human β chain. When multiple amino-acid substitutions involving a single codon were not compatible with single base changes, it was still sometimes possible to arrive at a unique sequence of base changes, involving a minimum number of steps, which would both explain the observed amino-acid interchanges and be compatible with accepted phylogenetic relationships[7].

By this method it was possible to derive probable codons for seventy-two of the hundred and forty-six amino-acid residues in the human β chain (Table 1). All three bases of the triplet could be specified for ten of these residues: valines 1, 113, 126, 98 (GUG), valines 54 and 67 (GUA), leucines 31 and 48 (UUG), glycine 29 (GGA) and alanine 62 (GCG). For the remaining sixty-two residues on which sufficient substitution data are available to restrict significantly the possible codon assignments, only the first and second elements of each codon triplet could be specified with certainty although some possible codons could be eliminated. In no case was support for mRNA heterogeneity in the human encountered, that is, no data required that multiple codons for a single amino-acid residue be postulated.

Besides limiting the possible codons at many positions, significant features of the coding for arginine and the amino-terminal valine are revealed. For all of the arginine residues in the β chain, the AGA–AGG codon set was unequivocally specified by the mutation pattern. This observation is consistent with nearest neighbour studies which suggest that C–G sequences are relatively rare in DNA[12]. Although inconclusive, results of incorporation of labelled amino-acids by fractionated arginyl-tRNA into rabbit β chains are consistent[3] with this conclusion. On the other hand, the CGN set of codons (where N specifies A, U, G or C) is used for arginines 92 and 141 in the α chain[3] of rabbit haemoglobin and these codons are almost certainly used in man as well[9]. There therefore exists the possibility of differential control of α and β chain synthesis by regulation of the availability of tRNA species which recognize the CGN set. Selective reduction of α chain synthesis by tRNA restriction has been demonstrated *in vitro*[13] and may be relevant to the mechanism of α thalassaemia in which α chains are structurally normal but their rate of synthesis is reduced.

Table 1. TRIPLETS FOR SOME AMINO-ACIDS IN HUMAN HAEMOGLOBIN β CHAIN

Amino-acid	Position	Possible codons
Alanine	62	GCG
	13, 128, 140	GCC, GCU
	142, 10 53, 70, 76, 86, 135, 138	GCA, GCG
	27	GCA, GCC, GCU
Arginine	30, 40, 104	AGA, AGG
Glycine	16, 56, 69, 136	GGC, GGU
	29	GGA
	46	GGA, GGG
	74	GGA, GGC, GGU
Leucine	3, 96	CUC, CUU
	32	CUN, UUA, UUG
	48, 31	UUG
	75	CUG, UUG
	68, 114, 105, 106, 110, 141	CUA, CUC, CUU, UUA
	78	CUA, CUG, UUA, UUG
	81, 28, 14, 88, 91	CUN
Proline	51, 58, 125	CCA, CCG
	100	CCA, CCC, CCU
Serine	9	UCU, UCC
	89	UCN
	44, 72	AGC, AGU
	49	UCA, UCG
Threonine	4, 84, 86	ACA, ACG
	12, 50, 123	ACC, ACU
Valine	1, 113, 126, 98	GUG
	11	GUA, GUC, GUU
	20, 33	GUA, GUG
	23, 34, 109, 111, 134, 137	GUC, GUU
	133	GUG, GUC, GUU
	54, 67	GUA

Perhaps the most interesting finding is that GUG is the most probable codon for the amino-terminal valine. In *Escherichia coli*, GUG codes for formylmethionine and at the 5′ end of mRNA serves as the initiator codon for protein synthesis[14]. The fact that valine rather than methionine is found in the N-terminal position of the β chain can be explained by the apparent absence of formylmethionyl-tRNA in the cytoplasm of cells of higher organisms[15]. These considerations predict that if formylmethionyl-tRNA were available it would initiate β chain synthesis on a mammalian ribosome and that formylmethionine would be substituted for the normal N-terminal valine.

I thank Dr I. B. Weinstein for criticisms and Dr L. M. Fink for discussions. This work was supported in part by a National Cancer Institute US Public Health Service grant.

KARL W. LANKS

College of Physicians and Surgeons,
Columbia University Institute
of Cancer Research,
Francis Delafield Hospital,
99 Fort Washington Avenue,
New York 10032.

Received October 2; revised November 24, 1969.

[1] DeWacter, R., and Fiers, W., W., *Nature*, **221**, 233 (1969).

[2] Gonano, F., *Biochemistry*, **6**, 977 (1967).

[3] Weisblum, B., Cherayil, J. D., Bock, R. M., and Soll, D. J., *J. Mol. Biol.*, **28**, 275 (1967).

[4] Tsugita, A., Inouye, M., Imagawa, T., Nakaniski, T., Okada, Y., Emrich, J., and Streisinger, G., *J. Mol. Biol.*, **41**, 349 (1969).
[5] Yanofsky, C., "Gene-Enzyme Relationships", in *The Bacteria* (edit. by Gunsalus, I. C., and Stanier, R. Y.), **5**, 373 (Academic Press, New York, 1964).
[6] Sarabhai, A., Stretton, A. O. W., Brenner, S., and Bolle, A., *Nature*, **201**, 13 (1964).
[7] Dayhoff, M. O., and Eck, R. V., *Atlas of Protein Sequence and Structure 1967–1968* (Silver Springs, Md., National Biomedical Research Foundation, 1968).
[8] Caskey, C. T., Beaudet, A., and Nirenberg, M., *J. Mol. Biol.*, **37**, 99 (1968).
[9] Lehmann, H., and Carrell, R. W., *Brit. Med. Bull.*, **25**, 14 (1969).
[10] Crick, F. H. C., *J. Mol. Biol.*, **19**, 548 (1966).
[11] Subak-Sharpe, H., Shepherd, W. M., and Hay, J., *Cold Spring Harbor Symp. Quant. Biol.*, **31**, 583 (1966).
[12] Anderson, W. F., *Biochem. Biophys. Res. Commun.*, **36**, 456 (1969).
[13] Clark, B. F. C., and Marcker, K. A., *J. Mol. Biol.*, **17**, 394 (1966).
[14] Gasper, J. B., and Darnell, J. E., *Biochem. Biophys. Res. Commun.*, **34**, 205 (1969).

Model for Quantitative and Qualitative Control of mRNA Translation in Eukaryotes

In a recent communication by Griffith and Mahler[1] dealing with a ticketing theory of memory, the authors mentioned that their model stemmed from a hypothesis previously advanced by us in order to account for certain features of the process of translation in eukaryotic cells. This hypothesis has been briefly noted[2] but not adequately described and is therefore presented here in detail.

The hypothesis was originally suggested by experiments designed to study the accumulation of a variety of enzymes at different stages of cellular slime mould morphogenesis. The results indicated that there is in each case a stoichiometric relationship between how much transcription is permitted and how much of a particular enzyme subsequently accumulates[3,4]. The RNA product of one of these periods of transcription, normally stable for 4–5 h before actual accumulation of the corresponding enzyme, was found to remain stable several hours longer if one added and then removed cycloheximide, a reversible inhibitor of protein synthesis. There was no significant reduction in the level of enzyme activity accumulating thereafter[5].

These findings prompted the postulate that every molecule of mRNA, at least in eukaryotic cells, has at its 5′-hydroxyl end a set of redundant base sequences. In Fig. 1, these are schematically designated as triplets but might be, and probably would be, much longer. Each of these sequences would serve as the attachment site for a ribosome. As the ribosome moved down the length of the molecule, one of the ribosomal proteins, an excising enzyme, would clip and release the first of the redundant sequences. This ribosome would then pass along the polynucleotide chain and, having reached an initiation codon, would begin polypeptide synthesis. Meanwhile, a second, third and fourth ribosome would successively attach and in turn excise the then terminal redundant sequence. After excision of the last redundancy, no additional ribosomes could attach, and the mRNA molecule would be open to degradation by nucleolytic attack. Two considerations follow from this model.

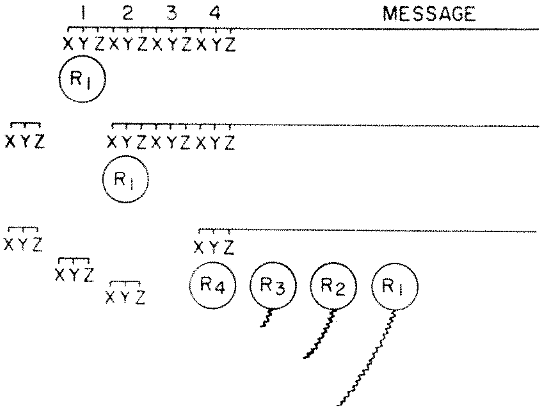


Fig. 1. XYZ represent purine or pyrimidine bases within a redundant trinucleotide sequence. In the example shown, four such sequences occupy the 5′ end of a molecule of mRNA. R_1 to R_4 represent ribosomes successively attaching to the mRNA. Each such attachment results in the excision of one of the redundant sequences.

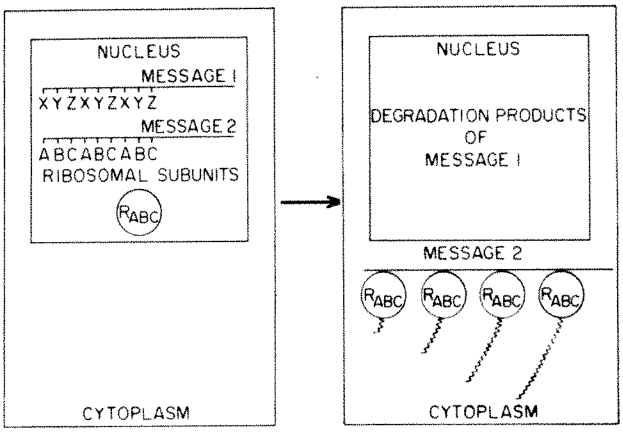


Fig. 2. Redundant sequences XYZ and ABC at the 5′ termini of mRNA molecules 1 and 2, represent attachment sites for different classes of ribosomes R_{XYZ} and R_{ABC} respectively. Only the latter are present in the cell. Only mRNA molecule 2 can therefore be conveyed into the cytoplasm and translated while mRNA molecule 1 remains in the nucleus until it is degraded.

First, the model would provide for automatic digital control over the level of a particular enzyme that accumulates in a cell when it follows a specific developmental programme. This is consistent with, for example, the patterns of enzyme accumulation observed during cellular slime mould morphogenesis[2] and in the cell growth and division cycle of *Saccharomyces cerevisiae*[6].

Second, mRNA molecules transcribed from different genes might contain different numbers of the redundant base sequence at their 5′ termini. This would enable a cell to possess mRNA molecules with different stabilities and thus automatically to accumulate different levels of the corresponding proteins. Mutations, deletions or additions in the redundant region could conceivably permit natural selection to operate, and in this manner regulate the quantity of a particular protein in a cell.

The model can be expanded to provide for qualitative as well as quantitative control of protein synthesis. As schematically illustrated in Fig. 2, more than one kind of redundant base sequence might occupy the 5′ termini of different mRNA molecules in a given cell. These could serve as specific, mutually exclusive attachment sites for different classes of ribosomes. Thus a cell might be potentially capable of engaging in either of two alternative developmental programmes, each leading to a specific differentiated state. mRNA molecules appropriate to one developmental programme might contain one kind of redundant sequence; those appropriate to the alternative programme might contain a second kind. Both classes of mRNA might be transcribed and be present in the nucleus. Fabrication of ribosomes, however, might be restricted to the kind capable of attaching only to the redundant sequence present in one class of mRNA molecules. The result would be that the favoured class of mRNA would migrate to the cytoplasm and be translated; the second class of mRNA, finding no ribosomes to attach to, would remain in the nucleus and eventually be degraded.

This expanded model can conceivably account for three facts which seem to have great significance for the process of cytodifferentiation.

Eukaryotes have highly redundant DNA sequences of the order of 500 nucleotide pairs which are scattered throughout the genome. These are present at frequencies generally ranging between 10^2 and 10^5 copies per genome[7]. Such redundancies might be at least partly accounted for as different classes (and lengths) of nucleotide sequences at the 5′ termini of structural genes.

In the cellular slime moulds, the growth and cell division cycle and the construction of organized multicellular fruiting bodies represent alternative developmental programmes. The second can be carried out in the absence of all exogenous nutrients. It has been found that during fruiting body construction most, if not all, of the ribosomes present in the vegetative cells are destroyed and are replaced by ribosomes made during the morphogenetic sequence[8,9]. This might reflect the need for a new class of ribosomes which could attach to the new class of mRNA molecules. In heterocaryons of HeLa cells and hen erythrocytes (whose cytoplasm had been allowed to leak out before cell fusion), it has been shown[10] that the previously quiescent erythrocyte nuclei synthesized RNA and turned it over for several days, but none was found in the cytoplasm. By contrast the RNA coming from the HeLa nuclei was observed in the cytoplasm. Only after nucleoli developed in the erythrocyte nuclei and ribosome synthesis resumed did hen RNA appear in the cytoplasm and this was followed by the accumulation of hen-specific proteins.

As much as 80–90 per cent of the RNA transcribed during a short pulse of radioactive precursor has been shown to remain within the nucleus, to be highly unstable and to be polydisperse[11,12]. This class of RNA could be accounted for as being the portion of the mRNA lacking the corresponding classes of ribosomes with which to attach.

In summary, the model provides for an automatic, quantitative control over the use of any given mRNA molecule in protein synthesis by assuming the existence of a specified number of ribosomal attachment sites in the form of redundant base sequences at the 5′ terminus, each ribosomal attachment resulting in the excision of one of the redundant sequences. In its expanded form the model provides for qualitative control of protein synthesis by assuming the existence of more than one kind of redundant sequence on different mRNA molecules and more than one class of ribosomes, each capable of recognizing and attaching to one kind of redundant sequence.

The assumption of controls operating at the level of mRNA transport and/or translation does not and need not exclude the existence of a network of transcriptional controls as well. Ingenious models of the latter have been postulated for eukaryotic cells engaged in developmental activities, assuming negative feedback[13], and assuming a positive feedback[14] patterned loosely after the hypothesis of Englesberg *et al.*[15].

This work was supported by a US National Institutes of Health career development award and by a National Science Foundation grant.

MAURICE SUSSMAN

Department of Biology,
Brandeis University,
Waltham, Massachusetts.

Received January 12, 1970.

[1] Griffith, J. S., and Mahler, H. R., *Nature*, **223**, 580 (1969).

[2] Sussman, M., and Sussman, R., *Proc. Nineteenth Symp. Soc. Gen. Microbiol.*, 403 (1969).

[3] Sussman, M., and Sussman, R., *Biochim. Biophys. Acta*, **108**, 463 (1965).

[4] Roth, R., Ashworth, J. M., and Sussman, M., *Proc. US Nat. Acad. Sci.*, **59**, 1235 (1968).

[5] Sussman, M., *Proc. US Nat. Acad. Sci.*, **55**, 813 (1966).

[6] Halvorson, H. O., *Proc. Fifteenth Symp. Soc. Gen. Microbiol.*, 343 (1965).

[7] Britten, R. J., and Kohne, D. E., *Science*, **161**, 529 (1968).

[8] Sussman, R., *Biochim. Biophys. Acta*, **149**, 407 (1967).

[9] Cocucci, S., and Sussman, M., *J. Cell Biol.* (in the press, 1970).

[10] Harris, H., Sidebottom, E., Grace, D. M., and Bramwell, M. E., *J. Cell. Sci.*, **4**, 499 (1969).

[11] Soiero, R., Birnboim, H. C., and Darnell, J. E., *J. Mol. Biol.*, **19**, 362 (1966).

[12] Attardi, G., Parnas, H., Hwang, M. L. H., and Attardi, B., *J. Mol. Biol.*, **20**, 145 (1966).

[13] Jacob, F., and Monod, J., in *Cytodifferential and Macromolecular Synthesis*, 30 (Academic Press, New York, 1963).

[14] Britten, R. J., and Davidson, E. H., *Science*, **165**, 349 (1969).

[15] Englesberg, E., Irr, J., Power, J., and Lee, N., *J. Bact.*, **90**, 946 (1965).

Hydrolysis of Phospholipid Esters localized in Lysosomes and Related Structures of Rat Muscle Cells in Culture

THE fusion of normal myoblasts during differentiation of muscle leads to a multinucleated striated muscle fibril. The fusion reaction between cells is not well understood. Almost as soon as contact is made between two myoblasts, the plasma membranes of the two cells fuse to become one continuous plasma membrane surrounding the cytoplasm of both cells. It is not known whether such normal fusion reactions between muscle cells involve hydrolytic enzymes for digestion of carbohydrates, proteins and lipids constituting these membranes at their sites of fusion. The fusion reaction as well as the extensive cytoplasmic changes during differentiation prompted us to investigate digestive functions in our cultured cells using phosphate esters of a variety of compounds. This is the first report of histological localization for hydrolysis of phospholipid esters by cultured cells. Previous cytochemical studies on acid phosphatase in our cultured muscle cells showed an increase in lysosomal reaction product as myotubes formed and have been reported as ancillary observations to biochemical studies[1,2]. Our *in situ* investigations for lipid phosphate hydrolysis were also prompted from reports by Tappel *et al.*[3,4] as well as Fowler and de Duve[5] on digestion of lipids and organelle membranes with isolated rat liver lysosomal particles.

Cytochemical examinations for hydrolysis of phosphate esters from phospholipids were carried out on primary rat muscle cell monolayers grown under the regulated conditions of culture. The cultures showed 50–70 per cent muscle cells interspersed with fibroblasts and the monolayers allowed for a clear localization of phosphatase in both cell types during the culture period from days 3–10. Light and electron microscopic studies were performed on the same cultures by originally plating cells on glass and fluoroplastic coverslips coated with gelatine. The phospholipid ester hydrolysis was found to be localized in lysosomes and in related vesicles after incubation of the cells with substrates at acidic *p*H. The coverslips, on which cells were growing, remained in Falcon plastic culture dishes for all the treatments. They were then mounted for light microscopy and subjected to further treatment for electron microscopic studies.

Cell cultures were washed free of growth medium with cold Pannett–Compton saline, *p*H 7·4. Cold 2·5 per cent glutaraldehyde fixative in 0·1 M cacodylate buffer *p*H 7·2 was then added for 10 min. The fixative was removed with two washings of cold 0·05 M acetate buffer *p*H 4·8. Five ml. of the fresh incubation medium was added to each culture dish. The incubation medium was made by adding 25 mg phospholipid sonically dispersed for 1 min in 5 ml. of glass distilled water to 10 ml. of 0·1 M acetate buffer at *p*H 4·8. Ten ml. of lead nitrate (0·1 per cent) was added to give a final lead concentration of 1·2 mM in 25 ml. completed medium. The following phospholipids were tested: egg lecithin (Mann Research), dipalmitoyl L-α-lecithin (synthetic, A. G. Fluka and Co.), dipalmitoyl-L-α-cephalin (Fluka); L-α-glycerophosphoryl choline (Sigma) was also used. Cytidine 5′ monophosphate was also tested at *p*H 4·8 with an incubation time of 45 min. Control media for long incubation periods were prepared by, first, omitting substrate; second, adding 0·01 M NaF to the medium; and third, using suitable buffers in media at *p*H 6·6 and 7·2 such as Tris-maleate and histidine. No positive reaction product was obvious in the cells in these conditions. The coverslip cultures in the incubation media were placed in a moisture controlled 37° C incubator. Because of the known latency of this type of lysosomal enzyme (see ref. 5), an overnight or 12 h incubation period was required to show the reaction with phase microscopy. After incubation, the cultures for light microscopy were rinsed in cold 0·05 M acetate buffer *p*H 4·8 and immersed in 1 per cent ammonium sulphide for 1 min, rinsed in cold distilled water and mounted in glycerol gel. For electron microscope studies, fluoroplastic coverslips were used[6]. After incubation, the coverslips were thrice washed with acetate–veronal buffer *p*H 7·4. The cells were then post-fixed for 10 min with osmium tetroxide (*p*H 7·4 in acetate–veronal buffer). The cells were dehydrated and embedded in epon–Araldite mixture[7].

With Zeiss phase microscopy, perinuclear localization of the reaction product was evident in fibroblasts of early (4 day) cultures. As the cultures became confluent, the location and apparent visible amount of reaction product did not change in the fibroblasts. In myotubes, the reaction product increased with the age at which the cultures were sampled. This increase in maturing myotubes occurred in lysosomal type deposits, rather than perinuclear deposits in the Golgi zone. As each variation of the procedure was investigated, the cultured cells were checked for reaction product at 2 h and 5 h as well as 12 h and 20 h. Optimal reaction time for light microscopy is at 12 h. No decrease in latency was noted when short fixation times (5 min) with glutaraldehyde were used. As shown in the accompanying figures for electron microscopy, there was deterioration in cell structure.

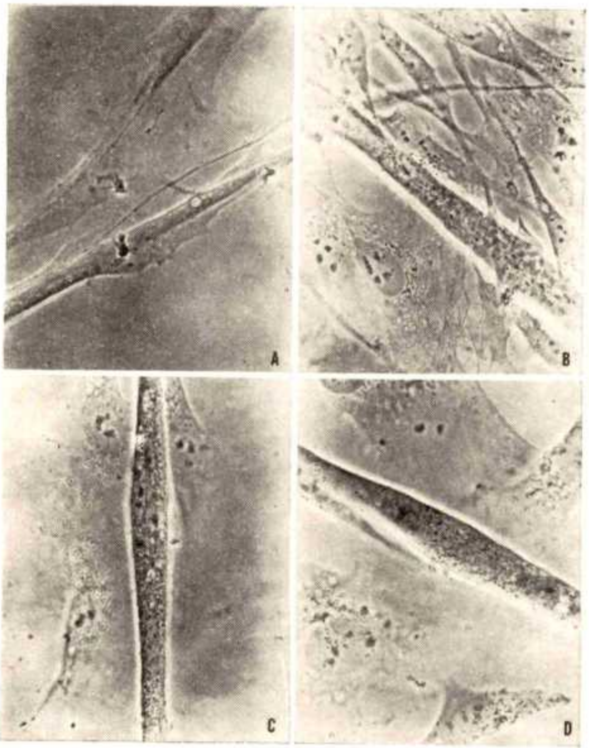


Fig. 1. Photomicrographs *A* to *D* (× 525) are from replicate plates of the same culture. *A*, Fibroblast and early myotube in a culture plate incubated without substrate but in the presence of 1·2 mM lead for 12 h at *p*H 4·8. The cells show no internal deposits; only external artefacts are evident. *B*, Egg lecithin was used as substrate. The deposits in the fibroblasts are perinuclear and in the Golgi region. Deposits in the myotubes are in localized regions of cytoplasm. *C*, Myotube after incubation with synthetic L-*α*-dipalmitoyl lecithin. *D*, Cytidine monophosphate was used as substrate *p*H 4·8, 45 min incubation.

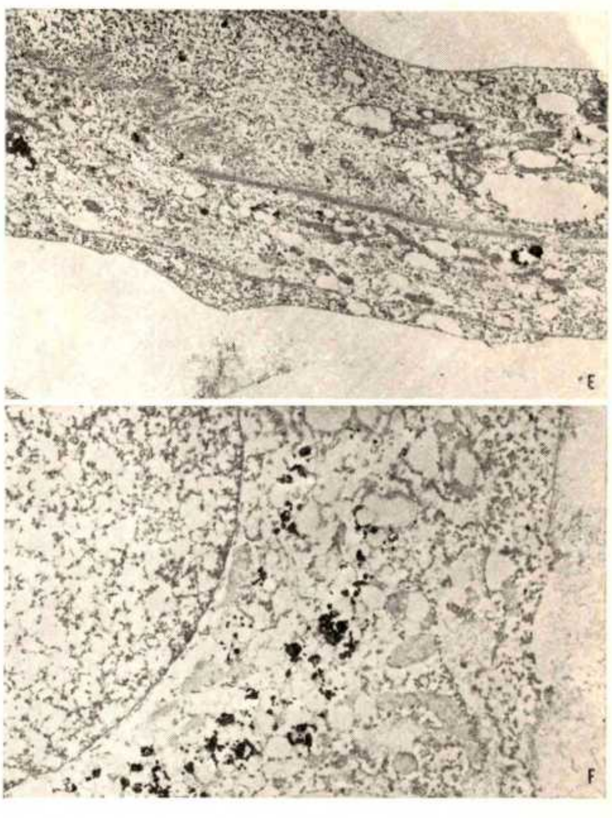


Fig. 2. Synthetic L-*α*-lecithin was used as substrate for the following electron micrographs from the same culture used for photomicrographs *A* to *D*. In *E*, two fusing myoblasts are shown. Note absence of reaction product at the plasma membranes at the common cell boundaries. Product is deposited in lysosomal vesicles. (× 5,000.) *F*, Showing perinuclear reaction product in fibroblast cell. Nuclear membrane shows no reaction. (× 11,000.) Cells were badly preserved through manipulations subsequent to short initial fixation time (5 min) used in this trial and the long incubation in the presence of phospholipid substrate.

Incubation of cells in incubation medium with 0·25 M sucrose was damaging to the enzyme reaction. No improvement was obvious when the incubation medium contained 25 per cent dimethyl sulphoxide. In experimental cell cultures, reaction product is absent in other organelles than lysosomes or Golgi type vesicles. Deposits of reaction product were absent from nuclear or plasma membranes. There was no increase in deposits apparent in regions where muscle cell fusion was taking place as in the accompanying electron micrograph (*E*) where plasma membrane between two fusing myoblasts is clearly seen.

The presence of hydrolytic activity in the Golgi zone and lysosomes of primary cultures of liver cells has also been successfully demonstrated in our laboratory and will be reported elsewhere. The enzyme is quite latent even in this well characterized cell type.

The failure to demonstrate any reaction product associated with membranes of fusing muscle cells with phospholipid substrate is unexpected. (We have had occasional experiments in which an unlocalized deposit in one limb of single muscle cells has been seen. At present we regard this as an artefact.) With a substrate such as ATP, membrane ATPase between adjacent cells can be demonstrated after only a 10 min incubation period. The fusion reaction between cells most likely involves energy of ATP hydrolysis. The fusion process between cells does not seem to involve "digestion" of plasma membranes in differentiating muscle cells.

The fusion process between gametes as well as that induced by viruses should also be investigated histologically. According to Okada *et al.*[8], energy is required for fusion of Ehrlich ascites tumour cells by HVJ virus. Does such a fusion process take place after hydrolysis of membrane constituents or because of involvement of the cell plasma membrane in construction of virus coat? The question with regard to fusion between gametes is all the more interesting because of involvement of the acrosome of the sperm. The origin of the acrosome is from the Golgi region which is one of the cell structures involved in digestion of phospholipid esters.

Electron microscope studies were performed by H. H. Mollenhauer.

GRACE NORRIS
MINOCHER REPORTER

C. F. Kettering Research Laboratory,
Yellow Springs, Ohio 45387.

Received October 3; revised December 15, 1969.

[1] Reporter, M., *J. Cell Biol.*, **35**, 112A (1967).
[2] Reporter, M., *J. Cell Biol.*, **31**, 93A (1966).
[3] Mellors, A., and Tappel, A. L., *J. Lipid. Res.*, **8**, 479 (1968).
[4] Mahadevan, S., and Tappel, A. L., *J. Biol. Chem.*, **243**, 2849 (1968).
[5] Fowler, S., and de Duve, C., *J. Biol. Chem.*, **244**, 471 (1969).
[6] Masurovsky, E. B., and Bunge, R. P., *Stain. Tech.*, **43**, 161 (1968).
[7] Mollenhauer, H. H., *Stain. Tech.*, **39**, 111 (1964).
[8] Okada, Y., Murayama, M., and Yamada, K., *Virology*, **27**, 175 (1966).

Non-enzymatic Hydrolysis of Adenosine Phosphates

ENERGY transfer in living systems usually involves reactions in which adenosine triphosphate (ATP) is converted to adenosine di (ADP) or monophosphate (AMP) and inorganic phosphate. Similar reactions were probably necessary to provide energy for prebiological evolution. Concentrations of these compounds are affected by non-metabolic destruction, principally hydrolysis releasing orthophosphate. Thus the rates of such hydrolysis are of interest with respect both to present living systems and to the origin of life. Hydrolysis of ATP and ADP has been studied previously at selected temperatures and *p*H, both in distilled water and in the presence of several inorganic ions which might act as catalysts[1,2]. The present work is concerned with reactions of ATP, ADP and AMP over a range of temperatures in distilled water, in 0·02 moles Ca^{2+} (previously reported to act as a catalyst), and in a synthetic seawater containing present oceanic concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, Sr^{2+}, Cl^-, SO_4, HCO_3^-, Br^- and $H_2BO_3^-$. The latter medium was chosen as being as reasonable a representation as possible of primitive seawater, because the composition of the primitive seas, though not exactly known, was probably not drastically different from that of the present ones[3].

The sample compounds were dissolved in the liquid medium, usually at 0·001 M concentration, and *p*H adjusted by addition of NaOH or HCl (0·1 moles). The solutions were then incubated at the desired temperatures and the rates of hydrolysis determined by analysing for inorganic orthophosphate by the method of Lowry and Lopez[4]. Less than 10 per cent of the initial compound was hydrolysed in each case, to ensure that the amount of phosphate split off would be linearly related to initial concentration and that little phosphate would be split from degradation products (for example, from ADP in tests on ATP). In some cases checks were run at different concentrations to ensure that the reactions were first order in organic phosphate.

Experimental results are shown in Fig. 1 in which the logarithm of the hydrolysis rate is plotted against the reciprocal of absolute temperatures (to give a straight line). The rate of hydrolysis of ATP was greatest at low ionic strength in the presence of 0·02 moles Ca^{2+} ions, as shown by curve 1. Hydrogen ions also catalysed the reaction as shown by the comparison of curve 2 in water at *p*H 4, with curve 4 in water at *p*H 8. Hydrolysis in seawater at *p*H 8 (curve 3) is only slightly greater than in pure water at the same *p*H, probably because the catalytic effect of ions like Ca^{2+} is reduced in the high ionic strength solution. ADP is slightly more resistant to hydrolysis at high temperatures than ATP, with very similar rates in water and in seawater, as shown by curves 5 and 6. AMP is much more resistant to hydrolysis than ADP, with a lower rate in seawater than in pure water, as shown by curves 7 and 8. In order to illustrate the relationship between life of these compounds and temperature, the scale on the right has been added, giving half-life for various reaction rates. The experimental results in the synthetic seawater have been extrapolated to temperatures encountered in the present environment. Of all the compounds, only AMP would be expected to survive in such solvents for more than a few years at temperatures near present ambient.

The composition of the synthetic seawater used is probably not exactly that of the primitive oceans. The facts that hydrolysis in this high ionic strength medium is very close to that in water and that some of the ions (for example, Ca^{2+}, which is the most abundant cation in fresh water) catalyse the reaction in low ionic strength solutions, however, indicate that rates of hydrolysis in the primitive oceans and lakes would have been of the same order of magnitude as those found. Thus a long term build-up of these compounds would not be expected in the prebiological environment. In addition the relatively short life at high temperatures indicates that the origin of life would have been facilitated by cold environments, at any rate as far as participation by ATP and ADP is concerned.

Also of interest is the extremely short life of ATP—of the order of a few hours or less—at temperatures above 90° C, even though bacterial growth has been reported at these temperatures[5]. Unless thermophilic organisms have intracellular environments capable of slowing down the reaction appreciably, a major limitation on the maxi-

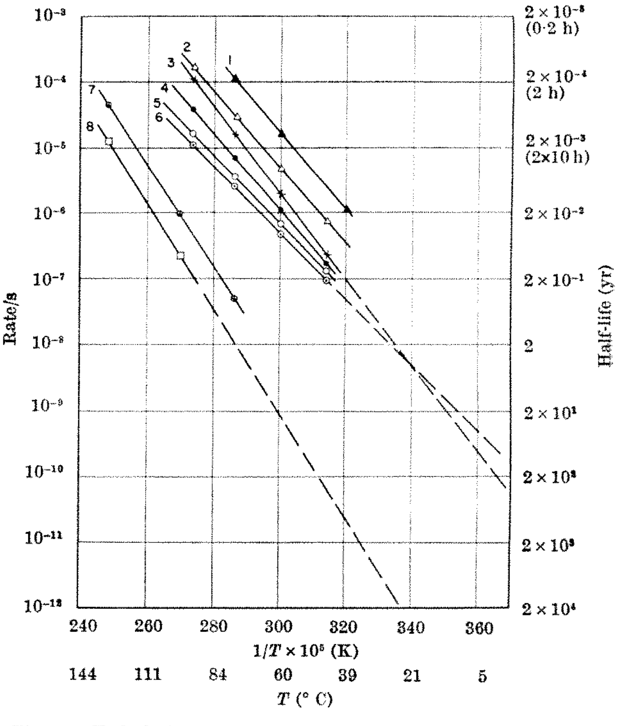


Fig. 1. Hydrolysis rates of nucleoside phosphate as a function of temperature. 1, ATP, *p*H 8, in 0·02 moles Ca^+; 2, ATP, *p*H 4, in H_2O; 3, ATP, *p*H 8, in synthetic seawater; 4, ATP, *p*H 8, in H_2O; 5, ADP, *p*H 8, in H_2O; 6, ADP, *p*H 8, in synthetic seawater; 7, AMP, *p*H 8, in H_2O; 8, AMP, *p*H 8, in synthetic seawater.

mum temperature at which life is possible may be non-enzymatic hydrolysis of ATP.

This work was supported by a NASA grant.

H. R. HULETT

Department of Genetics,
Stanford University School of Medicine,
Stanford, California 94305.

Received December 15, 1969.

[1] Titas, M., and Lowenstein, J. M., *Biochemistry*, **2**, 350 (1963).
[2] Miller, D. L., and Westheimer, F. H., *J. Amer. Chem. Soc.*, **88**, 1507 (1966).
[3] Cloud, jun., P. E., *Science*, **160**, 729 (1968).
[4] Lowry, O. H., and Lopez, J. A., *J. Biol. Chem.*, **162**, 421 (1946).
[5] Brock, T. D., *Science*, **164**, 1411 (1969).

Possible Basis for the Cariostatic Effect of Inorganic Phosphates

INORGANIC phosphates are effective in reducing dental caries when added as supplements to cariogenic diets fed to rodents[1], and when added to chewing gum[2] and to breakfast cereal eaten by children[3]. Because the caries process may be influenced by pellicle proteins[4], and salivary proteins adsorbed to human enamel are differentially eluted by water and orthophosphate solutions[5], we reasoned that the cariostatic effect of phosphate might involve one or both of the following processes: (*a*) differential elution of proteins adsorbed on enamel; (*b*) modification of the enamel surface resulting in an alteration of the acquired protein layer. To test this hypothesis in a model system we have studied the effects of phosphates on enzymes adsorbed to human enamel.

Samples (50 mg) of purified human enamel powder[6] were equilibrated at room temperature with 1 ml. of a 0·1 mg/ml. solution of purified amylase (from *Aspergillus oryzeae*). The supernatant was decanted and the enamel samples were washed with 1 ml. of water, or with 1 ml. of saturated $CaHPO_4$ solution (1·8 mM)[7], or with 1 ml. of 1·8 mM solutions of either NaH_2PO_4 or $Na_3(PO_3)_3$. All solutions were at *p*H 6·0. Each of four successive 1 ml. washings was assayed for desorbed amylase activity. (We have found that the presence of these phosphates at a concentration of 1·8 mM does not interfere with the normal expression of amylase activity.) The results are shown in Table 1.

Table 1. DESORPTION OF AMYLASE FROM HUMAN ENAMEL

Wash number	Units* of amylase desorbed from 50 mg of enamel powder by 1 ml. washes of:			
	H_2O	$CaHPO_4$	NaH_2PO_4	$Na_3(PO_3)_3$
1	0·24	0·60	1·53	2·05
2	0·19	0·30	0·64	0·73
3	0·00	0·13	0·18	0·17
4	0·00	0·08	0·10	0·00
Total desorbed in four washes	0·43	1·11	2·45	2·95

* 1 unit of amylase catalyses the release of 1 mM of maltose per min from a 1 per cent soluble starch solution at 25° C. The phosphate concentrations were 1·8 mM.

Water desorbed very little amylase. The activity in the first eluant portion is probably the result of amylase remaining in the interstitial fluid after removal of the supernatant. Desorption effectiveness of the solutions was in the order $Na_3(PO_3)_3 > NaH_2PO_4 > CaHPO_4 > H_2O$. Similar desorption studies were made with purified hen's egg white lysozyme. Lysozyme was not desorbed by any of the 1·8 mM phosphate solutions, but was desorbed by sodium orthophosphate at a concentration of 40 mM and by 1 per cent (by weight) sodium trimetaphosphate (TMP) solutions.

We investigated the possibility of surface modification by pretreating enamel with 1·8 mM phosphate solutions before adsorption of the enzymes. Only TMP altered the adsorption properties of the enamel. Amylase adsorbed to TMP treated enamel was easily removed by water, and TMP treated enamel adsorbed 2 to 3 times as much lysozyme activity as untreated enamel. The alteration of the enamel surface by trimetaphosphate was not a simple detergent effect. Pretreatment of enamel with either a 0·1 or 1·0 per cent (by weight) solution of 'Triton *X*-100' (isooctylphenoxypolyethoxyethanol, a biodegradable non-ionic, surface active agent) had no effect on subsequent enzyme adsorption by the enamel. In the mouth, salivary proteins adsorbed on the tooth surface[9] might protect enamel from alteration by phosphate. We found, however, that solutions of TMP produced the same effects on enamel powder which had first been equilibrated with centrifuged whole saliva.

While our results do not exclude the possibility of other mechanisms of action, they support the hypothesis that the basis of the cariostatic effect of inorganic phosphates is a modification of the composition of the protein layer adsorbed on enamel and, in the case of TMP, an alteration in the surface of the enamel with resulting change in the affinity of various salivary proteins for the enamel surface.

We thank Dr Charles Bugg of the Institute of Dental Research for initially drawing our attention to this problem and for discussion.

K. M. PRUITT
A. D. JAMIESON
R. C. CALDWELL

Institute of Dental Research and
Laboratory of Molecular Biology,
University of Alabama,
Birmingham, Alabama.

Received October 20; revised December 9, 1969.

[1] Nizel, A. E., and Harris, R. S., *J. Dent. Res.*, **43**, 1123 (1964).
[2] Finn, S. B., and Jamison, H., *J. Amer. Dent. Assoc.*, **74**, 988 (1967).
[3] Stookey, G. K., Carroll, P. A., and Muhler, J. C., *J. Amer. Dent. Assoc.*, **74**, 752 (1967).
[4] Dawes, C., Jenkins, G. N., and Tonge, C. H., *Brit. Dent. J.*, **115**, 65 (1963).
[5] Pruitt, K. M., and Caldwell, R. C., *J. Dent. Res.*, *IADR Abst.*, 174 (1968).
[6] Manly, R. S., and Hodge, H. C., *J. Dent. Res.*, **18**, 133 (1939).
[7] *Handbook of Physics and Chemistry* (edit. by Weast, R. C.), forty-ninth ed., B-187 (Chemical Rubber Co., Cleveland, 1968).
[8] Jamieson, A. D., Pruitt, K. M., and Caldwell, R. C., *J. Dent. Res.*, **48**, 483 (1969).
[9] Pruitt, K. M., Caldwell, R. C., Jamieson, A. D., and Taylor, R. E., *J. Dent. Res.*, **48**, Supplement to No. 5, 818 (1969).

Production of "Sulphation Factor" by the Perfused Liver

LONGITUDINAL growth of the mammalian skeleton occurs at the epiphyseal plate where chondrocytes proliferate and synthesize a matrix composed of collagen and a group of sulphated polysaccharides. In spite of the well known requirement for growth hormone to sustain longitudinal skeletal growth, a direct effect of the hormone on cartilage metabolism has not been demonstrated. The incorporation of sulphate into the organic matrix of cartilage is stimulated by a component of normal serum which is not growth hormone. This factor is absent in the serum of hypophysectomized animals, including man, but is restored by the administration of growth hormone. In addition to the stimulation of sulphate incorporation into polysaccharide, collagen synthesis and cell replication are also enhanced. Because the original assay for this component involved measurement of the incorporation of radioactive sulphate into the cartilage of hypophysectomized rats, the term "sulphation factor" was proposed by Salmon and Daughaday[1]. The source of this factor as well as its composition have been unknown.

The fact that liver is one of the few tissues which has been shown to respond to growth hormone *in vitro*[2] makes it a likely source of sulphation factor. The work reported here demonstrates that the liver is capable of producing sulphation factor during perfusion with bovine growth hormone (BGH).

The livers of normal adult rats were perfused *in situ* (F. Chavez-Peon, unpublished technique). The animals were kept alive until the perfusion started, thus reducing the time for development of ischaemia to less than 12 s. The medium used for both perfusions and controls was Waymouth's 'MB 752/1' with 0·5 per cent bovine serum albumin, 100 units/ml. penicillin and 100 μg/ml. streptomycin added. The perfusions took place in two stages: the liver was first washed through with the medium alone, then medium containing 10 μg/ml. of BGH was recirculated three times through the same liver. All solutions were kept at 37° C and the perfusion rate was 3·5–4·5 ml./min. The perfusates were collected separately at the end of each stage; red cells were sedimented in a clinical centrifuge; and the supernatant was passed through a 'Millipore' filter. Thus sterilized, the supernatant was then kept frozen until assayed.

The ability of these solutions to stimulate the uptake of radioactive sulphate into the costal cartilage of hypophysectomized rats was then tested using a modification of Salmon and Daughaday's sulphation factor assay[3]. The cartilage segments were incubated in the various media with radioactive sulphate for 48 h, after which they were washed thoroughly, scraped to remove excess fibrous tissue, weighed after blotting and hydrolysed in 23 M formic acid. An aliquot of the hydrolysate was added to a scintillation mixture and radioactivity was counted in a liquid scintillation counter. Separate experiments to provide histological material were carried out and autoradiographs were prepared with Kodak 'NTB-2' liquid emulsion.

The incorporation of ^{35}S from medium containing perfused BGH was compared with that in three controls: 1, medium with no additions, not perfused; 2, medium containing 10 μg/ml. BGH but not perfused through the liver; 3, plain medium perfused through the liver. It was necessary to recirculate the BGH through the liver three times to produce a measurable effect, but recirculation of medium without BGH failed to produce any increase in its effect.

The results are shown in Fig. 1. Each bar represents the mean of six determinations of ^{35}S uptake from each of six liver perfusions and their controls. The small increases seen with exogenous BGH and with perfusion of plain medium are not statistically significant whereas that seen with the perfused BGH is highly significant ($P < 0{\cdot}01$). These results are confirmed by the autoradiographs shown in Fig. 2. Little ^{35}S activity is seen in the controls, while intense labelling is evident in the matrix of cartilage incubated with medium containing perfused growth hormone.

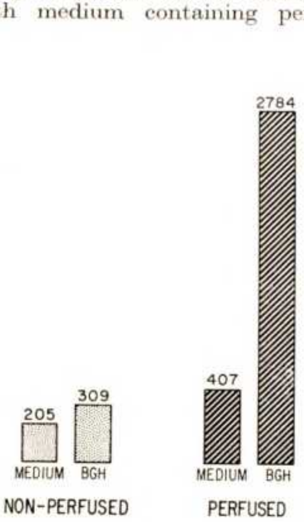


Fig. 1. The incorporation of $^{35}SO_4$ into the organic matrix of rib cartilage of hypophysectomized rats, measured in c.p.m./100 mg. The first two bars show the uptake from media with and without growth hormone, not perfused through the liver. The third and fourth bars show the result of perfusing these media through the liver.

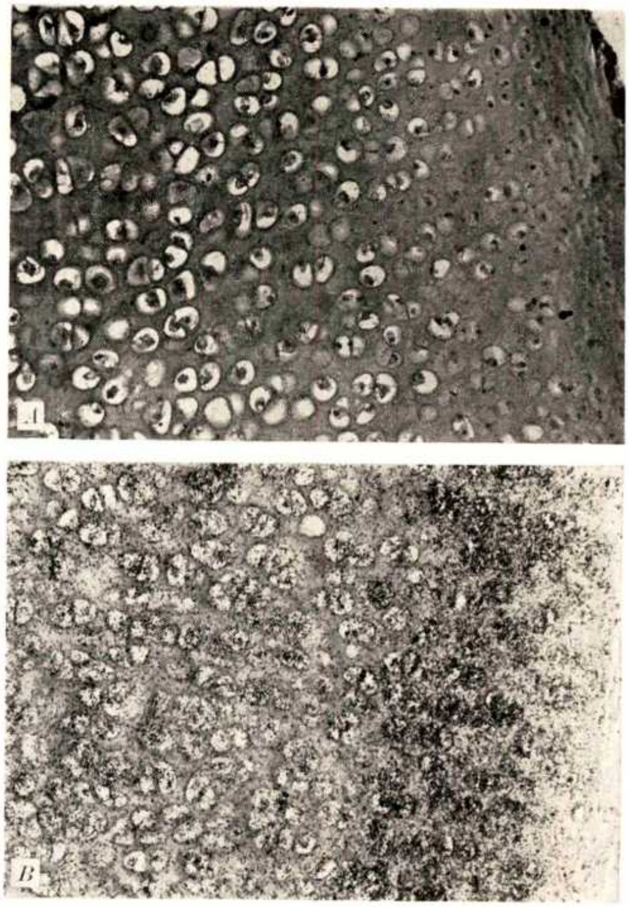


Fig. 2. ^{35}S autoradiographs of incubated cartilage. *A*, Perfused medium; *B*, medium plus BGH, perfused through the liver.

What is "sulphation factor"? Is it an altered form of growth hormone, the result of conjugation or degradation, or is it a second hormone released in response to growth hormone stimulation of the liver? We have no clear answers, but the reported increase in liver DNA and RNA following perfusion with growth hormone, in conjunction with our finding that fairly prolonged exposure of the liver to growth hormone is required, is compatible with the hypothesis that sulphation factor is a distinct hormone chemically unrelated to growth hormone.

Table 1. UPTAKE OF $^{35}SO_4$

	C.p.m./100 mg net weight cartilage			
	Not perfused		Perfused	
	Control	Growth hormone	Control	Growth hormone
Number	24	24	36	36
Mean c.p.m.	205	309	407	2,784
S.E.	±34	±34	±50	±199

We thank Miss Sonya Shortkroff and Miss Sandra Perlstein for assistance and Dr F. Chavez-Peon for performing the liver perfusions. BGH was supplied by Dr A. E. Wilhelmi. This work was supported by a general research support grant from the National Institutes of Health, US Public Health Service.

PADDY McCONAGHEY
CLEMENT B. SLEDGE

Department of Orthopedic Surgery,
Harvard Medical School,
Boston, Massachusetts 02114.

Received October 24, 1969.

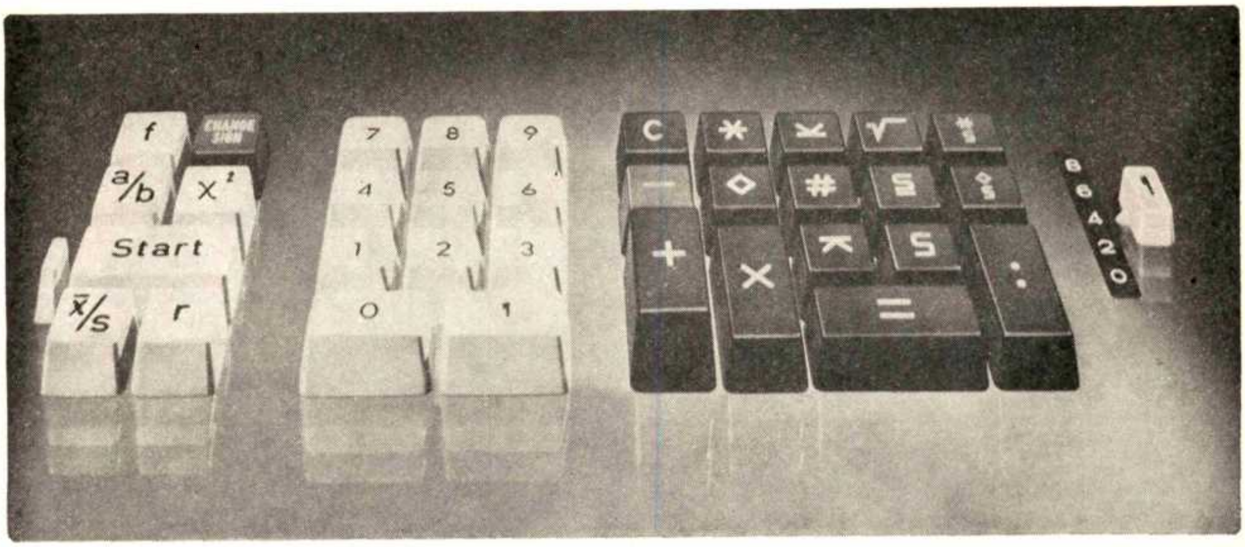
[1] Salmon, jun., W. D., and Daughaday, W. H., *J. Lab. Clin. Med.*, **49**, 825 (1957).

[2] Jefferson, L. S., and Korner, A., *Biochem. J.*, **104**, 826 (1967).

[3] Almquist, S., *Acta Endocrinol.*, **39**, 31 (1961).

DIEHL sigmatron

Electronic Calculating System with Pre-programmed Statistical functions



DIEHL sigmatron is the first calculating system specifically designed for Statistical calculations. By pre-programmed functions, Statistical calculations are reduced to one key operations. For instance, to obtain the Arithmetic Mean and Standard Deviation enter data on the keyboard and touch the pre-programmed key. After entry of the data is completed you need only touch the same pre-programmed key to obtain the results immediately. Linear Regression, Chi Square, Coefficient of Correlation are all reduced to the simplicity of one key operation. DIEHL sigmatron also gives you a powerful and effective calculating performance with ten separate memories, automatic square root, automatic decimal selection up to 8 places and a complete check of each figure, each step and each result. If you prefer to make your own programs, DIEHL combitron S has 10 Program/Memory registers providing a capacity of 120 steps with branching and looping. For external input of data, DIEHL dilector – punched tape reader – is available.

Please cut out!

DIEHL Calculating Systems 8500 Nürnberg 2 W · Germany

Please send me details of DIEHL Calculating Systems

Name ____________________ Title ____________________

Address ____

City ____________________ Country ____________________

Diehl

Quantitative information on cell structures, fast, with the Barr & Stroud Integrating Microdensitometer GN2 –in use all over the world

Cells stained by the Feulgen method absorb light in direct proportion to their mass. By mechanically scanning the image of a stained specimen, and electronically integrating the values for the whole image, an absolute measure of absorption in arbitrary units is obtained. Similar measurements can also be obtained on other cell properties.

The Barr & Stroud Integrating Microdensitometer type GN2 has been designed to use this principle to provide quick, accurate, quantitative information on cell structures. It has gained wide acceptance throughout the world, particularly for Cancer research, where large numbers of specimens must be examined quickly.

Write for pamphlet No. 1578/N

BARR AND STROUD

BARR & STROUD LIMITED

Anniesland, Glasgow W.3.
Tel: 041-954 9601 Telex: 778114
Kinnaird House, 1 Pall Mall East, London SW1
Tel: 01-930 1541 Telex: 261877

Binding Activity of Oestrogen Receptors destroyed by Iodination

WHEN 17β-oestradiol and other oestrogens enter a target cell they are bound by highly specific oestrophilic proteins in a strong but non-covalent manner ($K_{ass} \simeq 1{\cdot}3 \times 10^9$–$1{\cdot}5 \times 10^9$ M^{-1} for 17β-oestradiol)[1]. These oestrogen receptors are found in both nuclear and cytoplasmic fractions of uterus and other target organs to this hormone[1-11]. We now report (1) that iodination of calf uterus oestrogen receptors in alkaline media at lowered temperature destroys their ability to bind 17β-oestradiol; (2) that this effect cannot be ascribed to oxidation of SH groups; and (3) that iodination is ineffective when the binding site is interacting with the hormone, and is thus protected from a direct effect.

Oestradiol–receptor complexes from the cytoplasmic and nuclear fractions of the same calf uteri were prepared by salting-out with $(NH_4)_2SO_4$, as described elsewhere[7]. *In vitro* reversible dissociation of the oestradiol–receptor complexes was achieved by treatment for 5 min at 42°–45° C; *in vitro* formation of the complexes by incubation at 4° C for 90 min; and separation of oestradiol–receptor complexes from free hormone by chromatography on 'Sephadex *G*-25', as already described[1,6]. ^{3}H-17β-oestradiol (from NENC, Boston; specific activity 42 Ci/mmole; ≥97 per cent pure at time of experiment) was determined by liquid scintillation as described[1,6]. Iodination was carried out with 0·037 M I_2 in 0·214 M KI, which was standardized against $AgNO_3$ (ref. 12) and $Na_2S_2O_3$. When required, the iodide solution was labelled with carrier-free ^{131}I (NENC). The following conditions were used throughout: 0·2 mg protein of receptor preparation per ml. of 0·1 M Tris-HCl (*p*H 8·5), 10^{-2} M KCl, 10^{-3} M EDTA (Tris-K$^+$-EDTA medium); the *p*H remained unchanged during iodination, which was stopped by adding 5 moles of $Na_2S_2O_3$ per mole of I_2. ^{131}I was determined in a well type scintillation counter. Protein was determined by a modification of the biuret reaction[13]. Iodoacetate was recrystallized from petroleum ether before use. The activity of L-malate dehydrogenase from pig heart was measured with oxaloacetate as substrate by the decrease of absorbance at 366 nm arising from oxidation of NADH (ref. 14).

An inverse correlation was found between amount of organic iodine formed and ability of a preparation of oestrogen receptor from the nuclear fraction to bind 17β-oestradiol (Fig. 1). One can see that maximal iodination of the preparation corresponds to a virtually total loss of binding activity. Also, our data show that the formation kinetics of organic iodine coincide with the kinetics of inactivation of the ability to bind. I_2 also destroys the binding activity of receptor from the cytoplasmic fraction (Table 1).

Hughes[15] found that during iodination of a protein the sulphydryl groups are oxidized before any iodine is coupled; this reaction could not therefore account for the observed loss of ability to bind 17β-oestradiol with progressive iodination. SH reagents in a variety of experimental conditions were never found able to prevent formation of the oestradiol–receptor complex nor to destroy the preformed complex (Table 1). In an experiment with *P*-mercuribenzoate ion and iodoacetamide, sufficient L-malate dehydrogenase was added as an internal standard to receptor preparations; the enzyme was always strongly inhibited while receptor binding activity was unaffected. Differences from the data of others[16] could be explained by the finding that in our hands a concentration of 1 mM *p*-mercuribenzoate ion induces aggregation of receptor molecules; the aggregates sediment to the bottom of 5–20 per cent sucrose gradients after 12 h at 60,000*g*.

To determine whether the destruction of binding ability of receptor molecules on iodination results from a substitution reaction of a residue or residues within the binding site, or at a different site(s) with secondary effects on the conformation of the binding site, the effect of I_2 was also tested on preformed oestradiol–receptor complex, that is, with the binding site protected from a direct effect. This method is commonly used in covalent labelling of active sites of enzymes and antibodies[17]. The results show that the binding site is protected when interacting with the hormone (Table 1), and we conclude that inactivation of binding activity by iodination is a consequence of destruction of a residue or residues at or near the binding site.

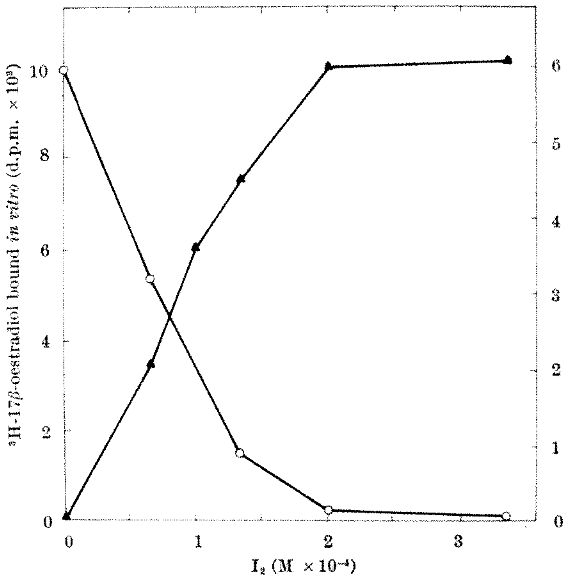


Fig. 1. Correlation between amount of organic iodine formed and ability to bind ^{3}H-17β-oestradiol by receptor preparation from calf uterus nuclear fraction. Aliquots containing 0·2 mg protein/ml. of Tris-K$^+$-EDTA medium (*p*H 8·5) were iodinated for 1 h at 2°–4° C with either unlabelled or labelled tri-iodide solution; after stopping iodination, 6×10^5 d.p.m. of 6,7-^{3}H-17β-oestradiol were added to the samples iodinated with unlabelled tri-iodide; after 90 min at 2°–4° C, unreacted reagents and free ^{131}I or ^{3}H-17β-oestradiol were removed by gel filtration on 'Sephadex *G*-25' columns of standard size (diameter 1·2 cm; total volume 20 ml.) at 4° C; the macromolecular, breakthrough peak was assayed for either ^{3}H (○—○) or ^{131}I (▲—▲) to assess iodine or hormone bound to macromolecules.

Table 1. EFFECT OF SH REAGENTS AND I_2 ON BINDING ACTIVITY OF CALF UTERUS OESTROGEN RECEPTORS WITH BINDING SITE EITHER PROTECTED BY 17β-OESTRADIOL OR UNPROTECTED

Reagent (mM)	Temperature (° C)	Incubation time (h)	*p*H	17β-Oestradiol binding activity (per cent of control)* Nuclear	Cytoplasmic
	Receptor (oestradiol-free)				
p-Mercuribenzoate (0·5)	4–19	1–2	7·8–8·5	93–106	92–101
N-Ethylmaleimide (1)	19	2–16	7·8–7·9	96–103	97–100
Iodoacetate (5)	19	1–16	6·7–8·9	95–102	96–99
Iodoacetamide (5)	19	1·5	7·8	102–104	—
I_2 (0·2)	4	1	8·5	6–14	12–14
	Oestradiol–receptor complex				
p-Mercuribenzoate (1)	4	1	8·5	96–113	94–96
N-Ethylmaleimide (1–5)	4	1	8·5	89–104	—
I_2 (0·2)	4	1	8·5	116–128	116–121

* At least three tests were carried out, and values of maximal variations from control in corresponding series are given.

Oestradiol-free receptor was obtained by heating of native complex for 5 min at 42° C, followed by filtration on 'Sephadex *G*-25' at room temperature to eliminate free oestradiol: the efficiency of this treatment was about 89 per cent of hormone removed. Sequences of treatments with SH reagents were as described in text for iodination, except that treatment by SH reagent substituted for the iodination step. Iodoacetate was freshly recrystallized from petroleum ether. The activity of SH reagents was checked on sensitive enzymes.

Histidyl residues may be as reactive toward I_2 as tyrosyl residues[15], and may be iodinated simultaneously[18]. I_2 is also known to oxidize tryptophyl residues, although tryptophan in general is destroyed after iodination of tyrosyl and histidyl residues[15], and this is in disagreement with the coincident formation of organic iodine and inactivation of binding activity. Furthermore, the relatively high pH used in these experiments is unfavourable to this reaction[19].

We therefore conclude that a tyrosyl and/or a histidyl residue at or near the binding site is essential for binding of 17β-oestradiol by oestrogen receptors; involvement of tryptophan is unlikely. These results agree with the theoretical expectation of Engel[20] that a tyrosyl residue is involved at or near the site of binding of the aromatic end of the steroid molecule.

This work was supported by the Consiglio Nazionale delle Ricerche, Roma. We thank Dr I. Covelli for help with iodination methods and G. Santarpia for technical assistance.

G. A. PUCA
F. BRESCIANI

Institute of General Pathology,
University of Messina,
and Regina Elena Institute for Cancer Research,
Rome.

Received September 5; revised November 10, 1969.

[1] Puca, G. A., and Bresciani, F., *Nature*, **223**, 745 (1969).
[2] Talwar, G. P., Segal, S. J., Evans, A., and Davidson, O. W., *Proc. US Nat. Acad. Sci.*, **52**, 1059 (1964).
[3] Noteboom, W. D., and Gorski, J., *Arch. Biochem. Biophys.*, **111**, 559 (1965).
[4] King, R. J. B., Gordon, G., and Inman, D. R., *J. Endocrinology*, **32**, 9 (1965).
[5] Jungblut, P. W., Hatzel, I., De Sombre, E. R., and Jensen, E. V., *Colloq. Ges. Physiol. Chem.*, **18**, 158 (Springer, Berlin, 1967).
[6] Puca, G. A., and Bresciani, F., *Nature*, **218**, 967 (1968).
[7] Puca, G. A., Nola, E., and Bresciani, F., *Atti Accad. Naz. Lincei* (*Rend. Sci. Fis.* Mat.Nat.), **46**, 268 (1969).
[8] Erdos, T., Gospodarowicz, D., Bessada, R., and Fries, J., *CR Acad. Sci.*, **266**, 2164 (1968).
[9] Korenman, S. G., and Rao, B. R., *Proc. US Nat. Acad. Sci.*, **61**, 1028 (1969).
[10] Rochefort, H., and Baulieu, E. E., *Endocrinology*, **84**, 108 (1969).
[11] Brecher, P. I., and Wotiz, H. H., *Endocrinology*, **84**, 718 (1969).
[12] Kolthoff, I. M., and Stenger, V. A., *Volumetric Analysis*, **2**, 273 (Interscience, New York, 1947).
[13] Zamenhof, S., and Chargaff, E., in *Methods in Enzymology* (edit. by Colowick, S. P., and Kaplan, N. O.), **3**, 702 (Academic Press, New York, 1957).
[14] Strominger, J., and Lowry, O. H., *J. Biol. Chem.*, **213**, 635 (1955).
[15] Hughes, W. L., *Ann. NY Acad. Sci.*, **70**, 3 (1957).
[16] Jensen, E. V., Hurst, D. J., De Sombre, E. R., and Jungblut, P. W., *Science*, **158**, 385 (1967).
[17] Singer, S. J., in *Adv. Protein Chem.*, **22**, 1 (1967).
[18] Covelli, I., and Wolff, J., *J. Biol. Chem.*, **241**, 4444 (1966).
[19] Hartdegen, F. J., and Repley, J. A., *Biochim. Biophys. Acta*, **92**, 625 (1964).
[20] Engel, L. L., Stoffyn, A. M., and Scott, J. F., in *Proc. First Intern. Cong. Hormonal Steroids*, **1**, 291 (Academic Press, New York, 1964).

Differential Inhibition of Rat Liver Cholesterol Biosynthesis by Plant Growth Retardants

To investigate the action of plant growth retardants more precisely, cell-free rat liver preparations have been used as models of plant systems[1]. At least some of the retardants inhibited the enzymes of the isoprenoid biosynthesizing pathway by which radioactive acetate and mevalonate are incorporated into cholesterol. Inhibition of the isoprenoid biosynthetic route[2] that leads to the formation of gibberellins[3] thus seemed to be one of the main effects of applying the retardants to plants[4]. I have expanded the earlier results and here summarize the effects of five plant growth retardants.

Male hooded and albino Wistar rats were used throughout and, after killing by stunning, the livers were excised, weighed, minced and homogenized. The homogenizing medium consisted of 28 mM nicotinamide, 7 mM $MgCl_2 \cdot 6H_2O$, 126 mM sucrose in 44 mM KH_2PO_4–K_2HPO_4 buffer (pH 7·4). A volume equivalent to twice the weight of the liver was used, and after homogenizing for 30 s in a chilled, loose-fitting, mechanically driven glass homogenizer, the homogenate was centrifuged at 3,000 r.p.m. (1,400g) for 5 min. Each 25 ml. incubation flask contained 2 ml. tissue supernatant, 3 ml. phosphate buffer, 1 mg each of ATP, NAD and NADPH, 5,000 µg retardant and either 10 µCi sodium ^{14}C-acetate (30 mCi/mmole) or 0·5 µCi ^{14}C-(+,−)-mevalonic acid lactone (5·03 mCi/mmole). The flasks were loosely stoppered and mechanically shaken at 35° C for 3 h.

Adding 2 ml. 30 per cent KOH terminated the reactions, and the mixtures were then saponified at 35° C for 16 h. The incubations were made 50 per cent with respect to ethanol, carrier cholesterol was added, and the contents were partitioned three times with 25 ml. portions of petroleum ether (b.p. 60–80°). The petroleum ether extracts were reduced to dryness under N_2 and the residues were dissolved in 3 ml. acetone–ethanol (1 : 1). Cholesterol was precipitated by adding 1·5 ml. of 1 per cent digitonin in 55 per cent ethanol, and 3 h later adding a further 2·5 ml. of 0·4 per cent digitonin in water. After 21 h at room temperature the solution was filtered on Whatman No. 1 and the precipitate was washed once with 10 ml. acetone–ether (1 : 1) and three times with 10 ml. ether. The filter papers were treated with 3 ml. pyridine for 1 h at 60° C and then extracted with 20 volumes (60 ml.) petroleum ether. The ether was removed under N_2 and the residue dissolved in 0·2 ml. chloroform–methanol (1 : 1) and 25 µl. aliquots (in Bray's scintillation mixture)[5] were counted. (In two thin layer chromatography solvent systems, 90–95 per cent of the radioactivity in the chloroform–methanol solutions moved with the cholesterol standards.)

Table 1. GROWTH RETARDANT-INDUCED INHIBITION OF PRECURSOR INCORPORATION INTO CHOLESTEROL BY CELL-FREE RAT LIVER PREPARATIONS

Retardant (1,000 µg/ml.)	Percentage inhibition of incorporation in 3 h incubation Acetate	Mevalonate
'Phosphon S'	97 (4)*	38 (2)
'Phosphon D'	99 (6)	89 (7)
'B995' (Alar)	0 (4)	0 (2)
'Amo 1618'	99 (5)	93 (3)
'CCC' (Cycocel)	30 (6)	0 (4)

* Number of replicates.

Table 1 contains the results of a number of experiments with five retardants at a single concentration (1,000 µg/ml.), with either acetate or mevalonate as cholesterol precursors. The retardants at this concentration differ significantly in their capacity to inhibit cholesterol biosynthesis from the two precursors. All retardants except 'B995' inhibit cholesterol formation from acetate. The incorporation of mevalonate (which is further along the biosynthetic pathway) is not, however, inhibited by this concentration of 'B995' or 'CCC', and the effects of 'Phosphon S' are appreciably reduced. It is interesting that substitution of the phosphonium group in 'Phosphon D' by a quaternary nitrogen ('Phosphon S') markedly decreases the effectiveness of the molecule for inhibitory action with mevalonate. Although 'B995' is not inhibitory at this concentration with either precursor, at double the concentration (2,000 µg/ml.) it also suppresses acetate and mevalonate incorporation. 'CCC', on the other hand, even at the higher dose level, does not inhibit the formation of cholesterol from mevalonate. No accurate compilation of dose–response relationships of these retardants on plants has been made, but their general phytoactivity seems to parallel roughly their capacity to inhibit the incorporation of precursors into cholesterol by cell-free rat liver preparations.

These plant growth retardants must now be recognized as cholesterol (and, consequently, steroid) biosynthesis inhibitors, making them comparable in action as well as structure to the animal steroid biosynthesis inhibitors such as 'SK and F 7997-A_3', 'SK and F 7732-A_3' and 'SK and F 525'[6]. The similarity in action between these two groups of compounds has been extended by the findings that 'SK and F 7997-A_3' and 'SK and F 525' exert retardant-like activity on peas[7], tobacco[8], wild cucumber[9] and the fungus *Gibberella fujikuroi*[10].

Although these results suggest a common mode of action for the retardants in plants and animals, it is unlikely to be their only action. It is also unlikely that all plants (and, for that matter, all animals) are equally sensitive. The pronounced differences in species specificity with respect to retardant application effect[11] amply support this conclusion. Differences in response may result from differences in the metabolism of accumulated precursors, or even from completely different types of effects[12]. In connexion with the latter point, unpublished information with J. Sabine shows that 'Phospon D' may inhibit the formation of long-chain fatty acids as well as isoprenoid derivatives.

In spite of the possible diversity of individual retardant effects, the universality of the general effect on isoprenoid metabolism is attested to by the responses of a wide range of organisms. Isoprenoid biosynthesis in the fungus, *Gibberella fujikuroi*, is inhibited[13]; similar inhibition has been recorded in higher plants[14]; reports of effects of retardants on reproductive physiology of houseflies[15], cotton leaf worms[16], aphids[17,18] and locusts[19] can also be easily interpreted as responses induced by inhibited steroid metabolism; and, finally, this communication and earlier results[1] extend this aspect of retardant action to a mammalian system.

The activity of retardants as inhibitors of steroid biosynthesis raises important problems with respect to their agricultural utility. Several of the retardants are not rapidly metabolized by plants[11], and at least one is accumulated and concentrated in particular plant tissues[20]. Unpublished work with J. Sabine also shows that at least two retardants are effective cholesterol biosynthesis inhibitors when applied to rat liver slice systems, as well as in cell-free liver preparations. These findings strongly support the necessity for caution in the large-scale use of retardants for agricultural purposes.

I thank Mr G. Leyson and Miss Nancy Joseph for their technical assistance and the Australian Barley Improvement Trust Fund for its continued support. Part of this work was made possible by a grant from the US National Science Foundation.

L. G. PALEG

Department of Plant Physiology,
Waite Agricultural Research Institute,
Glen Osmond,
South Australia 5064.

Received November 11, 1969.

[1] Paleg, L. G., and Seamark, R., *Biochemistry and Physiology of Plant Growth Regulators*, 389 (The Runge Press, Ottawa, 1968).

[2] Birch, A. J., Rickard, R. W., and Smith, H., *Proc. Chem. Soc.*, 192 (1958).

[3] Graebe, J., Dennis, D. T., Upper, C. D., and West, C. A., *J. Biol. Chem.*, **240**, 1847 (1965).

[4] Paleg, L. G., Kende, H., Ninnemann, H., and Lang, A., *Plant Physiol.*, **39**, 165 (1964).

[5] Bray, G. A., *Anal. Biochem.*, **11**, 279 (1960).

[6] Holmes, W. L., and Di Tullio, N. W., *Amer. J. Clin. Nutr.*, **10**, 310 (1962).

[7] Moore, T. C., and Anderson, J. D., *Plant Physiol.*, **41**, 238 (1966).

[8] Reid, W. W., *Phytochemistry*, **7**, 451 (1968).

[9] Dennis, D. T., and West, C. A., *J. Biol. Chem.*, **242**, 3293 (1967).

[10] Reid, W. W., *Biochem. J.*, **113**, 37P (1969).

[11] Cathy, H. M., *Ann. Rev. Plant Physiol.*, **15**, 271 (1964).

[12] Reed, D. J., Moore, T. C., and Anderson, J. D., *Science*, **148**, 1469 (1965).

[13] Kende, H., Ninnemann, H., and Lang, A., *Naturwissenschaften*, **50**, 599 (1963).

[14] Dennis, D. T., Upper, C. D., and West, C. A., *Plant Physiol.*, **40**, 948 (1965).

[15] Tahori, A. S., Zeidler, G., and Halevy, A. H., *Naturwissenschaften*, **52**, 400 (1965).

[16] Tahori, A. S., Zeidler, G., and Halevy, A. H., *J. Sci. Food Agric.*, **16**, 570 (1965).

[17] Tahori, A. S., Zeidler, G., and Halevy, A. H., *J. Sci. Food Agric.*, **16**, 568 (1965).

[18] Tahori, A. S., Zeidler, G., and Halevy, A. H., *Naturwissenschaften*, **52**, 16 (1965).

[19] Carlisle, D. B., Ellis, P. E., and McVeigh, L. J., *Nature*, **220**, 189 (1968).

[20] Zeevaart, J. A. D., *Plant Physiol.*, **41**, 856 (1966).

Soluble Nitrogenase from Vegetative Cells of the Blue–Green Alga *Anabaena cylindrica*

REPORTS of nitrogen fixation by blue–green algal species have, in the main, been restricted to members of the orders Nostocales and Stigonematales[1]. Unless they are grown in the presence of large concentrations of free ammonium ions, all the species produce characteristic types of cells called heterocysts. The correlation between the ability of a species to fix nitrogen and the possession of heterocysts has led to the suggestion that the heterocyst is the site of nitrogen fixation in blue–green algae[2].

Attempts to obtain active cell-free nitrogenase preparations from blue–green algae have previously resulted in preparations with low activities only, and the activity has been associated with a large particulate fraction[3,4]. Stewart *et al.*[4] were unable to obtain soluble nitrogenase from blue–green algae using sonic oscillation to break the cells. The heterocysts were not broken by sonic oscillation, and it was suggested that this was why enzyme activity was not obtained. The results were considered as evidence that the heterocyst is the site of nitrogen fixation. We have reinvestigated the nitrogenase activity of cell free extracts and have obtained active preparations using sonic oscillation, suggesting that nitrogenase is not restricted to the heterocysts but is also present in vegetative cells.

Anabaena cylindrica (obtained from Dr P. Fay of Westfield College, London) was grown in a medium free of combined nitrogen[5] which was bubbled continuously with 95 per cent N_2, 5 per cent CO_2 for four days. 40 l. of culture was harvested, resuspended in 8 l. of sterile medium and bubbled with 95 per cent argon, 5 per cent CO_2 for 40 h. The argon/CO_2 treatment increased the nitrogenase activity of the culture ten-fold, but did not qualitatively affect the results. The culture was then harvested and the cell paste was either used directly or stored frozen until required.

Extracts were prepared by suspending the cell paste 1 : 4 (w/v) in 0·02 MK N-2 hydroxyethyl piperazine N^1-2-ethanesulphonic acid (HEPES) buffer, *p*H 7·3. After gassing the suspension with nitrogen for 2 h, 1·0 µmole/ml. of $Na_2S_2O_4$ was added and the suspension was exposed to sonic oscillation for 5 min with a Dawe Soniprobe on setting 8; the initial temperature was 10° C. The suspension was then centrifuged for 10 min at 40,000*g* and the supernatant used without further treatment. Nitrogenase activity was assayed using the reduction of acetylene to ethylene[6].

Table 1. REQUIREMENTS FOR ACETYLENE REDUCTION BY EXTRACTS OF *A. cylindrica*

Treatment	nmoles ethylene formed
Complete	218
ATP omitted	0
$MgCl_2$ omitted	0
Creatine phosphate and creatine kinase omitted	0
$Na_2S_2O_4$ omitted	3

The reaction mixture contained, in a final volume of 1·5 ml., HEPES buffer potassium salt, *p*H 7·3, 100 µmoles; $MgCl_2$, 5 µmoles; ATP, 4 µmoles; creatine phosphate, 20 µmoles; creatine kinase, 50 µg; sodium dithionite, 2·5 µmoles; 40,000*g* supernatant fraction containing 6·5 mg protein. The gas phase was 90 per cent argon : 10 per cent acetylene. The reaction was carried out in 7·0 ml. glass vials sealed with 'Subaseal' rubber stoppers. After incubation for 20 min at 30° C the reaction was stopped by injecting 0·1 ml. 60 per cent $HClO_4$ and the ethylene assayed.

Table 1 shows the requirements for acetylene reduction by the 40,000*g* supernatant fraction of *A. cylindrica*. The system showed absolute requirements for adenosine triphosphate (ATP), for an ATP generating system and for a low potential reductant, $Na_2S_2O_4$. These requirements are identical to those previously found for bacterial nitrogenase systems[7].

Table 2 shows the distribution of nitrogenase activity in the different fractions from *A. cylindrica* obtained by sonic oscillation followed by centrifugation. About 20 per cent of the original activity of the whole cell suspension is obtained in the crude sonicate. After centrifugation for 10 min at 40,000*g* essentially all of this activity is recovered in the supernatant fraction, only about 5 per cent of the initial activity in the extract being in the sediment. These results are in contrast to those of Stewart *et al.*[4], who report an initial activity less than 1 per cent of that which we have found in our initial extract. Moreover, all of their nitrogenase activity was associated with a fraction sedimenting in 10 min at 5,000*g*, which may correspond to the low activity which we found to be sedimented in our experiments. The chief difference between our experiments and those of Stewart *et al.* seems to reside in the assay procedure used. The concentrations of reagents used in the assays reported in Table 1 were found to be optimal; greater concentrations of either ATP or dithionite were inhibitory to the soluble nitrogenase. Stewart *et al.* used double the ATP concentration employed by us and ten times the dithionite concentration; the latter concentration has been found to be completely inhibitory to the soluble nitrogenase.

Table 2. DISTRIBUTION OF NITROGENASE ACTIVITY IN EXTRACTS OF *A. cylindrica*

Fraction	nmoles C_2H_4/mg N/min	nmoles C_2H_4/ml. of suspension*/min	Percentage of activity of original suspension
Whole cell suspension	75	280	100
Crude sonicate	18	60	21·4
40,000*g* supernatant	19·5	54·5	19·5
40,000*g* sediment	5·5	2·7	1·0

* The concentration of each fraction was equivalent on a volume basis.

The activities of the extracts were assayed as in Table 1. For determination of the activity of the suspension the reaction mixture contained, in a final volume of 1·5 ml., HEPES buffer potassium salt, *p*H 7·3, 100 µmoles; $MgCl_2$, 5 µmoles and 0·1 ml. of cell suspension. After illumination for 30 min at 30° C at a light intensity of 5,500 lux, the reaction was stopped by injecting 0·1 ml. 60 per cent $HClO_4$ and the ethylene assayed.

In agreement with the findings of Stewart *et al.*[4], the sonication procedure used in these experiments was found to break the vegetative cells while leaving the heterocysts intact. Our extraction procedure resulted in a nitrogenase preparation which was not sedimented by centrifugation at 40,000*g* for 10 min and must be assumed to be released from the vegetative cells. The nitrogenase recovered in this fraction represented 20 per cent of the activity of the original cell suspension, which is similar to the soluble nitrogenase activity extracted from a number of bacteria[8]. We think our results imply that most of the nitrogenase activity of blue-green algae is in the vegetative cells and not, as suggested by Stewart *et al.*[4], in the heterocysts. A more detailed report of these experiments will be published elsewhere.

This work was supported by the Science Research Council and by the University of London Central Research Fund. We thank Miss J. E. Holder and Miss M. C. Lotson for technical assistance.

R. V. SMITH
M. C. W. EVANS

Department of Botany,
King's College,
London.

Received December 11, 1969.

---

[1] Stewart, W. D. P., *Proc. Roy. Soc.*, B, **172**, 367 (1969).
[2] Fay, P., Stewart, W. D. P., Walsby, A. E., and Fogg, G. E., *Nature*, **220**, 810 (1968).
[3] Cox, R. M., and Fay, P., *Arch. Mikrobiol.*, **58**, 357 (1967).
[4] Stewart, W. D. P., Haystead, A., and Pearson, H. W., *Nature*, **224**, 226 (1969).
[5] Allen, M. B., and Arnon, D. I., *Plant Physiol.*, **30**, 366 (1955).
[6] Dilworth, M. J., *Biochim. Biophys. Acta*, **127**, 285 (1966).
[7] Bulen, W. A., Burns, R. C., and LeComte, J. R., *Proc. US Nat. Acad. Sci.*, **53**, 532 (1965).
[8] Hardy, R. W. F., Holsten, R. D., Jackson, E. K., and Burns, R. C., *Plant Physiol.*, **43**, 1185 (1968).

Lack of Porosity of the Mouse Placenta to Maternal Cells

RECENT papers by Tuffrey, Bishun and Barnes[1,2] have evoked great interest, because they have challenged the hitherto conventional views on the integrity of the placental barrier to maternal cells. They first reported finding an often substantial proportion (up to 33 per cent) of cells of maternal origin in certain tissues of mice aged between 4 and 60 days, which had developed in the uterus of a foster mother after transplantation at the blastocyst stage. Subsequently, they found maternal type cells in offspring from normal pregnancies.

The importance of these findings is obvious. One group has already been unable to confirm the results[3], and, using a slightly different experimental system, I have also produced no evidence for the porosity of the placenta to maternal cells.

Mice of the genotype W^vW^v have a genetically determined macrocytic anaemia, which may be permanently cured by the introduction of normal haemopoietic cells[4,5]. Using allogeneic tissue bearing the T_6 chromosome marker, I have found that whether the cells are injected in the neonatal period[6], or into adults previously made tolerant, or into adults under immunosuppression with antilymphocytic serum (M. J. S., manuscript in preparation) the outcome is always the same—the bone marrow rapidly becomes completely replaced by cells of donor origin. Further, the spleen, thymus and lymph nodes become largely composed (70–90 per cent) of the donor-type cells. It would thus appear that the injected normal cells have a definite selective advantage over the defective host cells.

These mice are also affected in foetal life—the defect being detectable by the twelfth day of gestation when yolk sac erythropoiesis is operative, and it continues through the phase of liver erythropoiesis[7]. It was felt therefore that these W^vW^v mice would make excellent subjects for a study of the porosity of the materno-foetal barrier to cells, for it was postulated that even if only a few maternal cells got across to the foetus, with such a selective advantage for genetically normal cells they would be able to proliferate and be detectable.

Because it was considered that the technique of introducing blastocysts into the uterus of a foster mother could possibly itself give rise to chimaerism, it was decided not to use this method, but to rely on normal pregnancies. Because the W^vW^v mice are sterile and anaemic, however, they must be derived from matings between the heterozygotes (genotype W^v+) which are fertile. These mice are also anaemic, but less severely so than the homozygotes. I have also found that these mice may be cured by the transplantation of genetically normal tissue[8], and although the bone marrow is not totally replaced, a very high degree of chimaerism results in this tissue (90 per cent) and in other organs of the lympho-myeloid complex.

The experimental system was as follows. Newborn W^v+ female mice were injected with around $8–10 \times 10^6$ haemopoietic cells, derived from the livers of 16–18 day old CBA-*T6T6* embryos. When these mice were at least 11 weeks old (it being known from previous work that the cells would have implanted and proliferated in a large number of individuals so treated), the success of the transplant was determined by a study of the haemoglobins in the peripheral erythrocytes. The CBA haemopoietic tissue produces a haemoglobin which, on electrophoresis on cellulose acetate paper at *p*H 8·6, yields a diffuse type of pattern. This is easily distinguishable from the single type of haemoglobin in the W mice. Those W^v+ mice which possessed a strong diffuse haemoglobin pattern, and which thus had a large proportion of chromosomally marked cells in their myeloid and lymphoid systems, were then mated with untreated W^v+ males. The resulting W^vW^v offspring (the other genotypes also produced by such a mating, W^v+ and $++$, being ignored,

Table 1. PERCENTAGE OF CBA-*T6T6* CELLS IN THE MITOSES OF THE TISSUES OF W^vW^v OFFSPRING DEVELOPED IN W^v+ MOTHERS TRANSPLANTED WITH CBA-*T6T6* HAEMOPOIETIC TISSUE

Age (weeks)	No. of mice	Per cent CBA-*T6T6* cells in the mitoses of Bone marrow	Spleen	Thymus	Lymph node
1	3	0	0	0	—
2	3	0	0	0	0*
3	3	0	0	0	0
4	3	0	0	0	0
6	2	0	0	0	0

One hundred cells were scored in each tissue.
* 1 mouse only examined.

on the grounds that they were considered to be less propitious hosts for the multiplication of the maternal cells) were allowed to develop normally after birth and were then killed at intervals for chromosome studies. The bone marrow, spleen, thymus and lymph nodes were studied in each of three mice at the age of 1, 2, 3 and 4 weeks and in two mice at 6 weeks. One hundred metaphase plates were scored in each tissue, but it was not possible to obtain enough cells to study the lymph nodes at the age of 1 week or at 2 weeks in two of the mice. The W^v+ mothers were killed shortly after their offspring had been examined in order that their chimaeric state could be quantitated. Chromosome preparations were made 1·5 h after the mice had been injected with 'Colcemid', by an air dry method[10], and stained with 2 per cent toluidine blue.

The results of the chromosome studies in the W^vW^v offspring are presented in Table 1. There were never any cells with the *T6* marker chromosomes in any of the tissues of the fourteen individuals studied, a total of 5,100 mitoses being examined in the series. Table 2 shows the percentage of CBA-*T6T6* cells among the mitoses of the maternal tissues: they were chimaeric to a considerable degree.

Table 2. PERCENTAGE OF CBA-*T6T6* CELLS IN THE MITOSES OF THE TISSUES OF W^v+FEMALES TRANSPLANTED WITH CBA-*T6T6* HAEMOPOIETIC TISSUE

No. of mice	Percentage CBA-*T6T6* cells in the mitoses of Bone marrow	Spleen	Thymus	Lymph node
7	91	79	91	53

Fifty cells were scored in each tissue.

Thus no evidence could be found for the porosity of the placenta to maternal cells. These findings differ therefore from those of Tuffrey *et al.*[1,2]. It is possible that the particular strain combination used by these workers endowed some especial facility for the passage of maternal cells across the placenta. Their experiments and mine examined the actual component cells of the myeloid and lymphoid organs of the offspring, the basic progenitor cell of which is the stem cell. Presumably therefore we were both investigating the passage of this type of maternal cell through the placenta. The whole myelo-lymphoid complex is not a static but a dynamic system, and the stem cells circulate freely within the blood and between the blood and the cellular compartments. It is on this basis that the use of W^v+ mothers so heavily populated with marker cells in this complex was justified for such a study of placental permeability.

I thank Miss A. R. Harcourt for technical assistance. This work was supported by the Spastics Society.

MARY J. SELLER

Paediatric Research Unit,
Guy's Hospital Medical School,
London SE1.

Received November 28, 1969.

[1] Tuffrey, M., Bishun, N. P., and Barnes, R. D., *Nature*, **221**, 1029 (1969).

[2] Tuffrey, M., Bishun, N. P., and Barnes, R. D., *Nature*, **224**, 701 (1969).

[3] Billington, W. D., Kirby, D. R. S., Owen, J. J. T., Ritter, M. A., Burton shaw, M. D., Evans, E. P., Ford, C. E., Gauld, I. R., and McLaren, A., *Nature*, **224**, 704 (1969).

[4] Bernstein, S. E., and Russell, E. S., *Proc. Soc. Exp. Biol. Med.*, **101**, 769 (1959).

[5] Seller, M. J., and Polani, P. E., *Nature*, **212**, 80 (1966).

[6] Seller, M. J., *Nature*, **220**, 300 (1968).

[7] Russell, E. S., Thompson, M. W., and McFarland, E. C., *Genetics*, **58**, 259 (1968).

[8] Seller, M. J., *J. Med. Gen.*, **4**, 33 (1967).

[9] Ford, C. E., in *Tissue Grafting and Radiation* (by Micklem, H. S., and Loutit. J. F.), 197 (Academic Press, London, 1966).

Lymphocyte Cytotoxic Antibodies in Systemic Lupus Erythematosus

WE have observed lymphocytotoxic antibodies in sera of patients with connective tissue disorders. The antibodies are cytotoxic to lymphocytes from unrelated random donors using the microcytotoxicity technique[1,2]. The initial studies were done on 161 coded stored sera submitted to the clinical laboratory for routine immunoelectrophoresis, and confirmatory studies were done on forty-seven coded sera selected from patients followed in the clinic for connective tissue disorders. Results of testing of both series of sera are summarized in Table 1 and show that 74 per cent of all sera tested from patients with systemic lupus erythematosus and 14 per cent of sera from patients with rheumatoid arthritis contained lymphocytotoxic reactivity. Appropriate controls indicated that the cytotoxic effect of lupus sera was dependent on complement; fractionation of one lupus serum by anion exchange chromatography indicated that the cytotoxic factor resides among the IgG globulins. Because cytotoxic antibodies are not present in sera of normal persons except in some cases of immunization by multiple transfusions[3,4], pregnancy[5,6] or organ transplantation[7,8], the results suggest that lymphocytotoxic antibodies develop during the course of systemic lupus erythematosus.

Table 1. INCIDENCE OF LYMPHOCYTOTOXIC ANTIBODIES IN SERA OF PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS

Disease	Study	Number of patients: Total tested	Number of patients: Cytotoxic antibodies present	No. of sera: Total tested	No. of sera: Cytotoxic antibodies present
Systemic lupus erythematosus	Series 1	12	9 (75%)	18*	11 (61%)
	Series 2	24	19 (79%)	40	32 (80%)
	Total	36	28 (78%)	58	43† (74%)
Rheumatoid arthritis	Series 1	21	3 (14%)	21*	3 (14%)
	Series 2	7	1 (14%)	7	1 (14%)
	Total	28	4 (14%)	28	4† (14%)

* In series 1, the 122 remaining sera from patients with other diseases were also examined, of which seventeen (14 per cent) had cytotoxic activity. Positive reactions were seen in three patients with pulmonary fibrosis and one with myasthenia gravis. No clinical data were available on the remaining thirteen cytotoxic sera.

† The forty-seven lymphocytotoxic sera reacted with lymphocytes from varied numbers of donors. Twenty reacted with lymphocytes from 2 to 24 per cent, 14 with 25 to 49 per cent, and 13 with 50 to 100 per cent of the donors.

Readings in the microcytotoxicity tests were scored on the basis of the percentage increase in number of dead cells compared with negative controls: 0–9 per cent, negative (−); 10–14 per cent, doubtful (±); 15–24 per cent, weakly positive (+); 25–84 per cent, positive (+ +); 85–100 per cent, strongly positive (+ + +). Only the + + and + + + reactions were used in the calculations for this table.

Coefficient of correlation[9] (*r*) values were obtained between fifty-four sera of known HL-A specificity and the thirty-seven cytotoxic sera in series 2 which included the thirty-three strongly cytotoxic sera (Table 1) and in addition four other sera which showed only a weakly positive reaction. Reactions of two of the lupus sera correlated with sera of known HL-A specificity (Table 2). Reactions of thirty-three of the thirty-seven sera tested correlated inversely with those of HL-A specific sera. When these thirty-three sera from lupus and rheumatoid patients were compared among themselves, their reactions were divisible into three or more groups, suggesting that they may recognize three or more lymphocytic antigens distinct from the ten known HL-A antigens. It is possible that certain components of the lymphocyte cell membrane become antigenic as a result of yet unknown pathological processes in systemic lupus erythematosus. The individual may then form antibodies to these antigens which, in the presence of complement, cause the lymphocytes to lyse and release intracellular contents. Further studies are required to determine the importance of such a mechanism in the pathogenesis of the disease.

This study was supported by grants from the US National Institutes of Health to the General Clinical Research Center, the Cardiovascular Research and Training Center, and the Arthritis Training Program, the

Table 2. EVALUATION FOR HL-A SPECIFICITIES IN THIRTY-FIVE LYMPHOCYTOTOXIC SERA FROM PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND RHEUMATOID ARTHRITIS

Coefficient of correlation (r)*	Antigen groups of known antisera									
	HL-A1	HL-A2	HL-A3	HL-A9	HL-A5	HL-A7	HL-A8	Da4	4a	4b
Positive sera ($r \geq 0{\cdot}3$)	0	1	0	0	1†	1†	0	0	1†	0
Negative sera ($r \leq 0{\cdot}1$)	18	7	7	14	15	2	6	8	10	0

*r value considered only when correlation appeared with more than one antiserum of known HL-A specificity.

† Single serum showing these positive correlations.

The forty-seven sera of series 2 were examined with a panel of the same forty-three cells from unrelated random healthy donors: thirty-three were clearly positive (+ +, + + +) and four were weakly positive (+). The cytotoxicity reactions of these thirty-seven sera were compared with those of fifty-four antisera containing specificities for known histocompatibility antigens. Two of the thirty-seven sera reacted with lymphocytes from all donors and therefore gave no correlation. Entries in this table are based on the remaining thirty-five sera. Any one antigenic group was present on lymphocytes from a minimum of five and a maximum of thirty-nine of the donors.

Gulf Coast Arthritis Foundation, the Methodist Hospital and the Veterans Administration.

KAMAL K. MITTAL
ROGER D. ROSSEN
JOHN T. SHARP
MARTIN D. LIDSKY
WILLIAM T. BUTLER

Departments of Microbiology and Medicine,
Baylor College of Medicine,
Houston, Texas 77025.

Received December 29, 1969.

[1] Mittal, K. K., Mickey, M. R., Singal, D. P., and Terasaki, P. I., *Transplantation*, **6**, 913 (1968).
[2] Mittal, K. K., Mickey, M. R., and Terasaki, P. I., *Transplantation* (in the press).
[3] Dausset, J., *Vox Sang.*, **4**, 190 (1954).
[4] Payne, R., *Arch. Int. Med.*, **99**, 587 (1957).
[5] Payne, R., and Rolfs, M. R., *J. Clin. Invest.*, **37**, 1756 (1958).
[6] Rood, J. J. van, Eernisse, J. G., and Leeuwen, A. van, *Nature*, **181**, 1735 (1958).
[7] Walford, R. L., Carter, P. K., and Anderson, R. E., *Transplant. Bull.*, **29**, 106 (1964).
[8] Morris, P. J., Hume, D. M., and Terasaki, P. I., in *Histocompatibility Testing* (edit. by Curtoni, E. S., Mattiuz, P. L., and Tosi, R. M.), 339 (Williams and Wilkins, Baltimore, 1967).
[9] Dausset, J., Ivanyi, P., and Feingold, N., *Ann. NY Acad. Sci.*, **129**, 86 (1966).

Delayed Hypersensitivity in *Trypanosoma cruzi* Infection

THE *in vitro* method of studying delayed type hypersensitivity has never been applied to protozoal infections. This communication presents evidence that delayed type hypersensitivity can be demonstrated *in vitro* in mice immunized against *Trypanosoma cruzi*, the causative microorganism of Chagas' disease, and in mice infected with *T. cruzi*.

Delayed type hypersensitivity occurs in all intracellular infectious diseases[1]. Until recent years this phenomenon could be studied only *in vivo* state with skin tests. The development of a positive skin test of the delayed type is shown to be associated with the acquisition of cellular immunity by the animal[2,3]. In fact, these two terms are used interchangeably by many workers. *In vitro* methods of studying delayed type hypersensitivity have recently been introduced, and these methods have been applied to study this phenomenon in bacterial infections, principally infections by *Mycobacterium tuberculosis*, *Brucella*, *Streptococcus*, *Listeria monocytogenes*, and some fungal and viral infections. In protozoal diseases, skin tests of the delayed type are available for infections by *Leishmania*, *Trichomonas*, *Toxoplasma* and *Trypanosoma cruzi*[4,5].

Rich and Lewis first described *in vitro* migration of cells from spleen cell explant as an index of delayed type hypersensitivity[6]. George and Vaughan, and David *et al.*, modified and improved this test and put it on a semi-quantitative basis[7,8]. Recently Fauve and Dekaris described a simpler test, the macrophage spreading inhibition on glass surface[9]. It has also been shown that the degree of inhibition of macrophage spreading correlates well with the long established *in vivo* skin test for delayed type hypersensitivity[10].

Peritoneal washing was obtained from four groups of Carworth Farm Strain 1 (CF1) male mice of body weight 25–30 g. Group *A* were normal CF1 mice. Group *B* were mice given 10^6 cultural form *Trypanosoma cruzi*. The cultural flagellates gave rise to low grade and transient parasitaemia, and the mice developed premunition against future virulent challenge. Group *C* were immunized by subcutaneous injection of 5·0 mg of desiccated cultural *T. cruzi* incorporated in Freund's complete adjuvant (0·1 ml. of *T. cruzi* antigen in saline homogenized with 0·1 ml. of adjuvant for each mouse). Peritoneal washing was obtained from groups *B* and *C* 2 months after the inoculation. Group *D* were immunized as group *C*, but 1 month after the immunization the mice of group *D* were challenged with virulent mouse blood form of Peru strain *T. cruzi*. Peritoneal washings were obtained from the survivors 2 months after the challenge.

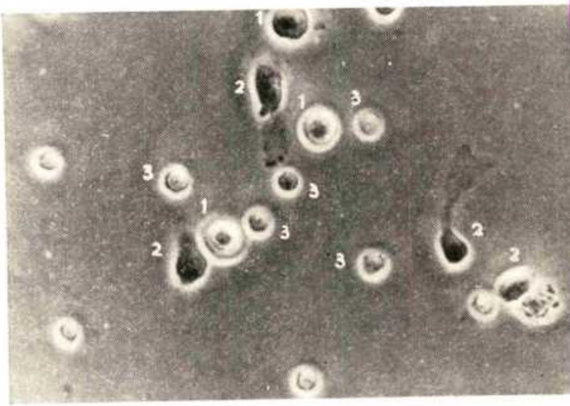


Fig. 1. Phase contrast photomicrograph of peritoneal cells after incubation for 30 min at 37° C. 1, Macrophages; 2, spreading macrophages; 3, lymphocytes.

The mice were exsanguinated by decapitation and the abdominal wall was opened and fur stripped back. Three ml. of tissue culture medium 199, with 5 units of heparin and 20 mg of bovine serum albumin per ml. added, were injected into the abdominal cavity along the mid-line. The abdomen was gently rocked to distribute the fluid throughout. The fluid was then withdrawn. Care was taken to avoid traumatic bleeding. This abdominal washing has an average cell count of 2×10^6 cells/ml. The proportion of lymphocytes and macrophages varies from 2 : 1 to 1 : 2. This is true in all groups of mice studied.

The macrophage spreading inhibition was determined by the method of Fauve and Dekaris[9]. The peritoneal washing was gently shaken and 1 ml. was put into each of two polyethylene tubes. To one tube was added 0·05 ml. of a saline extract of cultural form *T. cruzi* antigen (equivalent to 0·05 mg of lyophilized *T. cruzi*), and to the other was added 0·05 ml. of isotonic saline. After gentle shaking, the tubes were incubated in a water bath at 37° C for 30 min. After the incubation the tubes were gently shaken and a drop was put into a Thoma counting chamber. This was then incubated for another 30 min in a

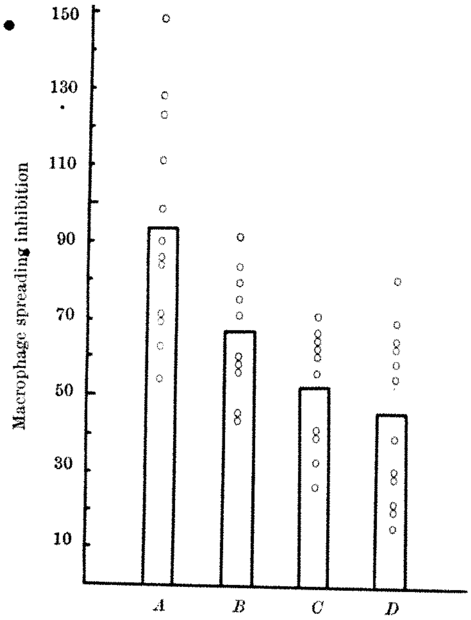


Fig. 2. Macrophage spreading inhibition of mouse peritoneal cells by *Trypanosoma cruzi* antigen. *A*, Normal mice; *B*, acute *T. cruzi* infection; *C*, immunized with lyophilized *T. cruzi* in Freund's complete adjuvant; *D*, immunized as in *C* and recovered from virulent challenge.

moist chamber at 37° C. A reading was taken with phase contrast microscopy using a Leitz Ortholux microscope with ×500 magnification. The cells in all four corner squares (white cell count chambers, each square having a volume of 1 mm^3) were counted and the number of cells that had spread was noted. The macrophages are larger and darker than the lymphocytes. Only the macrophages with spreading cytoplasm and irregular borders are considered to have spread. The photomicrograph (Fig. 1) shows the typical appearance of the spreading macrophages. The percentage of spreading cells varies from animal to animal, but it is usually in the neighbourhood of 20–40 per cent. Readings in duplicate preparations from the same mouse usually agree closely. The macrophage spreading inhibition is calculated from the following formula

$$\text{MSI} = \frac{\text{Average per cent spreading with antigen}}{\text{Average per cent spreading without antigen}} \times 100$$

Fig. 2 shows the results obtained in the different groups of mice. Each point represents the result from one mouse. It can be seen that there is a great variation of the MSI in each group. The horizontal bars represent the means of the groups. It can be seen that, compared with the normal group of mice (group *A*), there was significantly more inhibition of macrophage spreading in the presence of *T. cruzi* antigen in the acutely infected group (group *B*), the group immunized with killed *T. cruzi* incorporated in Freund's complete adjuvant (group *C*), and the similarly immunized group that had subsequently survived the virulent challenge (group *D*) (with 't' test $P<0{\cdot}05$, $P<0{\cdot}001$ and $P<0{\cdot}001$ respectively). The difference between groups *B* and *C* was minor and at the borderline of significance ($P=0{\cdot}05$). Groups *C* and *D* showed no significant difference in MSI in the presence of *T. cruzi* antigen. Not shown in the figure are the group of mice given Freund's complete adjuvant alone without *T. cruzi* antigen. The MSI of this group (mean MSI = 85) was not significantly different from the non-vaccinated and non-infected mice.

This work was supported by a fellowship from the Medical Research Council of Canada. I thank Professor A. W. Woodruff and Dr P. D. Marsden for providing the laboratory facilities.

Stanley Seah*
Department of Clinical Tropical Medicine,
London School of Hygiene and
Tropical Medicine, London WC1.

Received October 27; revised December 4, 1969.

* Present address: Parasitology Research Unit, Queen Mary Veterans' Hospital, Montreal, Quebec, Canada.

[1] Mackaness, G. B., *Brit. Med. Bull.*, **23**, 52 (1967).
[2] Lurie, M. B., *J. Exp. Med.*, **60**, 163 (1934).
[3] Mackaness, G. B., *J. Exp. Med.*, **116**, 381 (1962).
[4] Lumsden, W. H. R., in *Handbook of Experimental Immunology* (edit. by Weir, D. M.), 925 (Blackwell Scientific Publications, Oxford, 1967).
[5] Gonzalez-Cappa, S. M., Schmunis, G. A., Traversa, O. C., Yanovsky, J. F., and Parodi, A. S., *Amer. J. Trop. Med. Hyg.*, **17**, 709 (1968).
[6] Rich, A. R., and Lewis, M. R., *Bull. Johns Hopkins Hosp.*, **50**, 115 (1932).
[7] George, M., and Vaughan, J. H., *Proc. Soc. Exp. Biol. Med.*, **111**, 514 (1962).
[8] David, J. R., Al-Askari, S., Lawrence, H. S., and Thomas, L. J., *J. Immunology*, **93**, 264 (1964).
[9] Fauve, R. M., and Dekaris, D., *Science*, **160**, 795 (1968).
[10] Dekaris, D., Fauve, R. M., and Raynaud, M., *J. Immunol.*, **103**, 1 (1969).

Ion Dependent Conductance: Switching in Lipid Bilayers

The action of antibiotics on the ionic conductances of lipid bilayers has provided useful models of permeation mechanisms in biological membranes[1,2]. Voltage dependent conductance states are of special interest. They have been demonstrated for nerve[3] and are probably present, though with much slower kinetics, in other cell types[4,5]. There is a distinction between ion selective conductances and ion dependent switching of conductances which themselves may or may not be selective. For example, one has the well known effect of $[Ca^{2+}]$ on the Na^+ conductance[6] in nerve, and more recently $[K^+]$ has been shown to increase the inactivation parameter and time constant of this conductance[7]. I wish to describe two examples of the effect of $[Na^+]$ favouring a low conductance state even though the voltage dependence is opposite in each case and there is no ion selectivity for the conductances.

Fig. 1*A* shows a continuous record of the voltage–current relationship for phospholipid and equimolar phospholipid–cholesterol bilayers in the presence of the cyclic hexadepsipeptide, monamycin[8], after a steady state of incorporation has been reached. It reveals an asymmetry arising from the presence of Na^+ on one side and K^+ on the other in the sense that an Na^+ current causes a switch to a lower conductance state, from which the time constant for the recovery is approximately 10 s. For the phospholipid bilayer there is no ion selectivity for either the high or low conductivity; the almost vanishing zero-current potential is evidence for this. For the phospholipid–cholesterol bilayer, however, there is a substantial K^+ selectivity for the low conductance state which may be connected with the greater ratio of high to low conductances.

Fig. 1*B* shows the behaviour of the phospholipid bilayer in the presence of the cyclic nonadecapeptide, alamethicin, in the same conditions. Here, as has been reported for symmetrical NaCl solutions[9], there is a switch to a high conductance, the increment for an *e*-fold increase being 4·5 mV. In this case, however, the potential for a measurable conductance is shifted by at least 25 mV, or a factor 250 times, in favour of the K^+ current. In spite of this the bionic (zero current) potential is negligible (about −8 mV). The results for phospholipid–cholesterol are not significantly different.

For both antibiotics the apparent order *n*, of what is presumably a surface association, is high as judged by concentration dependence. For alamethicin[9] $n=6$; for monamycin one can estimate that it is the same by fitting

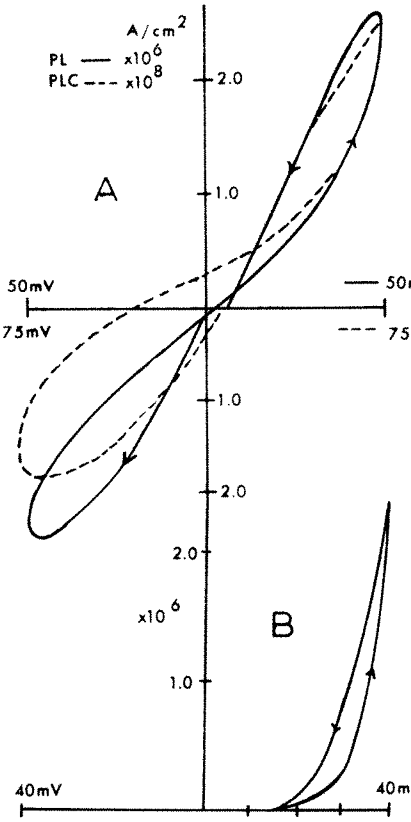


Fig. 1. Continuous voltage-current recording with voltage sweep frequency $f = 0{\cdot}06$ s^{-1}, 10 mM potassium and sodium phosphate buffer at pH 7·3 inside and outside respectively; positive current outwards. PL, phosphatidyl choline (from soya bean) 30 mg/ml. in 1 : 2 dodecane–octane; PLC, 20 mg cholesterol and 10 mg phosphatidyl choline in the same solution. *A*, 3×10^{-8} M monamycin both sides; *B*, 7×10^{-7} M alamethicin both sides.

the sigmoid kinetics of the low potential conductance to $(1 - e^{-kt})^n$; that is, a rate-limiting first order reaction followed by an nth order reaction which leaves an excess of reactants. A forward rate constant $k_1^+ \sim 0{\cdot}01$ s^{-1} for symmetrical K$^+$ solutions, and at least an order of magnitude smaller for symmetrical Na$^+$ solutions is then found. In the case of alamethicin and possibly also monamycin, k_1^+ is also voltage dependent (but in an opposite sense). This scheme of kinetics is formally the same as the Hodgkin–Huxley[3] phenomenological description of the K$^+$ channel in nerve. The analogy is further reinforced by the fact that adding 1 mM teraethylammonium to one side of symmetrical K$^+$ solutions, one can induce asymmetry similar to that observed in Fig. 1*A*. Because tetraethylammonium is quite impermeant in these conditions (judged by the large bionic potential against K$^+$ alone), this is a surface reaction and does not involve ionic current *per se*.

Although the tertiary structure of the above peptides is not yet known, similar results have been found for gramicidin *S* whose structure is completely known[10]. In each case there is a specific structural effect of cholesterol which seems to act on the equilibrium constant of the nth order reaction. When more of these structures are known it should be possible to elucidate this effect.

The biological significance of these results is that one has a control mechanism which, because it is non-linear with respect to potential, is somewhat more flexible than simply changing the ionic selectivity of conductance. Elsewhere I have shown that the latter effect occurs with the cyclic decapeptide, tyrocidine *B*, in the presence of amines (unpublished results of M. C. G.). That amines have this effect in these systems suggests that they can be models for the membrane mediated action of biogenic amines.

I thank Professor C. H. Hassall, University College of Swansea, for monamycin and Dr George B. Whitfield, Upjohn Company, for the alamethicin.

M. C. GOODALL

Institute for Biomedical Research,
American Medical Association—Education and
Research Foundation,
Chicago, Illinois 60610.

Received October 13; revised November 24, 1969.

[1] Mueller, P., and Rudin, D. O., *J. Theor. Biol.*, **18**, 222 (1968).

[2] Eisenmann, G., *Fed. Proc.*, **27**, 1249 (1968).

[3] Hodgkin, A. L., and Huxley, A. F., *J. Physiol.*, **117**, 500 (1952).

[4] Matthews, E. K., and Saffran, M., *Nature*, **219**, 1369 (1968)

[5] Morrill, G. A., and Kostellow, A. N., *Biophys. J.*, **9**, FAM-14 (1969).

[6] Frankenhaenser, B., and Hodgkin, A. L., *J. Physiol.*, **137**, 217 (1957).

[7] Adelman, W. J., and Palti, Y., *J. Gen. Physiol.*, **53**, 685 (1969).

[8] Hassall, C. H., and Magnus, K. E., *Nature*, **184**, 1223 (1959).

[9] Mueller, P., and Rudin, D. O., *Nature*, **217**, 713 (1968).

[10] Stern, A., Gibbons, W., and Craig, L. C., *Proc. US Nat. Acad. Sci.*, **61**, 734 (1968).

Strychnine, Glycine and Vertebrate Postsynaptic Inhibition

ON the basis of experiments performed on Mauthner cells of the goldfish, the suggestion has been made[1] that the competitive and reversible antagonism demonstrated in the feline spinal cord between glycine and strychnine[2–4], an alkaloid known to block spinal postsynaptic inhibition, does not necessarily support the proposal that glycine could be a spinal inhibitory transmitter in the cat.

In the goldfish, strychnine administered systemically in amounts adequate to suppress the late collateral inhibition of Mauthner cells has little or no effect on the inhibitory action of electrophoretically administered glycine[1]. In the cat the effect of glycine on spinal motoneurones and Renshaw cells has also been reported to be more resistant to antagonism by intravenously injected strychnine than the synaptically released transmitter[2–4], and an explanation has been offered for this in terms of the distribution of receptors activated by glycine. Inhibitory postsynaptic potentials are generated by the summed activity at a number of synapses distributed over the soma-dendritic membrane of the neurone. On the other hand, the hyperpolarization induced by electrophoretically administered glycine probably results from the more intense activation of a smaller area of the postsynaptic membrane. Thus, assuming glycine to be the transmitter, the concentration of this amino-acid in this region of the membrane is presumably much higher than that at the synapses generating an equivalent synaptic hyperpolarization. As a consequence, concentrations of a systemically injected glycine antagonist adequate to block the synaptic response could have minimal or no effect upon the action of locally administered glycine. As in the cat[2–4], elevation of the strychnine concentration in goldfish beyond that which blocks synaptic inhibition does result in a reduction of the effectiveness of glycine[1].

By contrast with these results, when administered electrophoretically from micropipette adjacent to that ejecting glycine, strychnine readily blocks the action of glycine on feline spinal neurones; it is, however, relatively ineffective as an antagonist of postsynaptic inhibition[2–4], as might be expected if the alkaloid fails to gain access to membrane sites located remotely from the micropipette. Similar arguments have been proposed to explain differences in the effectiveness of systemically and electrophoretically administered dihydro-β-erythroidine as an antagonist of acetylcholine, released electrophoretically or from axon collateral terminals, upon feline Renshaw cells[5].

It is thus possible that the apparent low sensitivity to systemic strychnine of the inhibition of Mauthner cells by glycine arises also from differences in local concentrations of glycine which achieve similar degrees of inhibition after electrophoretic or synaptic release. If this is so, the results obtained in the goldfish[1] provide no evidence for a presynaptic action of strychnine, and support a transmitter role of glycine, or a "glycine-like" amino-acid[2-4], at vertebrate strychnine-sensitive inhibitory synapses.

D. R. CURTIS
G. A. R. JOHNSTON

Department of Physiology,
Australian National University,
Canberra, Australia.

Received September 29, 1969.

[1] Roper, S., Diamond, J., and Yasargil, G. M., *Nature*, **223**, 1168 (1969).
[2] Curtis, D. R., Hösli, L., Johnston, G. A. R., and Johnston, I. H., *Exp. Brain Res.*, **5**, 235 (1968).
[3] Curtis, D. R., Hösli, L., and Johnston, G. A. R., *Exp. Brain Res.*, **6**, 1 (1968).
[4] Curtis, D. R., Duggan, A. W., and Johnston, G. A. R., *Brain Res.*, **14**, 759 (1969).
[5] Curtis, D. R., and Ryall, R. W., *Exp. Brain Res.*, **2**, 81 (1966).

Strychnine Antagonism and Glycine: a Reply

Drs Curtis and Johnston seem to have overlooked certain items in our experiments. The important question is, should one expect the concentration of strychnine which blocks the action of a synaptically released transmitter to cause a reduction in the action of the same substance delivered iontophoretically to the neurone? Our critics quote experiments in which they measured the inhibitory hyperpolarization, which is of course a function of the driving force on the ions, as well as the change in membrane resistance. In our experiments these facts must be stressed: (*a*) The physiological response we measured, the increased membrane conductance (a direct measure of transmitter action), was largely the result of synaptic action on the region of the cell from which we recorded, and the glycine was pipetted onto the same region of the cell (and indeed must sometimes have reached even more distant regions also). The contribution to our recorded conductance changes of physiological inhibitory activity more than about 200 μm along the dendrites would be small. We did not detail the evidence for these items, but simply quoted the work on which they are based[1]. (*b*) The strychnine concentration about the cell, as detected by the blocking of physiological inhibition, rose gradually until the effects of two independently activated inputs were totally prevented (we would have detected an inhibition as little as 2 per cent of the control). (*c*) At this critical blocking concentration of strychnine, glycine effects were unchanged over a range of doses from the just detectable to the obviously "saturating" (not only doses "which achieve similar degrees of inhibition after electrophoretic or synaptic release"). The smallest glycine dose whose effect we could reliably measure caused a 1–2 per cent inhibitory conductance change; this was less than 1/20 of that caused by a "saturating" dose. These small responses to glycine should be compared with the relatively enormous conductance change during synaptic activation (50–60 per cent increase). It is difficult to imagine that this threshold glycine dose was causing a "more intense activation of a smaller area of the postsynaptic membrane" than that involved in the full physiological response. Nevertheless, the direct effects of this small dose of glycine (and of the larger doses) were quantitatively unaffected, even though the very large physiological response was not just reduced but totally eliminated.

The point we wish to make is that experiments which attempt to answer the questions we asked are necessary in order to exclude the possibilities raised in our report. If the blocking action of strychnine is to be used as evidence, then the investigation must be made quantitatively, with regard to the critical concentration of strychnine, the dose-response relations of glycine, and the extent of cell activated both physiologically and pharmacologically. It is important to note the results of the analogous experiment performed at the neuromuscular junction: curare, in uniform concentration over the preparation, was somewhat more effective in reducing the end-plate potential than in reducing the iontophoretically applied acetylcholine potential, but the effects of small doses of acetylcholine seemed to be readily abolished[2]. These findings further strengthen our concern about accepting the qualitative observation that strychnine blocks both physiological and pharmacological inhibition as evidence for the identification of the transmitter. Our results still leave the possibility that a glycine-like substance (or perhaps a glycine complex) is a transmitter. But the postsynaptic action of strychnine, as revealed by glycine itself, seems to require larger concentrations of strychnine than are necessary to block the effects of physiological inhibition (at least on the Mauthner cell).

S. ROPER
J. DIAMOND

Department of Physiology,
University College,
London WC1.

Received December 4, 1969; revised January 9, 1970.

[1] Diamond, J., *J. Physiol.*, **194**, 669 (1968).
[2] Goldsmith, T. H., *J. Physiol.*, **165**, 368 (1963).

5-Hydroxyindoleacetic Acid Levels in the Cerebrospinal Fluid of Depressive Patients treated with Probenecid

ACCORDING to the serotonin (5-hydroxytryptamine, 5-HT) hypothesis there is a causal relationship between mental depression and 5-HT deficiency in the brain[1-3]. Some depressive patients—mainly those suffering from an endogenous depression—display the following clinical signs. (1) The concentration of 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) (refs. 4 and 5) and the urinary concentrations of 5-HT (ref. 6) and 5-HIAA (refs. 7 and 8) are unusually low. (2) The transformation of 5-hydroxytryptophan into 5-HT seems to be defective[9] (although in a later publication Coppen[1] reported his inability to reproduce these findings). (3) Precursors of 5-HT which readily enter the brain—5-hydroxytryptophan[10] and tryptophan[11]—can have a therapeutic effect. Finally, in suicide victims the cerebral concentration of indoleamines proved to differ from that in a control group and, according to Shaw *et al.*[12], the 5-HT concentrations were diminished. Bourne *et al.*[13] were unable to corroborate this but did find a diminished concentration of 5-HIAA.

These data pertain to absolute concentrations and offer insufficient information on the rate of metabolism of 5-HT. It is evident that suitable methods for the calculation of the turnover of 5-HT in the human brain are required before the serotonin hypothesis can be established. Neff *et al.*[14] have found, in rats, that the transport of 5-HIAA from the brain into the bloodstream is almost totally inhibited by *p*-(dipropylsulphamoyl)benzoic acid (probenecid). They also suggested that the rate of accumulation of 5-HIAA under the influence of probenecid could be equated with the rate of synthesis of 5-HT in the brain. In dogs, the concentration of 5-HIAA in the CSF increased following probenecid administration[15]. There are indications (albeit indirect) that acid amine metabolites in CSF are derived from brain amines and that changes in the CSF acid concentrations may reflect the metabolism of the cerebral amine precursor[16,17]. If this is true, then the rate of increase of the 5-HIAA concentration in the CSF under the influence of probenecid can be regarded as an index of the rate of synthesis of 5-HT in the brain.

Olsson and Roos[18] were the first to apply the probenecid test to human subjects: sufferers from Parkinson's disease. The purpose of our study was to establish whether the effect of probenecid in depressive patients supplies evidence of a reduced synthesis of 5-HT in the brain. We treated fourteen patients with "vital" (or "endogenous") depressions and eleven non-depressive controls. The diagnosis was established on the basis of the results of a structured standard interview[19]. The examination was performed between the fifth and the tenth days after arrival at the hospital. All test subjects had been without medication for at least a week. Age and sex distribution in the two groups were not significantly different.

The test was as follows (personal communication from B.-E. Roos). On the first day, a lumbar puncture was made between 0900 and 1000 h. On the second and third days, $4 \times 0·5$ g probenecid was given orally; a further 1 g probenecid was given on the fourth day, at 0730 h, followed 4 h later by the second lumbar puncture. The CSF was stored at $-20°$ C and the 5-HIAA concentration was determined by the method of Korf[20] within three days of collection. In the procedure described, the probenecid test merely gives an impression of the rate of accumulation of 5-HIAA. More exact determination requires that more than two points of the 5-HIAA rise curve are known; this, however, would require as many lumbar punctures, which was not considered justifiable.

In the vital depression group, the mean 5-HIAA concentration in the CSF before administration of probenecid was significantly lower than in the control group ($P < 0·01$, Wilcoxon test), which corroborates the observations of Ashcroft *et al.*[4] (Table 1). After probenecid administration the concentration of 5-HIAA in the CSF rose in all subjects in both groups. The mean rise in the control group corresponds with the values found by Olsson and Roos[18] (Table 1). The rise in the vital depression group was smaller than that in the control group ($P = 0·05$, Wilcoxon test), which argues in favour of the 5-HT hypothesis provided that: (1) the rates of synthesis of 5-HIAA and of 5-HT are in fact identical, and (2) probenecid absorption was the same in all test subjects. The possibility that low probenecid absorption in the intestine is the cause of a small effect of probenecid is now being investigated by carrying out a parenteral probenecid test as well in these cases.

Table 1. CONCENTRATION OF HIAA IN THE CSF IN DEPRESSIVE PATIENTS AND CONTROLS BEFORE AND AFTER PROBENECID ADMINISTRATION

Group	No. of test subjects	5-HIAA concentration in CSF (μg/ml.) Before probenecid	After probenecid	Difference
Vital depression	14	0·017 ± 0·017 (0·000 – 0·046)	0·039 ± 0·025 (0·030 – 0·067)	0·022 ± 0·020 (0·000 – 0·073)
Control	11	0·040 ± 0·024 (0·004 – 0·081)	0·074 ± 0·026 (0·021 – 0·098)	0·035 ± 0·018 (0·017 – 0·060)

Results are given as means ± *S.D.* (with range).

The vital depression group included seven patients whose 5-HIAA concentration in the CSF rose by less than 0·020 μg/ml. after probenecid administration; the control group included only one such case. The difference was statistically significant ($P < 0·05$, Fisher's exact probability test). Vital depressive patients with a normal and those with a subnormal probenecid effect were indistinguishable in terms of psychopathological manifestations. In particular there was no correlation with the patient's motor inhibition or agitation.

The significance of the wide variation of chemical parameters in psychiatric patients is not generally appreciated. We believe that vital depressive patients with a subnormal probenecid effect may be incidental variants of the normal, but they can equally well constitute a group *sui generis*. Assuming that the latter is the case, and that mood and 5-HT metabolism do correlate, we conclude that 5-HT deficiency plays a part in some vital depressions but not in others. The group of vital depressions, which tends to be homogeneous in psychopathological terms, is therefore heterogeneous in terms of pathogenesis[21].

H. M. VAN PRAAG
J. KORF
J. PUITE

Department of Biological Psychiatry and
University Psychiatric Clinic,
State University,
Groningen.

Received September 2; revised November 20, 1969.

[1] Coppen, A., *Brit. J. Psychiat.*, **113**, 1237 (1967).
[2] Lapin, T. P., and Oxenbrug, G. F., *Lancet*, i, 132 (1969).
[3] van Praag, H. M., *Pharmakopsychiat. Neuropsychopharmak.*, **3**, 151 (1969).
[4] Ashcroft, G. W., Crawford, T. B. B., Eccleston, D., Sharman, D. F., MacDougall, E. J., Stanton, J. B., and Binns, J. K., *Lancet*, ii, 1049 (1966).
[5] Denker, S. J., *J. Neurochem.*, **13**, 1545 (1966).
[6] Rodnight, R., *Internat. Rev. Neurobiol.*, **3**, 251 (1961).
[7] Praag, H. M. van, and Leijnse, B., *Psychopharmacologia*, **4**, 1 (1963).
[8] Praag, H. M. van, Korf, J., Woudenberg, F. van, and Kits, T. P., *Psychopharmacologia*, **13**, 145 (1968).
[9] Coppen, A., Shaw, D. M., and Malleson, A. J., *Brit. J. Psychiat.*, **111**, 105 (1965).
[10] Person, T., and Roos, B.-E., *Lancet*, ii, 987 (1967).
[11] Coppen, A., Shaw, D. M., Herzberg, B., and Maggs, R., *Lancet*, ii, 1178 (1967).
[12] Shaw, D. M., Camps, F. E., and Eccleston, E. C., *Brit. J. Psychiat.*, **113**, 1407 (1967).
[13] Bourne, H. R., Bunney, W. E., Colburn, R. W., Davis, J. M., Davis, J. N., Shaw, D. M., and Coppen, A. J., *Lancet*, ii, 805 (1968).
[14] Neff, N. H., Tozer, T. N., and Brodie, B. B., *J. Pharmacol.*, **158**, 214 (1967).
[15] Guldberg, H. C., Ashcroft, G. W., and Crawford, T. B. B., *Life Sci.*, **5**, 1571 (1966).
[16] Bowers, jun., M. B., and Gerbode, F., *Life Sci.*, **7**, 773 (1968).
[17] Guldberg, H. C., in *Metabolism of Amines in the Brain* (edit. by Hooper, G.), 55 (Macmillan, London, 1969).
[18] Olsson, R., and Roos, B.-E., *Nature*, **219**, 502 (1968).
[19] Praag, H. M. van, Uleman, A. M., and Spitz, J. C., *Psychiat. Neurol. Neurochirur.*, **68**, 329 (1965).
[20] Korf, J., and Valkenburgh-Sikkema, T., *Clin. Chim. Acta*, **26**, 301 (1969).
[21] van Praag, H. M., *Brit. J. Psychiat.*, **114**, 1195 (1968).

Abnormal Zygote Development in Mice after Paternal Exposure to a Chemical Mutagen

AFTER paternal exposure to mutagens and subsequent mating, adverse genetic effects are seen in the form of pre-implantation losses of fertilized ova and zygotes, early foetal deaths, and sterility and semi-sterility in F_1 progeny[1]. Early foetal deaths are a direct quantitative index of dominant lethal mutations. These are generally concomitant with pre-implantation losses, which thus constitute a presumptive but less specific measure of mutagenicity[1]. A practical, sensitive assay for mutagens, based on the induction of dominant lethal mutations in mammals, has been developed and applied to a wide range of environmental chemical pollutants and to drugs[2].

The development of mammalian zygotes is retarded, as a result of induced mutations and chromosome anomalies, after paternal irradiation[3-8], artificial insemination with spermatozoa treated *in vitro* with chemical mutagens[9], and paternal[10] or maternal[11] treatment with chemical mutagens. We report here quantitative data on fertilization and the development of zygotes in mice after paternal exposure to a chemical mutagen and subsequent mating during the entire spermatogenic cycle.

Random-bred ICR/Ha Swiss male mice, 8–10 weeks old, were injected intraperitoneally with single doses of 10 mg/kg of triethylenephosphoramide (TEPA) in 0·1 ml. of aqueous solution; untreated males served as concurrent controls. Each male was then caged for 1 week with three virgin females, 8–10 weeks old, after which females were replaced weekly and consecutively with fresh animals for a total of 8 weeks. Matings in weeks 1–3 and 4–8 after injection represent samplings of sperm exposed to TEPA in

Table 1. INCIDENCE OF RETARDED AND/OR MALFORMED OVA AFTER MATING WITH MALE MICE TREATED WITH TEPA

Mating week of treated males	60 h post-ovulation			Percentage of cleaved ova with specified characteristics				82 h post-ovulation			Percentage of cleaved ova with specified characteristics			
	No. of females	No. of ova collected	No. of ova fertilized	Normal cleavage, normal morphology	Normal cleavage, malformed	Retarded cleavage, normal morphology	Retarded cleavage, malformed	No. of females	No. of ova collected	No. of ova fertilized	Normal cleavage, normal morphology	Normal cleavage, malformed	Retarded cleavage, normal morphology	Retarded cleavage, malformed
1	17	172	166	22	38	16	24	14	149	142	5	26	4	66
2	13	141	130	7	37	18	38	10	86	83	11	49	0	40
3	11	130	121	38	36	13	13	12	102	98	51	31	1	17
4	10	122	108	82	4	10	4	13	126	126	100	0	0	0
5	9	101	94	99	0	1	0	11	89	86	99	0	0	1
6	12	135	61	48	1	44*	7	8	75	43	100	0	0	0
7	12	131	121	85	7	7	1	11	105	102	100	0	0	0
8	10	113	106	92	0	8	0	13	109	105	100	0	0	0
Composite controls	50	576	564	97	0	3	0	32	313	312	98	1	0	1

* Most of these ova were retarded by only one cleavage stage. If these are considered as normally developed, then the percentage of cleaved ova which are normal in all respects would be increased from 48 to 84 per cent.

post-meiotic and pre-meiotic phases of spermatogenesis respectively. Females were killed after detection of vaginal plugs to obtain ova at the stage of 10, 30, 60 and 82 h post-ovulation (±2). Six replicates, with concurrent controls, were studied to yield a range of eight to seventeen females at each stage of zygote development for each mating week. Tubal ova and blastocysts were collected by flushing oviducts and uterine cornua respectively with 2 per cent sodium citrate; recovered ova were fixed overnight with 10 per cent buffered formalin, and stained with 1 per cent lacmoid[12]. Ova were scored as fertilized if the spermatozoon or its tail was found in the vitellus, or if the second polar body was present. Zygotes were scored for numbers of blastomeres, morphological anomalies such as alterations in relative blastomere size, micronuclei and multinucleate blastomeres.

The following data relate to all mating weeks other than the sixth, which are presented separately. At 10 h, the overall incidence of fertilized ova, for all weeks of mating other than the sixth, was 97 per cent for both test and control mice. By 30 h, 98 and 100 per cent of fertilized ova in test and controls respectively had undergone first cleavage (Fig. 1). In ova at the two cell stage, taken from test animals in the first, second and third mating weeks, there were 72, 50 and 20 per cent of micronuclei respectively; in ova from females mated during weeks 4 to 8 after males had been treated with TEPA, and in control matings at all times, there were < 1 per cent micronuclei. Sixty and eighty-two hours after ovulation, retardation of cleavage and other abnormalities were evident in test zygotes (Table 1 and Fig. 1). Retarded zygotes comprised 40, 56 and 26 per cent of cleaved ova 60 h after ovulation from matings in the first, second and third weeks respectively; corresponding values after 82 h were 70, 40 and 18 per cent. More than 90 per cent of retarded ova had other anomalies, such as anisocytosis, and multinucleate blastomeres. Such anomalies were also seen at 82 h in unretarded test zygotes—84, 82 and 38 per cent, in matings from weeks 1, 2 and 3 respectively.

The incidence of fertilized ova from the sixth mating week was markedly reduced—42, 48, 45 and 57 per cent at 10 h, 30 h, 60 h and 82 h respectively (Table 1). Most of these were at the two cell stage by 30 h. By 60 h, about half the zygotes consisted of four cells, revealing a delay of one cleavage stage. But this slight retardation was no longer apparent by 82 h. No other morphological anomalies were observed at this mating period.

The biphasic effects of TEPA on epididymal spermatozoa and spermatids, and on spermatogonia, denote different mechanisms. With post-meiotic matings, fertilization apparently proceeds normally and is followed by retarded and abnormal development of zygotes. Spermatogonial effects induced by TEPA seem to be largely restricted to a marked reduction in fertilization; such infertility is probably a consequence of aspermia[13]. These effects, however, also have a genetic component, for they are associated with pre-meiotic and post-implantation zygote deaths[1].

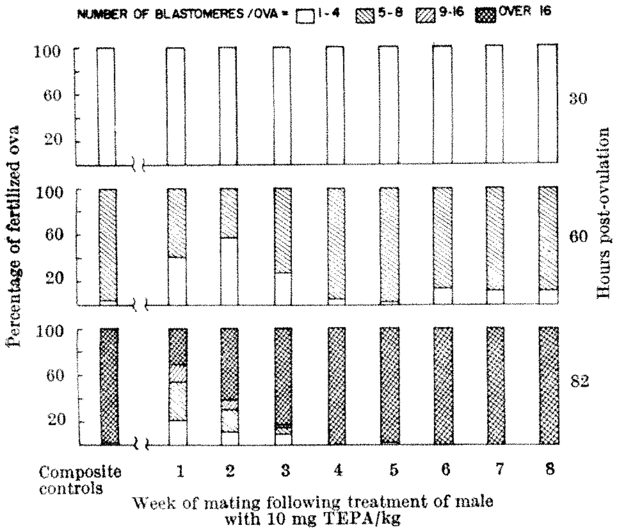


Fig. 1. Early embryonic development of fertilized ova in females mated with control males and males treated with TEPA.

This work was supported by grants from the US Public Health Service, and a contract from the National Air Pollution Control Administration.

SAMUEL S. EPSTEIN
SEWA R. JOSHI
ELSIE ARNOLD
ELAINE C. PAGE
YVONNE BISHOP

Laboratories of Environmental Toxicology and Carcinogenesis,
and
Laboratories of Biostatistics,
Children's Cancer Research Foundation;
Department of Pathology,
Harvard Medical School, and
Harvard School of Public Health, Boston, Massachusetts.

Received November 12, 1969; revised January 13, 1970.

[1] Epstein, S. S., Arnold, E., Steinberg, K., Mackintosh, D., Shafner, H., and Bishop, Y., *Toxicol. Appl. Pharmacol.* (in the press).
[2] Epstein, S. S., and Shafner, H., *Nature*, **219**, 387 (1968).
[3] Amoroso, E. C., and Parkes, A. S., *Proc. Roy. Soc.*, B, **134**, 57 (1947).
[4] Chang, M. C., Hunt, D. M., and Romanoff, E. B., *Anat. Rec.*, **129**, 211 (1957).
[5] Bedford, J. M., and Hunter, R. H. F., *J. Reprod. Fert.*, **17**, 49 (1968).
[6] Brenneke, H., *Strahlentherapie*, **60**, 214 (1934).
[7] Bruce, H. M., and Austin, C. R., *Proc. Soc. Study of Fert.*, **8**, 121 (1956).
[8] Edwards, R. G., *Proc. Roy. Soc.*, B, **146**, 469 (1957).
[9] Edwards, R. G., *Proc. Roy. Soc.*, B, **149**, 117 (1958).
[10] Cattanach, B. M., and Edwards, R. G., *Proc. Roy. Soc. Edinburgh*, **67**B, 54 (1958).
[11] Generoso, W. M., *Genetics*, **61**, 461 (1969).
[12] Chang, M. C., *J. Exp. Zool.*, **121**, 351 (1952).
[13] Fox, B. W., Jackson, H., Craig, A. W., and Glover, T. D., *J. Reprod. Fert.*, **5**, 13 (1963).

Effects of Hallucinogenic and other Drugs on the Nest-building Behaviour of Mice

WE have found that nest-building is ideally suited for the study of the effects of hallucinogenic drugs. Strains of laboratory mice differ in their nest-building activity. Males of the C57Bl/6j strain (Jackson Laboratory, Bar Harbor, Maine) are very reliable in daily testing and consequently well suited for experiment. Briefly, testing involves removal of the existing nest and provision of material for a new one. After a few days of adaptation to this procedure the mouse retrieves the material and builds the new nest within a few minutes after provision of the fresh material.

There are three primary components in nest-building: (*a*) retrieval of the nest material, (*b*) shredding behaviour (dismantling of the cotton dental balls), and (*c*) working the material together to form a single unit. We found that this sequence takes only 7 min, and the nest is in its final form within 45 min. Although nest-building may seem to be a cooperative endeavour in groups, our observations suggest that at least retrieval is competitive. In general, the pattern of behaviour is the same for isolated animals except for the occasional animal that may shred some of the cotton before retrieval. If animals are tested during their sleep period, they may only retrieve the material to make a mat, and the shredding and final shaping of the nest may not occur until the high activity period.

The drugs tested were chlorpromazine, chlordiazepoxide, desipramine, mescaline, 2,5-dimethoxy-4-methyl amphetamine (DOM), 2,5-dimethoxy-4-ethyl amphetamine (DOET), 3,4,5-trimethoxy amphetamine (TMA), D-amphetamine and LSD_{25}. Animals were placed individually in 26×35 cm wooden boxes with a wire floor at least 3 days before receiving nesting material, to allow for exploration and choice of a sleeping corner, which usually became the nest site. Twenty animals, 60 days old at the beginning of treatment, received a wide range of doses of each drug. All injections were of 0·1 ml., administered intraperitoneally at intervals of at least 4 days. Each animal served as its own control, receiving saline injections at the beginning and the end of each set of drug treatments.

On a test day, an animal was injected with drug or saline, returned to its box, and the old nest was removed. Thirty minutes after injection, forty tightly packed fresh dental balls, 6 mm in diameter, were placed randomly on the floor of the box and we recorded the behavioural pattern, state of activity relating to retrieval, shredding, time required to complete retrieval, number of cotton balls retrieved and the quality of the completed nest. Twenty-four hours after administration of the drug, animals were tested without injection to determine whether recovery from the drug was complete.

There was no readily observable effect of chlorpromazine, chlordiazepoxide and desipramine on the primary components of nest-building except in doses which obviously impaired motor activity. In these cases there was only partial retrieval of the cotton balls, or the behaviour was completely inhibited, presumably until the effects of the drug had subsided.

Mescaline and the amphetamine compounds with known hallucinogenic properties, however, produced considerable changes in behaviour. Effects were most pronounced during the retrieval phase. All drugs induced a stereotyped behavioural pattern that competed with and disrupted the normal pattern. The duration of the competing behavioural pattern was dependent on the dose and type of drug and was typically followed by a period of inactivity before the onset of the normal retrieval pattern. Descriptions of the various induced behavioural characteristics observed during the time that retrieval of the material to the nest site would have occurred normally are presented in Table 1. A drug is not included in the matrix unless the behavioural characteristic was observed in at least 50 per cent of the animals.

Table 1. QUALITATIVE ANALYSIS OF THE DRUG EFFECTS AFTER INITIAL PRESENTATION OF NESTING MATERIAL

	Dose in mg/kg								
	0·25	0·50	1·0	2·0	4·0	8·0	10·0	20·0	25·0
Actively avoids cotton, runs between or jumps over			AMPH	AMPH	AMPH				
Shreds intensively						AMPH*			
Explores cotton with minimal handling		DOET	DOET	DOET					
Explores cotton vigorously, repeated stereotypic manipulation of dental balls	DOM	DOM	DOM	DOM*	DOET*	TMA	TMA* MESC	MESC	
Paroxysms of ear and neck scratching		DOET	DOET	DOET DOM*	DOET*		TMA*	MESC	MESC*
Mild tremors				DOM*	DOET*	AMPH*			MESC*
Inhibition of retrieval of nest material	DOET DOM	DOET DOM AMPH	DOET DOM AMPH	DOET DOM* AMPH	DOET* AMPH	TMA AMPH*	TMA* MESC	MESC	MESC*

* Maximum dose used.

DOM, TMA and mescaline produced the same behavioural profile with a potency relationship similar to that reported for humans[1]. DOET and D-amphetamine induced behavioural patterns unique to each drug and easily differentiated from the effects produced by the other drugs. Four mg/kg of DOET had an effect similar to that of DOM, however. Likewise, the behaviour induced by 8 mg/kg of D-amphetamine changed from apparent avoidance of the cotton to intensive fine shredding.

We found that the animals were shredding the tightly packed dental balls a single strand at a time. Avoidance of the dental cotton balls after injection with D-amphetamine is not merely the result of increased activity. All drugs used increase activity, without the same end result. Furthermore, other experiments with morphine, resulting in high activity, did not induce active avoidance of the

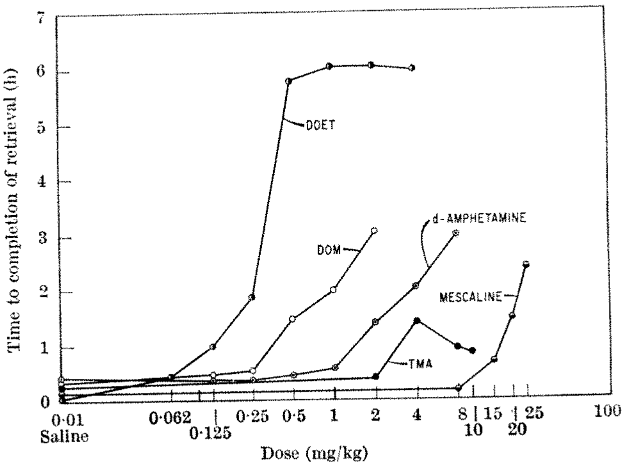


Fig. 1. Curves showing the time passed before retrieval of cotton to the nest site. Each curve is based on the mean of twenty animals.

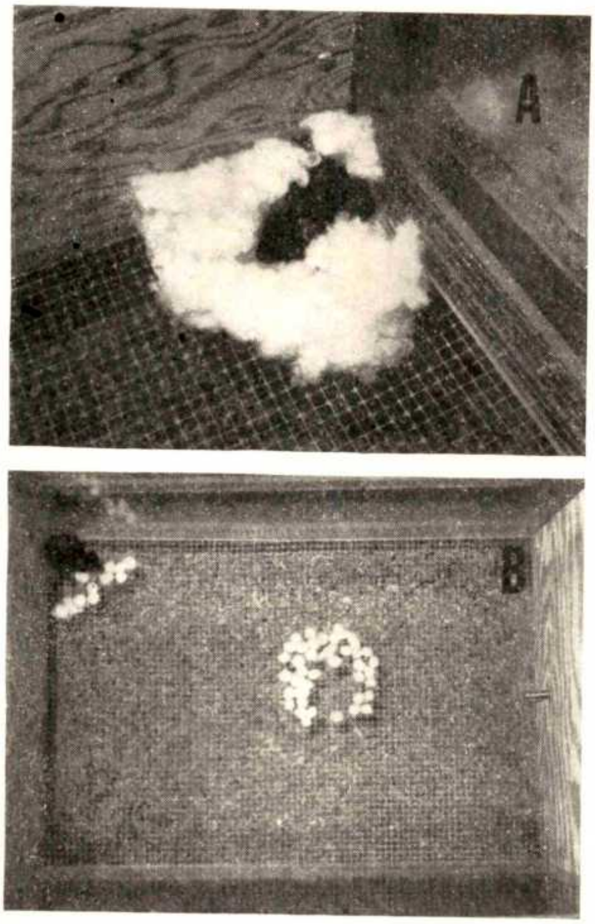


Fig. 2. *A*, Picture of a saline control taken 2 h after injection. *B*, Same animal 5 h after an injection of 100 μg/kg of LSD. The animal surrounded itself with cotton, sat in the centre for 1 h, moved to the nest site, placed the remaining cotton across the front of the area and remained in the same position for 6 h before completing the nest.

cotton balls. All drugs except D-amphetamine produced paroxysms of ear and neck scratching that were concurrent with the other competing responses, although they did not seem to interfere with the behaviour.

Table 2. PROPORTION OF ANIMALS IN WHICH VARIOUS DOSE LEVELS OF DRUGS INDUCED COMPETING RESPONSES INHIBITING RETRIEVAL OF NESTING MATERIAL

Dose level	DOM	DOET	D-Amphetamine	TMA	Mescaline
0·062	0·00	0·10	0·00	—	—
0·125	0·40	0·30	0·20	—	—
0·250	0·85	0·70	0·40	—	—
0·500	1·00	1·00	0·65	—	—
1·000	1·00	1·00	0·85	—	—
2·000	1·00	1·00	1·00	0·00	—
4·000	—	1·00	1·00	0·40	—
8·000	—	—	1·00	0·55	0·00
10·000	—	—	—	0·75	0·60
20·000	—	—	—	—	0·75
25·000	—	—	—	—	1·00

Table 2 shows the proportion of animals displaying competing responses at each dose. DOET and DOM are clearly the most potent of the drugs used. DOET is also much longer lasting, as indicated by the length of time before completion of the nest (Fig. 1). The curve for TMA (Fig. 1) suggests that the animals were developing a tolerance to the drug. But this did not seem to alter the quality of the competing response, although it shortened the duration. Tolerance was not observed with any of the other drugs.

Because our supply of LSD_{25} was limited, the results, while compelling, must remain tentative. Six animals were tested with 25, 75 and 100 μg/kg and the behavioural effects observed contained none of the characteristics of the stereotyped response induced by mescaline and the amphetamine compounds. Retrieval of the cotton may begin normally but is interrupted by frequent periods of inactivity. Some animals retrieved cotton to corners other than the nest site. Animals were observed carrying the same cotton ball from one corner to another continuously. Marked alterations in the topography and location of the nest (Fig. 2) were common. Behaviour was always altered by 100 μg/kg, and only two animals revealed any effect at smaller doses.

We thank Rose Eng and Sally Evans for technical assistance and Paul Pietsch for critical comments.

C. W. SCHNEIDER
M. B. CHENOWETH

Biochemical Research Laboratory,
The Dow Chemical Company,
Midland,
Michigan 48640.

Received September 9, 1969.

[1] Shulgin, A. T., Sargent, T., and Naranjo, C., *Nature*, **221**, 537 (1969).

Antennae: Another Wind-sensitive Receptor in Locusts

FIVE pairs of wind-sensitive hair beds at the upper part of the head control flight posture and wing movement of locusts[1-7]. But the antennae of flies and bees are airflow receptors which regulate the insect's flight speed relative to the surrounding air and stabilize the flight course in the horizontal plane[8-12]. During tethered flight these insects hold their antennae in a characteristic flight position. Exposed to the air current of a wind tunnel, they will move their antennae actively against the direction of the air current. This movement is considered as an antennal positioning reaction, which adapts the operating range of the receptor to different air speeds[10-12].

A similar forward movement of the antennae can be seen in locusts during tethered flight in the air current from a wind tunnel. It is of interest whether this antennal movement is induced passively by aerodynamic forces or actively by the animal itself, and whether this movement is connected with flight control.

The antenna of *Locusta migratoria* consists of the basal scape, the pedicel and a number of smaller segments forming the flagellum. The joint between head capsule and scape and the joint between scape and pedicel may be moved actively by muscles[13,14], whereas the morphological arrangement of the joint between pedicel and flagellum makes possible only slight passive movements caused by exterior forces acting on the flagellum. These passive movements are perceived by mechanoreceptors of the pedicel: the organ of Johnston, another chordotonal organ and campaniform sensillae[14-16].

In locust at rest the antennae may be held in different directions. Uvarov[17] has noted that "the antennae during flight are kept in a very rigid state at an angle to the body and may serve to record changes in the direction of air currents". For the exact evaluation of antennal movements I photographed the head region of locusts in tethered flight from above, some seconds after removal of the support (subdued red light; exposure time 1 s). The "antennal angle" (γ = angle between antenna, projected in horizontal plane, and longitudinal axis of the wind tunnel) was measured on the films. The antennal position of locusts in tethered flight depends on the speed of the frontal air current: without air current the antennal angle is about 50°. With increasing air speed the antennal angle will decrease (Fig. 1, *n*).

To find out whether this decrease is a direct effect of the air current or a reaction of the animal to stimulation by air current, I had to eliminate the receptors which are possibly controlling the antennal movements. It was obvious to suggest that the receptors are the mechanical sense organs of the pedicel, because the corresponding sense organs in flies and bees control the antennal positioning reaction and the flight movements[10-12]. Moreover, Uchiyama and Katsuki have pointed out[18] that the mechanoreceptors of the pedicel of locusts can be stimulated by contact stimuli or by air current applied to the flagellum. These receptors were therefore eliminated by immobilizing the pedicel–flagellum joint with wax colophony. Neither the active movement of the head–scape joint nor those of the scape–pedicel joint were directly impaired by this operation. The antennae were able to move freely in all directions. In tethered flight, however, the antennal angle was not significantly reduced with increasing air speed (Fig. 1, *i*), as occurs in unoperated locusts (Fig. 1, *n*), but remained nearly independent from the air current. Thus in normal animals the mechanoreceptors of the pedicel indicate the air speed and control the antennal angle by means of the antennal muscles of head and scape.

To determine whether there are wind-sensitive receptors in the pedicel that influence the flight movements, the wings were photographed in the stroke plane, which was perpendicular to the optical axis of the camera. The stroke angle of the forewings (φ), as it appeared on the film, was evaluated as a convenient measure of the flight reaction. In normal animals the stroke angle decreased by about 48° after air speed had been increased from 0 to 4 m/s (Fig. 2, *n*). After immobilizing the pedicel–flagellum joint the stroke angle decreased only by about 29° (Fig. 2, *i*; the difference of decrease is significant; $P < 0.0027$). Consequently, the great decrease of the stroke angle in normal locusts during frontal air current must have been partly induced by the wind-sensitive receptors of the pedicel that were blocked during the last experiment (*i*). The decrease of the stroke angle by 29° persisting in this experiment may be caused by the wind-sensitive hair beds on the head and perhaps by other wind-sensitive receptors[3] or by aerodynamic forces.

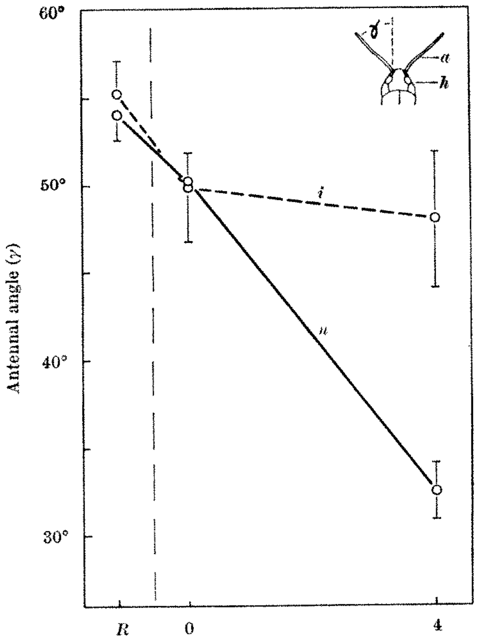


Fig. 1. Dependence of the antennal angle (γ) on the air speed of the wind tunnel. (0 m/s means that there was no air current from the wind tunnel; there was, however, an air current caused by the animal's flight movements which acted on the antenna.) Each circle represents the average value of 120 measurements (twelve for each of ten females in tethered flight). Vertical bars indicate the standard error (± *S.E.*); *a*, antenna; *h*, head; *n* normal animals; *i*, pedicel–flagellum joint immobilized; *R* marks the antennal position of the animals at rest.

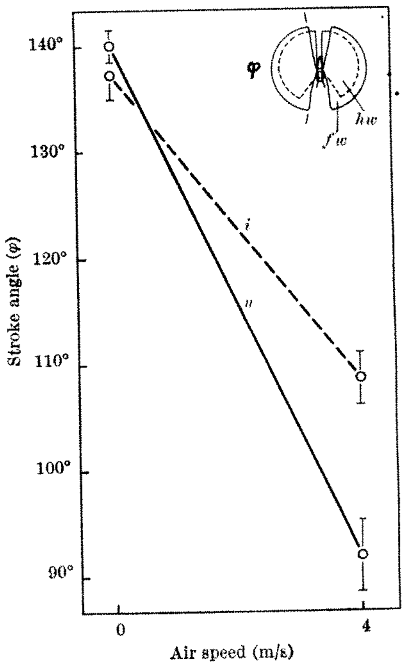


Fig. 2. Stroke angle (φ) of the forewings (*fw*) at different air speeds of the wind tunnel. *hw*, Hindwing. For other explanations see Fig. 1.

The active adjustment of the antennal angle therefore has a similar function to that of flies and bees. In all these cases it works as an antennal positioning reaction for the wind-sensitive receptors of antennae that control flight movements. Because of the active diminution of the antennal angle, the drag from frontal air current and, consequently, the bending of the flagellum with respect to the pedicel are kept relatively small. By means of the antennal positioning reaction the antennae are kept in the optimal position for a particular flight speed. Thus the wind-sensitive receptors of the pedicel are capable of fulfilling their function of measuring within a favourable range even at high flight speeds of free flying locusts.

M. GEWECKE

Institute of Zoology,
University of Düsseldorf,
Germany.

Received October 3; revised December 8, 1969.

[1] Boyd, K., and Ewer, D. W., *S. Afric. Sci.*, **2**, 168 (1949).
[2] Weis-Fogh, T., *Nature*, **164**, 873 (1949).
[3] Weis-Fogh, T., *Phil. Trans. Roy. Soc.*, B, **239**, 553 (1956).
[4] Haskell, P. T., *Proc. Fifteenth Int. Cong. Zool., London, 1958*, 960 (1959).
[5] Wilson, D. M., *J. Exp. Biol.*, **38**, 471 (1961).
[6] Guthrie, D. M., *Quart. J. Micros. Sci.*, **105**, 183 (1964).
[7] Camhi, J. M., *J. Exp. Biol.*, **50**, 335 (1969).
[8] Hollick, F. S. J., *Phil. Trans. Roy. Soc.*, B, **230**, 357 (1941).
[9] Burkhardt, D., and Schneider, G., *Z. Naturforsch.*, **12b**, 139 (1957).
[10] Heran, H., *Z. vergl. Physiol.*, **42**, 103 (1959).
[11] Burkhardt, D., and Gewecke, M., *Cold Spring Harbor Symp. Quant. Biol.*, **30**, 601 (1965).
[12] Gewecke, M., *Z. vergl. Physiol.*, **54**, 121 (1967).
[13] Albrecht, F. O., *The Anatomy of the Migratory Locust* (Athlone Press, London, 1953).
[14] Jannone, G., *Boll. Lab. Ent. agr. Portici*, **4**, 1 (1940).
[15] Eggers, F., *Zool. Bausteine*, **2**, 1 (1928).
[16] Debauche, H., *Proc. Fifth Int. Locust Conf. Brussels*, 417 (1938).
[17] Uvarov, B., *Grasshoppers and Locusts*, **1** (Cambridge University Press, 1966).
[18] Uchiyama, H., and Katsuki, Y., *Physiol. Comp. (Oecol.)*, **4**, 154 (1956).

Book Reviews

AGEING CONTROL

The Immunologic Theory of Aging

By Roy L. Walford. Pp. 248. (Munksgaard: Copenhagen, 1969.) Dkr. 97.50.

Dr Roy Walford is one of the pioneers of immunology as applied to age processes, and this book is a most powerful and critical presentation of his case. It is a tribute to the author that the book contains so much and such well organized material that, although it does present a theoretical case, it can equally be read as a general textbook of gerontology: it contains one of the best reviews of current theory and experiment to be published so far.

The association of immunological changes with age seems to be undoubted, so far as mammals are concerned. The question is whether they represent a point of access to the control of ageing—whether, in other words, ageing could be slowed or prevented clinically by slowing or preventing them. As Walford points out, several models are possible. They might represent a constant end-effect of several error processes whereby cells or molecules become not-self, or whereby, on the lines of Burnet's theory, immunocyte clones are mis-specified; they might represent one major outcome of a basic "clock" process; or they might constitute only a single group of dyshomoeostases in a general homoeostatic loss. Walford inclines to the first view—that autoimmunity cannot be ruled out as a main, if not the main, mode through which age information-loss is expressed. The possible syntheses between this outcome and the currently fancied error theories (mutation, cross-linking, free-radical attack on various systems, "error crisis" in synthetases, and clonal divergence) are all carefully explored in the light of what evidence there is.

With immunosuppression a growth-stock, it could be argued that theorizing now gives hostages to fortune. If an effective check on both humoral and tissue immunity appears shortly, the argument can probably be settled by direct trial. Of available agents, azathioprene and cyclophosphamide are highly toxic, though Walford has shown a life-prolonging effect for suitably chosen doses. The probable effects of anti-lymphocytic serum on the ageing process cannot yet be assessed, and it is in any event not as effective against humoral as against cellular immunity. If ageing autoimmune effects reflect clonal divergence, or some analogous error process, complete immunosuppression might be expected to suppress some manifestations of ageing—as anti-inflammatory agents suppress some overt pathologies—but aggravate the basic divergence. In order to control ageing, it would on this model be necessary to increase the body's censorship against mis-specified cells or molecules. Walford does not deal fully with this possibility, but it is implicit in the choice of models he advances. His reply to the argument that, autoimmune disease being apparently more prevalent in women, female life should on the autoimmune-ageing model be the shorter, is based chiefly on the selectivity of our statistics: a better case could perhaps be made on the adaptation of the female mammal to life with a half-foreign foetus. But these are perhaps the only omissions, and they derive from lack of space rather than from any reluctance of the author to deal fully and frankly with opposing arguments. In a vast body of background reading, errors are very few—perhaps the only questionable statement is that on page 150, that Tannenbaum's experiments on mouse dietary restriction gave negative results.

Our detailed knowledge of the immunology of old as against young mammals is limited, and much of what is known is due to a handful of workers, notably Walford himself and Makinodan. Many of the experimental results are, moreover, confusing to the theorist. Walford here reviews them fully, covering humoral and tissue immune mechanisms, transplantation immunity, analogies between syndromes such as amyloidosis, runt disease and progeria and the observable course of natural ageing, and the compatibility of immune models with observed or purporting modifiers of ageing rate such as radiation and calorie restriction. The actuarial and physiological criteria of ageing are fully discussed, and the author's approach to generalist theories of senescence which cover fruitflies, spiders and *Daphnia* as well as the more "immunological" phyla is robust and sensible. What might have been an indigestible or a dogmatic line is made consistently acceptable by a good prose style and a sense of proportion. In consequence the most partisan advocate of another theory of senescence is unlikely to emerge uninstructed or dissatisfied.

This is a brilliant and highly instructive performance. Gerontologists will read it with profit as a review of the contribution of immunology to their subject: immunologists and other biologists with even greater profit for the overview it gives of modern attempts to control ageing. It is rare to find a textbook which combines so fully and critically a well-argued hypothesis with a universalist survey of the entire field. Alex Comfort

HORMONES OF INVERTEBRATES

The Comparative Endocrinology of the Invertebrates

By Kenneth C. Highnam and Leonard Hill. (A Series of Student Texts in Contemporary Biology.) Pp. ix + 270. (Arnold: London, December 1969.) 80*s* boards; 40*s* paper.

This is probably the first text which covers systematically what is known about the endocrine systems of all invertebrate animals. It is an introductory text intended for the sixth form in schools and for undergraduate courses; and the authors stress the ready availability of many of these animals, notably the insects, and of some of the hormonally active chemicals, the effects of which can be demonstrated by simple class experiments. Presumably they are writing on the basis of personal experience. The book is clear and lucid throughout. About half deals with the insects which lend themselves well to hormonal studies and in which the subject is most advanced. But no attempt is made to force the endocrine system of other invertebrates into the pattern created by the insects. It is noteworthy that completely novel arrangements of great interest are beginning to be uncovered in other groups; such as the work of Burnett and Diehl on the role of neurosecretory cells in growth, regeneration and the development of sexuality in species of *Hydra*; and the work of Cheat on the release of neurosecretory substance from the radial nerves of starfish into seawater whence it is absorbed by the tube feet of the same and other individuals to precipitate the shedding of gametes.

The authors compare the neurosecretory process with the liberation of neuromuscular transmitter substances at the axon termination of ordinary neurones. But perhaps a truer comparison would be with the continuous downward streaming of the axoplasm of all neurones (carrying with it mitochondria that pile up when the axon lumen is constricted) which presumably must be liberated somewhere. So far as the insects are concerned, the section dealing with the hormonal control of reproduction is particularly good. This is the subject with which the authors' own researches have been chiefly concerned.

It is a difficult subject because the parts played by the different components of the endocrine system differ greatly even between closely related insects; but the authors manage to give a very clear and persuasive account of the whole problem. The hormonal control of growth and metamorphosis is not quite so successful. Thus they reproduce an account of the main experiments which demonstrate that the pattern of the adult body is often already laid out in invisible form in the larva—but they do not succeed in conveying the implications of these results to the reader. They produce a useful generalization about the evolution of the endocrine system, derived from the Sharrers, which must not, of course, be pursued too far: that in the more primitive invertebrates the endocrine system consists only of secreting neurones; that in the higher invertebrates (insects) there is often a two-tier system in which the neurosecretory products act on epithelial endocrine glands; and that in the vertebrates a three-tier system may occur in which the epithelial endocrine gland can exert a trophic influence on a second epithelial gland.

Altogether, this book provides a stimulating introductory account of the subject. V. B. WIGGLESWORTH

POPULATION REGULATION

Population Dynamics

By Maurice E. Solomon. (Institute of Biology's Studies in Biology, No. 18.) Pp. 60. (Arnold: London, November 1969.) 14*s* boards; 8*s* 6*d* paper.

M. E. SOLOMON's contribution to the "Studies in Biology" series is a welcome companion to its other ecological texts. Population dynamics is a difficult topic to deal with in a booklet of only 60 pages. The author's success in providing a readable and informative introduction to the field is achieved partly by clarity of expression aided by careful selection of diagrams but also by a rather restricted treatment. This is a controversial field in which rival views are strongly held and often acquire the status of dogma. The author avoids both controversy and dogma by largely limiting his discourse to density dependent processes in which negative feedback between numbers of animals (or plants) and the competitive demands of the population on some limiting resource is considered to be the main mechanism of population regulation.

The first three chapters give an account of the nature and properties of populations as units of ecological study. The concepts of population age structure, birth rate and death rate are discussed and the general notion of a potential for exponential increase resisted by limiting environmental resources is introduced.

Chapters four and five are concerned with observed fluctuations in numbers and with the mechanism underlying them. Easily understood graphs are used to show how birth and death rates and the intensity of parasitism or predation are related to population density. These density dependent processes, set in the context of a variable physical environment, are built into a generalized framework showing how the density of field populations may be determined. The inexperienced reader of this section may find some difficulty in deciding where demonstrable fact ends and informed hypothesis begins.

The parallels and contrasts made between plant and animal populations add little of conceptual value but at least serve to emphasize the current view of ecology as a corporate discipline involving the study of both animals and plants.

In spite of its brevity and hence rather restricted scope, this book will admirably serve its purpose in arousing interest in the field of population dynamics among school and first year university students. The final chapter, dealing with economic and social applications of population dynamics, is particularly opportune in this respect.

F. B. O'CONNOR

NATURAL PRODUCTS

Cyclopentanoid Terpene Derivatives

Edited by W. I. Taylor and A. R. Battersby. (Organic Substances of Natural Origin: A Series of Monographs.) Pp. x+432. (Dekker: New York, December 1969.) $26.50.

THIS publication is one of those rare volumes which I read avidly with delight and enthusiasm and was unable to put down until the last page.

It is the first comprehensive review of a field of chemistry which has developed very rapidly since 1958 when the structure of the first member of the group was elucidated. Since that time, knowledge of the cyclopentanoid monoterpenes and their derivatives has accumulated quickly. Ten years ago, the chemistry of these compounds consisted of various fragmentary areas with only tenuous interconnexions, but during the past five years there has been a rapid expansion and integration in this area of natural products. These developments have been catalysed by various events, but particularly by the demonstration that loganin and its closely associated derivatives are implicated as the central building unit of numerous indole alkaloids.

The volume includes chapters on the following topics: the iridoid glycosides and similar substances; picrotoxin; insect terpenoids and nepetalactone; monoterpene alkaloids; lower furanoterpenes; and sesquiterpene alkaloids.

This book is delightfully and tightly written in excellent English by authors who are active contributors and internationally recognized experts in the areas concerned. There are few excess words, but the essential information in each field is concisely presented. The formulae are numerous, clearly drawn and, in contrast to many publications, are generally placed immediately proximate to the relevant text. The standard of production and presentation is of a very high order and I noticed very few typographical errors: however, I have two minor criticisms. In chapter two there are intermittent examples of confusion between the singular and plural of the word "spectrum". In the same chapter and also in chapter four, there are several rather infelicitous statements as, for example, on page 254, line 4, where we have the comment that "these two protons appear as a singlet". But this is a minor caveat.

The authors and editors are to be complimented on a singular service to chemistry and biochemistry in general and to natural products in particular, while the publishers are to be congratulated on the speed of production. Although the official date of publication is 1969 there are even a few references from that year: references from 1967 and 1968 are numerous. If only some other publishers would follow this commendable example! This book may be recommended unreservedly to all who have any interest in this area, although the high price may regrettably deprive it of a well merited place on many private bookshelves. W. B. WHALLEY

PIONEERING RADIATION PHYSICS

The Photographic Action of Ionizing Radiations in Dosimetry and Medical, Industrial, Neutron, Auto- and Microradiography

By R. H. Herz. (Wiley Series on Photographic Science and Technology and the Graphic Arts.) Pp. xviii+629. (Wiley (Interscience): New York and London, December 1969.) 215*s*.

EVER since the first radiograph was taken in 1895, the photographic plate or film has probably been the most widely used method of detecting and measuring ionizing radiations. For specific purposes, many other methods of detection and measurement have been developed, but wherever it is necessary to integrate the effects of radiation over a period or to produce a permanent record, the photographic emulsion has remained supreme. Since 1945 there has been a spectacular rise in the available range of ionizing radiations and particles and in their applications in science and technology. This has called for new and more sophisticated measurement techniques and, alongside the developments of electronic and solid state methods, the photographic emulsion has surprisingly kept pace with the demands.

With most, if not all, of the post-war advances in the production of new photographic materials and their use with ionizing radiations, the name of Herz of Kodak has been associated. Now, in his "retirement", Dr Herz has brought together in this volume a remarkable résumé of a lifetime's activity in this field. This is not a single textbook but effectively several textbooks in one. The first five chapters give an elementary outline of radiation physics, photography and the photographic effects of ionizing radiations. They could well serve as an introductory text for any radiologist, radiographer or physicist entering the field. Chapter six, on photographic dosimetry, could be a valuable handbook for workers in radiation monitoring and so-called health physics. Chapters seven to ten represent a 220 page textbook of radiographic technique which many examination candidates would find useful. The final chapters on neutron radiography, autoradiography and microradiography summarize most impressively the more advanced fields in which the back-room work of Herz has been most effective. Throughout the book, although phenomena are well described and theories discussed, the emphasis is on the practicalities of techniques and operations. As might be expected from such a source, the diagrams and plates are excellent and, in particular, the microradiographs and autoradiographs would bring joy to photographer, scientist or artist.

In some ways it is a pity that this one volume covers so much ground, in that no single user could possibly become involved in all the sections. Nevertheless, it is a substantial monument to the work of a great pioneer in the field. J. E. ROBERTS

PHYSICAL BIOCHEMISTRY

Theoretical Biochemistry

Physico-chemical Principles of Vital Processes. By Hans Netter. Translated and edited by J. H. Ottaway and F. M. Irvine. Pp. xx + 928. (Oliver and Boyd: Edinburgh, 1969.) 300*s*.

THE design of courses on physico-chemical topics for biochemists presents many problems. The basic material with its formalisms can only be presented as physical chemistry. At that stage too many asides on biochemical applications, which are usually rather more complicated, only confuse the students. For a course of physical chemistry applied to biochemical systems one should accept as read a good first course in physical chemistry. Unfortunately this idealized situation, which would permit the presentation of sophisticated treatments of the analysis of biochemical systems, is not usually possible. Many students of biochemistry have a totally inadequate mathematical and physical background and are unable to cope with the complexities of the physical chemistry of biological problems. As a result most courses, including my own, have to revise basic physical chemistry and fit in applications where they can. The textbooks follow suit.

The text under review covers an enormous range of topics from atomic and molecular structure to biological organization by way of static and dynamic equilibria and kinetic control. Every chapter is interspersed with a certain amount of information about biological systems. One might expect the subject matter to be dealt with in some depth in 900 pages. Alas, this hope is frustrated by heavy dilution with irrelevant historical material and a rather confused presentation of the results of out of date experiments. My overall impression was that, though the book is out of date, the problems it poses are still with us. It so happens that an old copy of Sir William Bayliss's *Principles of General Physiology* in the Kendal public library inspired me 30 years ago with an interest in the application of physical methods to the study of biological systems. The book under review has the same philosophy behind it. It is somewhat more molecular in its approach, but the molecules are still very rigid.

Major deficiencies of the translation are, first, out of date and inaccurate data and references and, second, the often quite painful English.

In spite of my many criticisms I find it very valuable to have this detailed account of Professor Netter's presentation of "Theoretical Biochemistry". It will certainly help me in the preparation of some lectures and it should be useful for advanced students. The book is, however, not a substitute for some of the texts on special topics in physical biochemistry. Several of these could be bought for the price of the text under review. H. GUTFREUND

PROPERTIES OF MATTER

The Mechanical Properties of Matter

By M. T. Sprackling. (The Bridge Series.) Pp. xii + 144. (English Universities Press: London, February 1970.) 26*s*.

THIS is a very nicely written and clearly illustrated book covering a selected set of properties of matter at a level suitable for sixth forms in schools and first year undergraduates in some universities. The topics covered in seven chapters are: elastic properties of matter, plastic deformation of solids (crystals), fracture (of crystals), liquid viscosity, viscosity of gases and surface effects. The whole contains a very clear exposition with apposite choice of experimental data to illustrate the theory, and with a carefully selected set of examples at the ends of the chapters.

In a book of this size it is difficult to include everything necessary for the student, so that some sections will remain obscure to the tyro: for example, the difference between total and free energy of a surface. As I read the book I felt myself continually urging the author to go further, with the same clear exposition. This is a measure of its quality: I am sure that the student will be inspired to do so himself. A. KELLY

HIGH LEVEL FLOW

Flow Through Porous Media

Edited by Roger J. M. De Wiest. Pp. xiii + 530. (Academic Press: New York and London, October 1969.) 210*s*.

THIS book consists of a collection of state-of-the-art reviews on a variety of topics connected with fluid flow through the upper layers of the Earth's crust. Its contents are derived from lectures presented at an institute in hydrology for college teachers sponsored by the National Science Foundation, held at Princeton University in the summer of 1965. The original material for each of the eleven chapters has been revised and brought up to date for publication.

An essential ingredient of a multi-author volume of this type is a comprehensive opening chapter, which lays the foundations for the succeeding material. The editor's chapter one ("Fundamental Principles of Ground-Water Flow") is disappointing in this respect. Too many topics are covered too briefly, giving the book the appearance of a collection of essays rather than a coherent whole.

With the exception of chapter two by S. N. Davis ("Porosity and Permeability of Natural Materials"), which contains a summary of the available experimental information on these parameters for different rock types, the book demands a sound grasp of mathematical physics on the part of the reader. The chapters vary considerably in length. Chapter three by R. R. Rumer ("Resistance to Flow through Porous Media") and chapter five by E. S. Simpson ("Velocity and the Longitudinal Dispersion Coefficient in Flow through Porous Media") form short yet comprehensive treatments of their subjects. Chapter seven by D. Dicker, chapter nine by G. de Josselin de Jong and chapter ten by the editor are longer, and require a good mathematical background to be appreciated fully.

In dealing with unsteady flows in a compressible porous medium in chapter eight, A. Verruijt reviews material drawn from the literature of both soil mechanics and hydrology. A similar interdisciplinary approach is adopted by H. J. Morel-Seytoux in chapter eleven which draws upon experience in the petroleum industry.

Chapter six by D. Swartzendruber ("The Flow of Water in Unsaturated Soils") presents an interesting blend of theory and experimental results; in this respect, this is the most balanced contribution in the book. Finally, in introducing the longest chapter, J. Bear (chapter four) takes care to point out that his review of hydrodynamic dispersion is incomplete, a testimony to the literature explosion on this aspect of porous media flow.

In general, this type of book is prone to lack of uniformity in both notation and the level of competence required of the reader. As the editor, Dr De Wiest is to be congratulated on avoiding these pitfalls. The level of presentation, however, is very high—postdoctoral rather than postgraduate. The standard is certainly above that of most one-year MSc courses, irrespective of basic discipline, and the appeal of the book seems limited to research workers in this particular field. The prospect of a small circulation is reflected in the price. M. J. HALL

COLLECTED EARTHWORKS

The Seismicity of the Earth

1953–1965. By J. P. Rothé. (Earth Sciences, No. 1.) Pp. 336. (Unesco: Paris, 1969.) 48 francs; $12; 72*s*.

THIS is, against all the odds, a splendid book and one which seismologists may well feel they need themselves as well as having access to in a library. I must admit that at first glance I feared this book would be of little value. It is unashamedly a successor to Gutenberg and Richter's classic *Seismicity of the Earth*, and re-runs do not normally arouse much enthusiasm. Further, the recent compilation of US Coast and Geodetic Survey epicentres since 1961 by Barazangi and Dorman might seem to have saturated the subject. Not at all.

The study of seismicity is more than just a process of geographical cataloguing. Variation in activity with time, loss of life and damage to property, sorting of events by magnitude and the merging of seismic data into tectonic concepts—these are all important aspects of seismicity studies, and Professor Rothé has achieved a good balance of hard facts and general speculation. He has drawn on a wide variety of sources and his bibliography is superb—nobody studying local seismicity should venture on an area until he has followed up all Rothé's leads. Further, he makes wide use of the seismological notes of the *Bulletin of the Seismological Society of America*—an invaluable compilation which earns too little credit for its editor.

After an intelligent introduction surveying global seismic activity, the regions of the world are reviewed in turn, maintaining the same layout as Gutenberg and Richter. Bilingual (English and French) comments follow the listing of all earthquakes between 1953 and 1965 with a magnitude greater than about 6·0. The magnitude criterion is relaxed, however, when small earthquakes have occurred in unusual locations or have wrought considerable damage. At the end of the book a set of maps show locations, together with some notable faults and nuclear tests.

The whole is well produced and handsomely printed. The translation is first-rate. It is difficult to find fault with this invaluable work of reference, which is going to save hours of thumbing through journals, maps and cards. D. DAVIES

TRIANGULAR PUZZLES

Geometric Inequalities

By O. Bottema, R. Ž. Djordjević, R. R. Janić, D. S. Mitrinović and P. M. Vasić. Pp. 151. (Wolters-Noordhoff: Groningen, 1969.) $4.90.

THE book consists of a list of some four hundred inequalities on triangles and a further sixty on quadrilaterals, polygons and circles. A good many have a proof or some indication of a proof and nearly all are elementary in nature. Some, however, would provide testing exercises for any mathematician, even though the majority would be of most interest to secondary school students. Hardly any of the examples require any previous knowledge. Many are of the type which says that there is a maximum or minimum in the most symmetric case. For example, if A, B, C are the angles of a triangle, then

$$\sin A+\sin B+\sin C\leq 3\sqrt{3}/2;$$

or, if h_a, h_b, h_c are the altitudes of a triangle, then

$$(3r)^3\leqslant h_a h_b h_c\leqslant(3R/2)^3$$

where r and R are the radii of the incircle and circumcircle respectively; or, the area of an n-gon inscribed in a unit circle is at most that of the regular one, namely, $(n/2)\sin(2\pi/n)$. It is mentioned that the last example was set in a high school students' competition in China in 1957. The book would be a useful addition to any school library although, it must be said, it does not greatly extend the imagination, which a book of puzzles ought to do. More precisely, even though most of the book is devoted to triangles, the more interesting examples are in the last three sections of the book. For example, if a regular hexagon of area F contains non-intersecting circles of radii $r_1, \ldots, r_n$, then

$$(r_1+\;.\;\;.\;\;.\;+r_n)^2\leq nF/\sqrt{12}$$

References to all the results are given. Finally, the authors are to be admired for their assiduity in achieving such a complete collection. JAMES HIRSCHFELD

Obituaries

Dr G. S. Carter

GEORGE STUART CARTER was born on September 15, 1893, the son of the Rev. G. C. Carter and Hilda E. Keane. His schooling was at Marlborough College and it was probably there that he gave his first scientific paper, as evidenced by the entry "G. S. Carter . . . Ants" in the record of the Marlborough College Natural History Society for February 5, 1912. This youthful endeavour was the precursor of a lifetime's work in biology.

He went up to Gonville and Caius College, Cambridge, and read for Part I of the Mathematics Tripos in 1913. The First World War then called him to serve until 1919, first in the Leicestershire Regiment and later in the Royal Engineers. Returning to Cambridge he read for Part II of the Natural Sciences Tripos in Zoology, gaining a First and being awarded the Frank Smart Prize and Studentship in 1921. His highly productive career started with the publication, while he still held the studentship at Gonville and Caius, of papers on the structure and movement of cilia in *Mytilus* and on the early development of the echinoderm egg. Two other papers on veliger cilia followed after his move to a lectureship at Glasgow, and it is clear that this early work was influenced by Sir James Gray, who was two years his senior and held the Balfour Studentship at Cambridge during Carter's time as a Part II student. His work from Glasgow at the Millport Marine Station, where he was much encouraged by Richard Elmhirst, and at Naples, led to a further nine papers on sperm and fertilization in *Echinus* and *Asterias* and concluded the first recognizable phase in his work, largely concerned with invertebrate physiology. He returned to a lectureship in Cambridge and a fellowship at Corpus Christi College in 1930, holding the former to his retirement in 1960 and the latter until his death.

A new facet of Carter's wide ranging biological interests opened up with his expeditions in the 1920s and 1930s to Brazil, the Paraguayan Chaco and to British Guiana, and subsequently in the middle 1950s to Jinja and the papyrus swamps on Lake Victoria. Substantial papers with Beadle on the South American work, a biological review, and other papers of his own followed, covering all aspects of that fascinating environment, the tropical swamp, and dealing with adaptations largely of fish and oligochaetes to this taxing mode of life. It would seem clear that his great interest in evolution arose during this work. The material collected on these expeditions was worked upon by Gurney, Lowndes and Jepps, among others, and was of wide influence.

It was perhaps in his final and maturest phase, as a writer of substantial general texts, that Carter exerted his most profound influence on the development and teaching of zoology. His *General Zoology of the Invertebrates*, first published in 1940 and running to four editions by 1961, remains a model of readable analysis and instructive information, and his *Animal Evolution* (1951) and *A Hundred Years of Evolution* (1957) were of equal value to the student and the general reader respectively. He continued writing until the beginnings of his final illness and happily saw *Structure and Habit in Vertebrate Evolution* published in 1967. This late work is perhaps the best tribute to his versatile and enquiring mind, for it forms a synthesis of physiological, structural and behavioural knowledge in this field which shows most clearly his ability to develop a new and profitable approach to an old problem in the light of a lifetime's experience of enquiry.

Although this is meant primarily to be an account of his published contributions to knowledge, the moment cannot be allowed to pass without also recording the appreciation of generations of undergraduates and colleagues who were alike given his stimulating, critical but kindly advice and encouragement. He was much loved and will be much missed by many friends.

Correspondence

Should Slides be seen Blind ?

SIR,—I fully endorse the views expressed by Roe *et al.*[1] on the subject of histological examination using a "blind" technique.

In this sort of examination the pathologist often has to decide whether the abnormality he finds could have been the result of a natural disease process rather than of a specific insult from administration of the test substance. Such a distinction may be difficult or even impossible without a knowledge of necropsy findings and of lesions in the other animals on the same regimen of treatment.

Assessment of minor degrees of change resulting from toxic damage is one of the principal objects of safety evaluation tests. Such minor changes often cannot be readily distinguished from variations in the normal appearance of tissues, or from a processing artefact, without information relating to level of treatment, state of health of the animals, and necropsy findings.

In the clinical field the pathologist relates his findings and opinion to the clinical history and biochemical results. Only by following the same procedure in experimental animal studies can a sound pathological opinion be given of any lesions.

Efforts to examine pathological material from toxicity tests "blind" have been made in the past and they have been met with unqualified disfavour by pathologists. Dr A. A. Nelson, one of the pioneers in the field of pathological examination of animal tissues from toxicity tests, when asked whether he would advise this sort of procedure[2] replied, ". . . my own feeling is that a person that couldn't give a reliable opinion if he had the data would give a worse one without it. . . . The truly blind and random reading, I think, will result in the pathologist having wider limits of normality than he otherwise would have, and eventually what is actually a mild but definite effect will be passed off as within those broad normal limits"[1].

Yours faithfully,

P. GRASSO

Chief Pathologist,
British Industrial Biological
Research Association,
Woodmansterne Road,
Carshalton, Surrey.

[1] Roe, F. J. C., Carter, R. L., Cotchin, E., and Bonser, G. M., *Nature*, **225**, 1081 (1970).

[2] Nelson, A. A., in *The Pathology of Laboratory Animals: the Recording and Reporting of Pathological Data* (edit. by Ribelin, W. E., and McCoy, J. R.) (1965).

Another *Acanthaster* Disaster

SIR,—Possible biological consequences of establishing an open waterway between the eastern Pacific and the Caribbean have recently been pointed out by several scientists[1-5]. These consequences include interactions between closely related species as well as between unrelated species. Conceivably the results of either could, from man's viewpoint, be detrimental or beneficial. Generally biologists have warned of possible bad effects of uncontrolled biological exchange between the two oceans, although Topp[4] offers a guarded opinion that the characteristics of the fish faunas, at least, will not be drastically altered.

Introductions of foreign species leading to undesirable results are well known, and to ignore the probability of a plethora of serious problems resulting from free migrations through a sea-level canal is the height of folly, but unfortunately it is difficult to predict which organisms will cause trouble in new environments. Without intensive study, only obviously inimical species can be singled out.

Weathersbee[5] has valid fears about the possible introduction of the poisonous sea snake, *Pelamis platurus*, into the Caribbean through a sea-level canal, but *Acanthaster ellisi* (Gray), the eastern Pacific crown-of-thorns starfish, may present an even greater potential danger to the Caribbean. Wholesale destruction of coral reefs by the Indo-Pacific crown-of-thorns, *A. planci*, has received considerable attention recently[6-9] and the problem has become so acute that Chesher[6] has expressed fears for the future of Pacific reefs. *Acanthaster ellisi* is so similar to its Indo-Pacific relative that separation of the two species has been questioned (personal communication with J. Halpern, University of Miami). Presumably it eats coral, although nothing is known about its biology. It is thought to be rare[10], and population growth is possibly limited by a lack of suitable coral growth in the eastern Pacific.

The known distribution of *A. ellisi* includes the Gulf of California and nearby Socorro and Clarion Islands[11,12]. The adults do not appear to extend to the Isthmus, but their distribution is not well known.

If the crown-of-thorns were to invade the Caribbean it would find a lush habitat to exploit in the present extensive coral biotope. A population explosion on a scale comparable with that of its Indo-Pacific relative would be disastrous.

An invasion of the Caribbean by *A. ellisi* is entirely possible should a sea-level canal be constructed without a barrier to the free distribution of marine animals. The alternative to risking invasions into either ocean by potentially dangerous foreign species is patently clear; construction of a suitable biological barrier in any new trans-isthmus canal.

Yours faithfully,

Lowell P. Thomas

Institute of Marine and
Atmospheric Sciences,
University of Miami,
Florida, USA.

[1] Rubinoff, I., *Nat. Hist.*, **74**, 69 (1965).
[2] Briggs, J. C., *Science*, **162**, 511 (1968).
[3] Briggs, J. C., *Bioscience*, **19**, 44 (1969).
[4] Topp, R. W., *Science*, **165**, 1324 (1969).
[5] Weathersbee, C., *Science News*, **94**, 578 (1968).
[6] Chesher, R. H., *Science*, **165**, 280 (1969).
[7] Barnes, J. H., *Austral. Nat. Hist.*, **15**, 256 (1966).
[8] Williamson, D. E., *Skin Diver*, **17** (3), 26 (1968).
[9] Harding, J., *Sea Frontiers*, **14**, 258 (1968).
[10] Ziesenhenne, F. C., *Zoologica*, **22**, 209 (1937).
[11] Madsen, F. J., *Vidensk. Medd. dansk naturh. Foren. Kbh.*, **117**, 179 (1955).
[12] Caso, M. E., *Ann. Inst. Biol. Mex.*, **32**, 313 (1962).

Pulses, Units, Pitts and Hertz

Sir,—I refer to Stark's letter[1] proposing a new unit of frequency, pitts, for nerve pulses per second, which would simplify the use of neurophysiological papers and graphs. I wish to point out that the results of radioactivity measurements are likewise expressed in pulses (counts) per unit time, and that some confusion exists concerning units of frequency, because no distinction is made between periodic and aleatory phenomena.

Frequencies of periodic phenomena are expressed in Hertz units (with dimension s^{-1}), an accepted derived unit in the International System of Units (SI). The same dimension applies to averaged frequencies of aleatory phenomena like those mentioned in the preceding paragraph; the word "averaged" is intended to imply that the duration of the measurement is appreciably larger than the statistical frequency of the aleatory phenomenon. According to the latest ICRU report on radiation quantities and units[2], the unit for activity, the Curie (accepted by SI), is defined as $3{\cdot}7\times10^{10}$ nuclear transformations (that is disintegrations or isomeric transitions) per second. However, in accordance with the former definition of the Curie as a unit of quantity of a radioactive nuclide, the presently accepted definition of the Curie includes the words ". . . nuclear transformations occurring in a *quantity* of radioactive nuclide per second", although the italicized part is irrelevant since the dimension of the Curie unit is s^{-1}.

Until a treaty for the non-proliferation of scientific units (and journals) has been signed, I support Stark's proposal[1], with the proviso that frequencies of periodic phenomena should be expressed in Hertz, results of absolute measurements of radioactivity in Curie, and averaged frequencies of aleatory phenomena (including results of relative measurements of radioactivity) in a new unit (all these three units have s^{-1} as dimension). It would have been better to have only two frequency units, one for periodic and the other for aleatory phenomena, but this seems to be no longer possible.

Yours faithfully,

Alexandru T. Balaban

Institute of Atomic Physics,
Bucharest,
Roumania.

[1] Stark, L., *Nature*, **225**, 394 (1970).

[2] *Radiation Quantities and Units*, Report No. 11 of the International Commission on Radiation Units and Measurements (Washington, DC, September 1, 1968).

University News

Dr Donald G. Rea, NASA, has been appointed to the newly created post of assistant laboratory director for science at the Jet Propulsion Laboratory, **California Institute of Technology.**

Professor C. R. O'Dell has been reappointed chairman of the Department of Astronomy and Astrophysics and director of the Yerkes Observatory, and **Professor Eugene N. Parker** has been appointed chairman of the Department of Physics, in the **University of Chicago.**

Dr Eli Chernin has been appointed professor of tropical public health in the Faculty of Public Health, **Harvard University.**

Professor Richard H. Greenwood, University of Queensland, has been appointed to the second chair of geography, at the **University College of Swansea.**

Appointments

Professor J. Cruickshank, University of Sussex, has been appointed to membership of the **University Grants Committee,** and **Professor C. E. H. Bawn,** University of Liverpool, **Professor R. C. Cross,** University of Aberdeen, and **Professor J. Diamond,** University of Manchester, have been reappointed.

Corrigendum. At a meeting of the Parkinson's Disease Society on February 11 the impression arose that patients involved in trials of L-dopa would have to stop taking the drug when trials are completed. (*Nature*, **225**, 675; 1970.) Professor J. N. Walton, chairman of the MRC working party on L-dopa, has pointed out that this is not correct, and that patients who benefit from L-dopa have been guaranteed a continuing supply after completion of trials.

International Meetings

April 2–3, **Creativity in Engineering,** Birmingham (C. Hearn Buck, University of Aston in Birmingham, Gosta Green, Birmingham 4).

April 7–9, **Statistical Techniques for Library and Information Work,** London (Education Officer, Aslib, 3 Belgrave Square, London SW1).

April 7–10, **Special Library and Information Work,** London (Education Officer, Aslib, 3 Belgrave Square, London SW1).

April 7–10, **Bio-Medical Engineering,** Oxford (J. D. Gasking, Department of Pharmacology, The Medical College of St Bartholomew's Hospital, Charterhouse Square, London EC1).

April 9–10, **Metabolism and Biological Functions of Polyamines,** New York (New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021, USA).

April 14–16, **Handling Current Records,** London (Education Officer, Aslib, 3 Belgrave Square, London SW1).

Sabbatical Itinerants

In the hope of providing some practical assistance in the good cause of mobility between laboratories, *Nature* advertises the needs for housing of families about to take up periods of sabbatical leave. To begin with, no charge will be made for advertisements like this. It is hoped that a period of experiment will show what form these advertisements could most usefully take and whether they are effective.

Wanted: Furnished, centrally heated house or flat in Hampstead or vicinity for medical sociologist, wife and three children, for one year beginning September 1970. Please contact Dr Robert S. Weiss, Department of Psychiatry, Harvard Medical School 58 Fenwood Road, Boston, Massachusetts 02115, USA.

Wanted: Partially or fully furnished house or flat with 3 bedrooms and central heating in north central London, for a period of 10 months to 1 year beginning August 15 to September 1, 1970. Please contact Professor James A. Olson, c/o The Rockefeller Foundation, GPO Box 2453, Bangkok, Thailand.

Wanted: 3- or 4-bedroomed, furnished house with a small garden for an Oxford botanist and family, near the University of Berkeley, California, from January 12 to the end of August 1971. Please contact Dr B. E. Juniper, Botany School, Oxford University, South Parks Road, Oxford, OX1 3RA.

Vacant: 4- or 5-bedroomed detached modern house, fully automatic central heating, fully furnished, with double garage and large garden, 4 miles from centre of Oxford. Available December 15, 1970, to the end of August 1971. Please contact Dr B. E. Juniper, Botany School, Oxford University, South Parks Road, Oxford, OX1 3RA.

Vacant: Centrally heated house, near Harpenden, Hertfordshire. Good rail service (30 minutes) to London. 3 bedrooms, study (or fourth bedroom), living room, dining room, kitchen, bathroom, double garage and easily maintained garden. Available from end of September 1970, for one year. Please contact J. C. Gower, Department of Statistics, Rothamsted Experimental Station, Harpenden, Hertfordshire.

Vacant: From mid-June to mid-September, 2-bedroomed house in Cambridge; fully furnished, central heating, garage. Please contact Dr N. L. Malcolm, 255 Campkin Road, Cambridge.

Vacant: From early July to early September 1970, 5-roomed groundfloor flat in London, WC1. Central heating, key to private garden with tennis court. Please contact Dr J. A. Edgington, 19 Mecklenburgh Square, London WC1 (telephone 01-837 7800).

Vacant: 3-bedroomed furnished house in Bethesda, Maryland, within walking distance of the National Institutes of Health. Available June to September 1970. Central air-conditioning. Please contact Dr Gerald Ehrenstein, Biophysics Laboratory, NIH, Bethesda, Maryland, 20014, USA.

Vacant: From July 23, 1970, until end of August 1971, furnished, centrally heated detached house with 4 bedrooms, garage and garden. 32 minutes by rail from London; close to country. Please contact Dr Robert Spence, Department of Electrical Engineering, Imperial College, Exhibition Road, London SW7.

British Diary

Monday, March 30

Rubber Blank Preparation (6.45 p.m.) Mr P. D. Lanham, Institution of the Rubber Industry, at the Waldorf Hotel, Cooper Street, Manchester 2.

Tuesday, March 31

Yield, Deformation and Fracture of Polymers (four-day symposium) Society of Chemical Industry; jointly with the British Polymer Physics Group, British Society of Rheology; Institution of the Rubber Industry; Plastics Institute; Materials Science Club; Institute of Physics and the Physical Society, at Churchill College, Cambridge.

Wednesday, April 1

Chemical Engineering in the Pharmaceutical Industry (2 p.m. symposium) Institution of Chemical Engineers, at Heriot-Watt University, The Grassmarket, Edinburgh.

Direct Digital Measurement of Physical Quantities (6 p.m. discussion) Institution of Electronic and Radio Engineers, at 9 Bedford Square, London WC1.

Electrical Measurement on Acoustic Surface Wave Devices (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

The Interaction of Pesticides and Herbicides with Biochemical Systems; and Scientific Papers (two-day meeting) Biochemical Society, at the University College of Wales, Penglais, Aberystwyth.

The Use of Aluminium Cables in Industry (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Thermodynamics (four-day international conference) Institute of Physics and the Physical Society, at University College, Cardiff.

X-ray Analysis—Past, Present and Future (three-day Bragg symposium) Royal Society's National Committee for Crystallography; Institute of Physics and the Physical Society; and the International Union of Crystallography, at the Royal Institution, 21 Albemarle Street, London W1.

Thursday, April 2

Can Economic Control Models ever Work in Real Life? (5.30 p.m. discussion) Institution of Electrical Engineers, jointly with the Institute of Measurement and Control; and the Automatic Control Group of the I.Mech.E., at Savoy Place, London WC2.

Measurement Audits (Laboratory Inter-comparisons) (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Moulding to Tolerance (6.30 p.m.) Mr T. R. Weal, Plastics Institute, at Imperial Chemical Industries, Ltd., Britannia House, 50 Great Charles Street, Birmingham 3.

Friday, April 3

Accurate Methods of Analysis for Major Constituents (two-day meeting) Society for Analytical Chemistry; and the Analytical Chemistry Section of the Koninklijke Nederlandse Chemische Vereniging (Royal Dutch Chemical Society), at Imperial College, London SW7.

Aeronautical Communication by Satellite (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Cultured Human Cells and the Biology of Senescence (4.30 p.m.) Professor Leonard Hayflick, National Institute for Medical Research, at Mill Hill, London NW7.

Implementation of Digital Filters (5.30 p.m.) Professor R. Boite, Institution of Electrical Engineers, at Savoy Place, London WC2.

The Engineer and His Development in Society (9.30 a.m. symposium), Society of Engineers, on board the *M.V. Suerita*, Westminster Pier, London SW1.

Monday, April 6

Bio-Engineering 1970 (six-day conference) Biological Engineering Society, at the University of Oxford.

Modern Electronic Instrumentation (five-day vacation school) Institution of Electrical Engineers, at the University College of North Wales, Bangor, North Wales.

Multi-Element Phased Arrays (5.30 p.m. discussion) Institution of Electrical Engineers, at Savoy Place, London WC2.

Operational Research in the Chemical Industry (6.30 p.m.) Society of Chemical Industry, at 14 Belgrave Square, London SW1.

Post-graduate School on Absorption, Distribution and Metabolism of Drugs (5 days) Pharmaceutical Society of Great Britain, at Chelsea College (University of London), Manresa Road, London SW3.

Scientific Papers on Aspects of Mammalian Development (two-day meeting) British Society for Developmental Biology, in the Department of Zoology, University of Cambridge.

Spring Conference (three days) Association for High Speed Photography, jointly with the Shock Tube Liaison Group, at the University of Liverpool.

The Chemistry and Mineralogy of Meteorites and Extraterrestrial Matter (three-day symposium) Mineralogical Society of Great Britain and Ireland; the Meteorological Society and the International Association of Geochemistry and Cosmochemistry, at the British Museum (Natural History), London SW7.

Reports and Publications

(*not included in the monthly Books Supplement*)

Great Britain and Ireland

University Grants Committee. Non-recurrent Grants—Notes on Procedure, 1969. Pp. iii+56. (London: HM Stationery Office, 1970.) 10s (50*p*) net. [131

Educational Publications Limited. Wallcharts, C 1043: Bees (Hymenoptera). 30 in. × 40 in. 15*s*. C 1044: The Honey Bee (Apis Mellifera).

30 in. × 40 in. 15*s*. (East Ardsley, Wakefield and London: Educational Productions Limited, 1969.) [141

University of Cambridge, School of Agriculture Memoirs. Memoir No. 41: A summary of the papers published and research in progress by the members of the Staff of the School of Agriculture and its Associated Research Organizations during the period October 1st, 1968–September 30th, 1969. Review Series No. 24: The Commonwealth Bureau of Plant Breeding and Genetics. Pp. 24. (Cambridge: The University, 1970.) 3*s*. [141

Greater London Council—Scientific Branch. Annual Report of the Scientific Adviser for 1968. Pp. 88. (London: The Greater London Council, 1970.) 15*s* (postage extra). [141

The Settlement of International Disputes. By F. S. Northedge. (David Davies Annual Memorial Lecture, London, 24 November, 1969.) Pp. 21. (London: David Davies Memorial Institute of International Studies, 1969.) 8*s*. [151

Ministry of Technology. Industrial Research and Development in Government Laboratories—a New Organization for the Seventies. Pp. 17. (London: HM Stationery Office, 1970.) 2*s* 6*d* (12·5*p*) net. [151

The Wellcome Foundation Limited. Annual Report and Accounts for the year ending 31st August, 1969. Pp. 32. (London: The Wellcome Foundation Limited, 1970.) [151

Diamond Research 1970. Pp. 27. (London: Industrial Diamond Information Bureau, 1970.) 10*s*. [161

The Zoological Record. Vol. 103, Section 19, 1966: Mammalia. Compiled by Jean Ingles, John Fitzgibbon, Hazel Bartlett and Michael Dadd. Pp. vi + 377. UK and Eire, 60*s*; Overseas 70*s* ($US 8.40). Vol. 104, Section 7, 1967: Brachiopoda. Compiled by Patrick D. Armitage and Margaret O'Hanlon. Pp. 20. 15*s*. Vol. 105, Section 8, 1967: Bryozoa (Polyzoa) and Entoprocta. Compiled by Patrick D. Armitage and Eileen D. Hatch. Pp. 13. 12*s* 6*d*. (London: The Zoological Society of London, 1969.) [161

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 256, No. 810 (15 January, 1970): Responses and Acclimation to Salinity in the Adults of some Balanomorph Barnacles. By B. A. Foster. Pp. 377–400. 12*s* 6*d*; $1.60. Vol. 156, No. 811 (15 January, 1970): The Structure and Function of a Connective Chordotonal Organ in the Cockroach Leg. By D. Young. Pp. 401–428 + plates 77–88. 34*s*; $4.40. (London: The Royal Society, 1970). [161

Other Countries

US Department of the Interior: Geological Survey. Bulletin 1272-A: Phosphate Rock in Colombia—a Preliminary Report. By James B. Cathcart and Francisco Zambrano O. Pp. iv + 77 + plates 1–3. $1. Bulletin 1272-F: Gold Investigations in Precambrian Clastic and Pelitic Rocks, Southwestern Colorado and Northern New Mexico. By Fred Barker. Pp. iii + 22. $0.25. (Washington, DC: Government Printing Office, 1969.) [3012

Smithsonian Contributions to Zoology, No. 20: Five Species of Deep Water Cyclopoid Copepods from the Plankton of the Gulf of Alaska. By Gayle A. Heron and David M. Damkaer. Pp. 24. (Washington, DC: Smithsonian Institution Press, 1969. For sale by US Government Printing Office.) $0.40. [3012

Australian Mineral Development Laboratories. Ninth Annual Report, 1968/1969. Pp. 64. (Adelaide: Australian Mineral Development Laboratories, 1969.) [3012

World Health Organization. International Work in Cardiovascular Diseases, 1959/1969. Pp. 51. (Geneva: World Health Organization; London: HM Stationery Office, 1969.) 3 Sw. francs; 6*s*.; $1. [3112

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Memoir 358: Geology of the Annapolis—St. Mary's Map-Area, Nova Scotia. By F. C. Taylor. Pp. 65. (6 plates.) $2.50. Paper 68–64: Reflectivity and Vickers Microhardness of Ore Minerals. Prepared by C. R. McLeod and J. A. Chamberlain. 3 tables and chart. $1. Paper 69–5: Current Research in the Geological Sciences in Canada, 1968/1969. Compiled by J. F. Henderson. Pp. v + 211. $2. Paper 69–31: Stratigraphy and Engineering Description of the Soils Exposed on a Section of the Welland Canal By-Pass Project (Contract 743), Ontario, Canada. By E. B. Owen. Pp. 22. $1.50. Paper 69–33: Retreat of the Last Ice Sheet from the Maritime Provinces—Gulf of St. Lawrence Region. By V. K. Prest and D. R. Grant. Pp. 15. $1.50. (Ottawa: Queen's Printer, 1969.) [11

Cleaning Our Environment: The Chemical Basis for Action. Pp. ix + 249. (Washington, DC: American Chemical Society, 1969.) $2.75. [11

Iso-Antigenic Systems of Human Blood Platelets: a Survey. By Arne Svejgaard. (Series Haematologica, Vol. II, 3, 1969.) Pp. 87. (Copenhagen: Munksgaard, 1969.) D.Kr. 54. [21

International Atomic Energy Agency. Technical Report Series, No. 100: An International Neutron Data System. (Findings of a Panel on Neutron Data Compilation organized by the IAEA, and held in Brookhaven, 10–14 February 1969.) Pp. 33. (Vienna: IAEA; London: HM Stationery Office, 1969.) 53 schillings; 16*s* 8*d*; $2. [21

Annales Academiae Scientiarum Fennicae. Series A. III. Geologica—Geographica. Nr. 103: The Indian Ocean and Ancient Gondwanaland. Appendix: The Asian Continent and The Suboceanic Ridges. By Aarno Niini. Pp. 36. (Helsinki: Suomalanen Tiedeakatemia, 1969.) [51

US Department of Health, Education and Welfare. Highlights of Research Progress in Human Development. Pp. 11. (Bethesda, Md.: Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, 1969.) [51

The Invisible University: Postdoctoral Education in the United States. (Report of a Study conducted under the auspices of the National Research Council.) Pp. xxiii + 310. (Washington, DC: National Academy of Sciences, 1969.) $10. [51

Population Bulletin, Vol. XXV, No. 5 (Nov. 1969): A Sourcebook on Population. Pp. 52. (Washington, DC: Population Reference Bureau, Inc., 1969.) [51

US Department of the Interior: Geological Survey. Water-Supply Paper 1535-N: Occurrence and Distribution of Molybdenum in the Surface Water of Colorado. By Paul T. Voegell, Jr., and Robert U. King. Pp. iii + 32 + 1 plate. $0.65. Water-Supply Paper 1868: Sediment Transport by Streams in the Walla Walla River Basin, Washington and Oregon, July 1962–June 1965. By B. E. Mapes. Pp. iv + 32 + plate 1. $0.60. (Washington, DC: Government Printing Office, 1969.) [51

US Department of Agriculture. Miscellaneous Publication No. 1140: Distribution of Insect Pests in Cottonfields. By R. E. Fye, R. O. [illegible]uehl and C. D. Bonham. Pp. 32. (Washington, DC: Government Printing Office, 1969.) $0.30. [51

International Commission on Radiation Units and Measurements. ICRU Report 13: Neutron Fluence, Neutron Spectra and Kerma. Pp. viii + 57. (Washington, DC: ICRU Publications, P.O. Box 4869, 1969.) $3. [51

Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 138, No. 4: The Subfamily Tellinae in South African Waters (Bivalvia, Mollusca). By Kenneth J. Boss. Pp. 81–162. (Cambridge, Mass.: Museum of Comparative Zoology, Harvard University, 1969.) [51

World Health Organization. Technical Report Series, No. 433: Parasitology of Malaria—Report of a WHO Scientific Group. Pp. 70. (Geneva: World Health Organization; London: HM Stationery Office, 1969.) 4 Sw. francs; 8*s*.; $1.25 [51

Ninth Annual Report of the OECD Halden Reactor Project, 1969. Pp. 179. (Paris: OECD, European Nuclear Energy Agency, 1969.) [51

US Department of the Interior: Geological Survey. Water-Supply Paper 1921: Surface Water Supply of the United States 1961–65. Part 7: Lower Mississippi River Basin. Vol. 2: Arkansas River Basin. Pp. ix + 878. Water-Supply Paper 1935: Quality of Surface Waters of the United States 1964. Parts 5 and 6: Hudson Bay and Upper Mississippi River Basins, and Missouri River Basin. Pp. xi + 462. $2. Water-Supply Paper 1957: Quality of Surface Waters of the United States 1964. Parts 7 and 8: Lower Mississippi River Basin and Western Gulf of Mexico Basins. Pp. xii + 602. $2.50. (Washington, DC: Government Printing Office, 1969.) [61

Papers presented at a Symposium on Natural History Collections: Past—Present—Future. Edited by Daniel M. Cohen and Roger F. Cressey. (Proceedings of the Biological Society of Washington, Vol. 82. Pp. 559–762.) (Washington, DC: Biological Society of Washington, National Museum of Natural History, 1969.) $4. [61

Field Museum of Natural History. Fieldiana: Zoology. Vol. 51, No. 13 (September 30, 1969): New Birds from Camiguin South, Philippines. By Austin L. Rand and D. S. Rabor. Pp. 157–168. (Chicago: Field Museum of Natural History, 1969.) [61

Science, Technology, and Public Policy: a Selected and Annotated Bibliography. Vol. 2: Articles in Journals. Edited by L. K. Caldwell, assisted by W. B. DeVille and G. W. Lindesmith. (Prepared for the National Science Foundation by the Program of Advanced Studies in Science, Technology and Society, Department of Government, Indiana University.) Pp. xii + 544. (Bloomington, Indiana: Indiana University, 1969.) [61

Australia. Commonwealth Scientific and Industrial Research Organization. Annual Report of the Wool Research Laboratories, 1968/1969. Pp. 70. (Melbourne: CSIRO, 1969.) [71

Republic of South Africa. 35th Annual Report of the Director of Sea Fisheries for the calendar year 1967. (Reprint from *Commerce and Industry*.) Pp. 35. (Sea Point, Cape Town: Department of Industries, Division of Sea Fisheries, 1969.) [71

Institute of Applied Science of Victoria. Report of Activities 1967/1968. Pp. 24. (Melbourne: Institute of Applied Science of Victoria, 1969.) [71

Twenty-fourth Annual Report of the Council of the Queensland Institute of Medical Research for the year ended June 30, 1969.) Pp. 20. (Brisbane: Queensland Institute of Medical Research, 1969.) [71

US Department of the Interior: Geological Survey. Professional Paper 562-D: Response of a Laboratory Alluvial Channel to Changes of Hydraulic and Sediment-Transport Variables. By R. E. Rathbun, H. P. Guy and E. V. Richardson. Pp. iii + 32. $0.40. Professional Paper 614-C: The October 1963 Eruption of Kilauea Volcano, Hawaii. By James G. Moore and Robert Y. Koyanagi. Pp. iii + 13 + plate 1. Professional Paper 614-E: Distribution of Thorium, Uranium, and Potassium in Igneous Rocks of the Boulder-Batholith Region, Montana, and Its Bearing on Radiogenic Heat Production and Heat Flow. By Robert I. Tilling and David Gottfried. Pp. iii + 29. $0.40. Paper 627-A: The Precipitation Regime of Long Island, New York. By J. F. Miller and R. H. Frederick. Pp. vi + 21 + 1 plate. Professional Paper 680-B: Petrology of the Basalt Cores from Midway Atoll. By Gordon A. Macdonald. Pp. iii + 10. $0.25. (Washington, DC: Government Printing Office, 1969.) [91

HOW TO BUY NATURE

Volumes start in January, April, July and October, but subscriptions may begin with any issue.

The direct postal price per subscription for one copy of NATURE each week is:

12 MONTHS (52 issues and 4 indexes)

Great Britain and Eire.................................**£14 0 0** each
United States of America........(by air freight)..........**$48** each
Canada............................(by air freight)......**$52** each
Elsewhere overseas by surface mail...................**£14 0 0** each
(Charge for delivery by air mail on application)
*Shorter periods *pro rata*. (Minimum three months.)

Editorial and Publishing Offices of "NATURE"
MACMILLAN (JOURNALS) LIMITED
4 LITTLE ESSEX STREET, LONDON WC2
Telephone Number: 01-836 6633. Telegrams: Phusis LondonWC2
Subscription Department
MACMILLAN (JOURNALS) LIMITED
BRUNEL ROAD, BASINGSTOKE, HANTS
Telephone Number: Basingstoke 5431
Advertisements only should be addressed to
T. G. SCOTT & SON, LIMITED
1 CLEMENT'S INN, LONDON WC2A 2ED
Telephone 01-242 6264/01-405 4743
Telegrams: Textualist London WC2
Registered as a newspaper at the Post Office
Copyright © Macmillan (Journals) Limited, March 28, 1970

NEW TECHNICAL AND SCIENTIFIC DICTIONARIES

RUSSIAN-ENGLISH SCIENTIFIC AND TECHNICAL DICTIONARY

Two volumes

M. H. T. and V. L. ALFORD

Contains approximately 100,000 entries covering some 50 subjects. Grammatical and subject information is included where relevant and these volumes include a table of equivalent letters, prefixes, transliteration schemes and inflexions.

Volume 1 780 pages Volume 2 652 pages—£15 set

POLYTECHNICAL DICTIONARY

Volume 1—English/German
Volume 2—German/English

Edited by RUDOLF WALTHER with the co-operation of MARTIN STRAUSS

Featuring some 100,000 terms and words this remarkable comprehensive dictionary bridges the gap between the general, non-technical and specialized subject dictionaries.

Volume 1 1,256 pages £10 Volume 2 1,054 pages £10

RUSSIAN-ENGLISH SPACE TECHNOLOGY DICTIONARY

MICHAEL M. KONARSKI

Provides more than 10,600 terms in the fields of satellite and other spacecraft design and exploitation.

928 pages £10 hard cover

Set with Russian–English Dictionary of Modern Terms in Aeronautics and Rocketry *£15 hard cover*

FOUR-LANGUAGE TECHNICAL DICTIONARY OF DATA PROCESSING, COMPUTERS AND OFFICE MACHINES English/German/French/Russian

ERICH BURGER with the co-operation of Wolfgang Schuppe

An invaluable multilingual dictionary presenting some 13,000 terms in the rapidly developing areas of Data Processing, Computers and Office Calculating Machines. Arranged in four sections, each giving a side-by-side comparison of the terms in all four languages, employing the alphabetical order of each language in turn.

1,464 pages £12 hard cover

ALSO AVAILABLE

MULTI-LINGUAL

FOUR-LANGUAGE DICTIONARY OF ELECTRONICS

English/German/French/Russian

Compiled by P. NEIDHARDT

1,664 pages £14 10s.

FIVE-LANGUAGE DICTIONARY OF SURFACE COATINGS, PLATINGS, PRODUCTS FINISHING, CORROSION, PLASTICS AND RUBBER

English/Czech/Russian/German/French

ROBERT W. SANTHOLZER

582 pages £8

DICTIONARY OF CHEMISTRY AND CHEMICAL TECHNOLOGY —2nd Edition

English/German/Polish/Russian/French/Spanish

Compiled by H. STEPHEN, T. STEPHEN and Z. SOBECKA

1,108 pages £11 12s.

DICTIONARY OF NUCLEAR PHYSICS AND TECHNOLOGY

English/French/German/Russian

Contains 17,000 terms.

Compiled by R. SUBE

1,600 pages £11 12s.

DICTIONARY OF AUTOMOTIVE ENGINEERING

English/German/Russian/French

Includes 10,000 terms

Edited by W. MÜLLER

860 pages £11 12s.

INTERNATIONAL DICTIONARY OF REFRIGERATION

English/French/German/Russian/Spanish/Italian

Compiled by the Institut International du Froid

264 pages 81s.

TECHNICAL DICTIONARY OF HIGH POLYMERS

English/German/French/Russian

W. DAWYDOFF and H. HOWORKA

968 pages £11

BI-LINGUAL

CONCISE RUSSIAN/ENGLISH SCIENTIFIC DICTIONARY FOR STUDENTS AND RESEARCH WORKERS

Compiled by A. BLUM,
Imperial College, London

124 pages 20s hard cover
12s 6d flexi-cover

SHORTER TECHNOLOGICAL DICTIONARY

English/Polish, Polish/English

Edited by S. CZERNI and M. SKRZYNSKA

400 pages 81s.

Please write for a fully descriptive brochure on all Dictionaries available, to the **Marketing Manager, Pergamon Press, Headington Hill Hall, Oxford, OX3 0BW.**

PERGAMON PRESS

Classified Advertisements

All copy is subject to the approval of the Publishers, who retain the right to refuse or withdraw advertisements at their discretion. The Publishers will not be liable for any loss occasioned by the failure of any advertisement to appear from any cause whatever, nor do they accept liability for printers' errors, although every care is taken to avoid mistakes. Semi-displayed £7 4s. per single column inch. Minimum £3 12s., each additional 1/12th of an inch 12s. Full page £190, Half page across £96. Colour (orange) £25 extra. 2/6 is charged for the re-direction of replies to advertisement with a box number.

ADVERTISEMENTS SHOULD BE ADDRESSED TO: T. G. Scott and Son, Limited, 1 Clement's Inn, London, WC2A 2ED. Telephone: 01-405 4743. Telegrams: Textualist, London,W.C.2.

APPOINTMENTS VACANT

THE UNIVERSITY OF LEEDS

Applications are invited for posts in the following departments:

SCHOOL OF DENTISTRY-BIOLOGICAL RESEARCH UNIT

RESEARCH LECTURER IN ORAL BIOLOGY

The person appointed to the post should possess a Ph.D. degree or other higher qualification and will be required to carry out research work in the wide and expanding field covering bone, teeth and associated tissues. The appointment will be open to younger graduates who wish to undertake a career in this field.
Reference number 60/7/D.
Closing date May 31, 1970.

PHYSICS

EXPERIMENTAL OFFICER

The successful applicant will be responsible for the day to day operation of cosmic ray recording apparatus, including data reduction using the University's electronic computer. Qualifications: Degree or equivalent in Physics or Electrical Engineering. The post is open to students graduating this year.
Reference number 22/3/D.
SALARY ON SCALE: £995 to £1,460.
Applications (three copies), stating age, qualifications and experience, and naming three referees, should reach the **Registrar, The University, Leeds, LS2 9JT** (from whom further particulars may be obtained), not later than April 6, 1970. Please quote appropriate reference number.

(1260)

CAMBORNE SCHOOL OF METALLIFEROUS MINING (CORNWALL)

Appointment of

LABORATORY TECHNICIAN

for the Mining Laboratories.

Candidates should have had experience in a soil and/or rock mechanics laboratory. Assistance in other aspects such as ventilation will be required. Salary: Technician Grade II, £835 to £965 per annum—it is expected that the appointee would start at the maximum of the scale. The School provides a three-year course leading to the award of an Associateship diploma (A.C.S.M.) which is a degree equivalent.

Applications to be addressed to the Registrar. Tel.: Camborne 2167/2511.

(1243)

ULSTER MUSEUM

Zoological Technician —Taxidermist

Applications are invited for the above post in the Zoology Section of the Natural Sciences Department.

Duties are concerned with the preparation, preservation, and mounting of material for exhibition and for the study collections. Experience of fish-casting in fibre glass an advantage.

At present the Natural Sciences Department is working towards the opening of its new galleries.

Candidates should have Museum Technical Certificate and/or 4 G.C.E. 'O' levels including Zoology or Biology.

SALARY SCALE:

Museum Technician

Grade V—£860 (age 21), £885 (age 22), £910 (age 23), £935 (age 24).
Grade IV—£960 × £990 × £1,020.
Grade III—£1,040 × £1,085 × £1,135 × £1,190.
Grade II—£1,210 × £1,255 × £1,305 × £1,355.

Commencing salary will be decided on experience and age. A Candidate appointed on a lower scale will be eligible for promotion to the higher grades as shown and may eventually be considered for Museum Technician Grade I which has a maximum salary of £1,550.

Application forms may be obtained from the

ADMINISTRATIVE OFFICER,
Ulster Museum, Belfast BT9 5AB.

CLOSING DATE APRIL 30, 1970.

(1242)

SHEFFIELD REGIONAL HOSPITAL BOARD

SHEFFIELD NO. 3 GROUP OF HOSPITALS

REGIONAL MEDICAL PHYSICS DEPARTMENT

Applications are invited for posts in the Basic Grade as PHYSICISTS in the Sheffield Regional Medical Physics Department which is expanding on the commissioning of the new Weston Park Hospital. The posts involve work in radiation physics, radioisotope diagnostic techniques and medical instrumentation. Candidates should possess a good honours degree in physics or its equivalent. The salary scale for the Basic Grade is £1,134 to £1,937 with starting point depending on qualifications and experience. Candidates graduating this summer will be considered.

Applications, naming two referees, to: Chief Physicist, Regional Medical Physics Department, 21 Claremont Crescent, Sheffield, S10 2TA, from whom further details and application forms can be obtained.

(1228)

UNIVERSITY OF TORONTO

DEPARTMENT OF ZOOLOGY

The Department of Zoology invites applications for a post in General and/or Comparative Physiology at the rank of Assistant or Associate Professor, depending upon the applicant's qualifications. The successful candidate will be expected to teach and to develop an active research programme. Salary floors are: Assistant Professor, $10,500; Associate Professor, $12,300.

Inquiries accompanied with a curriculum vitae, may be directed to: Professor F. E. J. Fry, Ramsay Wright Zoological Laboratories, University of Toronto, Toronto 181, Ontario, Canada.

(1173)

ASSISTANT EXPERIMENTAL OFFICER required to collaborate with the Section Head in a detailed study on the effects of herbicides on the microflora of the soil. The candidate will handle a large variety of micro-organisms, using modern microbiological and some biochemical techniques. New microbiological laboratories with good facilities and some technical assistance available. A degree in microbiology or an equivalent qualification is required. Salary in A.E.O. grade (£872 at 21 to £1,208 at 26 to £1,454). For application form and further particulars write to Secretary, W.R.O., Sandy Lane, Yarnton, Oxford, quoting 6/70. Closing date for applications April 18. (1275)

TECHNICIANS or LABORATORY INSTRUMENT MAKERS

with experience of producing experimental apparatus are needed by the Apparatus Section of our Research Department. Inventive capability and a desire to participate in development is called for. The work ranges from devising gadgets and extemporized experimental equipment to constructing precise and complex instruments.

A scientific background is necessary and qualifications to HNC or ONC standard would be advantageous.

Conditions of service include a 5-day week of 37½ hours, Pension Fund, Profit-Sharing Scheme, Restaurant and Recreational Club. Temporary lodging allowances and generous assistance towards removal expenses are offered to married men.

Please apply, quoting reference N/2505 to:
O. Cheesmer, Personnel Department, Imperial Chemical Industries Ltd., Plastics Division, Bessemer Road, Welwyn Garden City, Hertfordshire.

ICI **PLASTICS DIVISION**

(1229)

SCIENTIFIC RESEARCH

Government of Canada

A. **RESEARCH LEADER** (Food Additives and Contaminants)—Salary to $23,077—Food Division, Food and Drug Directorate, Department of National Health and Welfare

An experienced scientist of proven ability and leadership is required to direct and supervise a research program concerned with analysis and biochemical properties of food additives and trace contaminants potentially hazardous to man. Present studies include analytical schemes for emulsifiers and flavouring agents, interaction of processing aids with foods, characterization and analysis of fungal toxins, insect metabolites, naturally occurring carcinogens and trace metals.

B. **RESEARCH SCIENTIST** (Poultry Genetics)—Salary to $19,866—Animal Research Institute, Canada Department of Agriculture, Ottawa, Ontario

The preferred candidate will have completed post-graduate training to the Doctorate level in Genetics with particular emphasis on quantitative genetics. Duties will involve collaboration with other scientists on research in poultry egg production, genetics and breeding and on the genetics aspects of a cooperative program on breeding for resistance to poultry leucosis disease complex.

Public Service of Canada

Applications, résumés or related correspondence quoting reference 70-100-01 and the position of interest, should be directed immediately to the:

**BIO-PHYSICAL SCIENCES PROGRAM,
PUBLIC SERVICE COMMISSION OF CANADA,
OTTAWA 4, ONTARIO.**

(1248)

ICI

PHARMACEUTICALS DIVISION

RESEARCH BIOCHEMIST

We have a vacancy in our Biochemistry Department for a biochemist holding a degree or equivalent qualification. The work would be with a senior biochemist, on problems connected with the Central Nervous, or Cardiovascular Systems. Previous laboratory experience would be a distinct advantage.

The modern, well-equipped Alderley Park research laboratories are set in pleasant parkland yet within easy road and rail access to Manchester and London.

Conditions of service, career prospects and assistance available to married men in moving home are designed to attract and retain staff of high calibre, and would be discussed at interview.

Applications, giving brief details of age, qualifications and experience should be sent to:

Mr. P. M. J. Eyre,
Personnel Officer,
IMPERIAL CHEMICAL INDUSTRIES LIMITED,
Pharmaceuticals Division,
Mereside, Alderley Park, Nr. Macclesfield, Cheshire.

THE QUEEN'S AWARD TO INDUSTRY 1966 1967 1968 1969

(1240)

ICI

PHARMACEUTICALS DIVISION

Cardiovascular Research

We are looking for a person to initiate research on aspects of cardiovascular disease. His original discipline may be pharmacology, physiology, or pathology. Candidates may or may not be medically qualified, but must have considerable research experience, and preferably hold a Ph.D. or similar qualification.

The successful candidate will become a member of an inter-disciplinary research project team engaged in work on different facets of Ischaemic Heart Disease and Hypertension, their prevention and treatment, and will be expected to develop independent research within the group.

The research staff at Alderley Park is representative of all scientific disciplines pertaining to research on medical and veterinary drugs. Original and fundamental research is encouraged and much freedom is given to the publication of scientific papers.

Conditions of service, prospects, and the assistance given to married men in moving home are designed to attract and retain staff of this high calibre, and would be discussed in detail at interview.

Applications giving brief details of age, qualifications and experience should be sent to:

Mr. P. M. J. Eyre,
Personnel Officer,
IMPERIAL CHEMICAL INDUSTRIES LIMITED,
Pharmaceuticals Division,
Mereside, Alderley Park, Nr. Macclesfield, Cheshire.

THE QUEEN'S AWARD TO INDUSTRY 1966 1967 1968 1969

(1241)